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Suboptimal cART Adherence is Associated with Higher Levels of Inflammation Despite HIV Suppression

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Summary: We observed higher serum levels of biomarkers of inflammation and immune activation in HIV-infected individuals who reported less than 100% antiretroviral adherence, even though they were virally suppressed (<50 HIV RNA copies/mL) at the time of biomarker measurement.

Abstract

Background: HIV-infected individuals exhibit residual inflammation regardless of virologic suppression. We evaluated whether suboptimal adherence to combination antiretroviral therapy (cART) is associated with greater residual inflammation compared to optimal adherence despite virologic suppression.

Methods: Longitudinal self-reported cART adherence data and serum concentrations of 24 biomarkers of inflammation and immune activation were measured at the same study visit in HIV RNA-suppressed (<50 copies/mL) HIV-infected men in the Multicenter AIDS Cohort Study from 1998 to 2009. Associations between dichotomized 6-month (<100% vs. 100%) and categorized 4-day (<85%, 85-99% and 100%) cART adherence with biomarker concentrations were evaluated.

Results: A total of 912 men provided 2816 person-visits with documented plasma HIV RNA suppression. In adjusted models, person-visits at which <100% cART 6-month adherence was reported had higher concentrations of interleukin (IL)-2, IL-6, IL-10, interferon-□, tumor necrosis factor-□□(TNF-□) and C-reactive protein compared to person-visits at which 100% cART adherence ($p<0.05$) was reported. These same differences were observed in person-visits reporting <85% vs. 100% 4-day cART adherence, but not in visits reporting 85-99% vs. 100% cART adherence. After adjusting for multiple comparisons, TNF-α remained significantly higher (11%, $p<0.001$) in person-visits at which <100% adherence was reported.

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Conclusions: Higher concentrations of inflammatory biomarkers were observed among HIV RNA-suppressed men who reported <100% cART adherence compared to more adherent men. Residual HIV replication (*i.e.*, below the limit of detection), more likely among men with suboptimal adherence, is a plausible mechanism. Whether improving cART adherence could impact residual inflammation and associated morbidity and mortality should be investigated.

Introduction

Sustained use of effective combination antiretroviral therapy (cART) is essential to achieve maximal efficacy in HIV treatment [1, 2]. Systemic ART exposure is directly related to host factors including age, gender, weight, diet, genetics and drug-drug interactions; however, the dominant factor impacting long-term drug exposure is adherence [3]. While modern cART regimens are more forgiving of suboptimal (*i.e.*, less than daily) drug intake, adherence remains the main predictor of HIV outcomes among cART-treated persons [3, 4]. Despite this, little is known regarding non-AIDS biological and clinical consequences associated with variations in adherence.

Initiation of cART and achievement of viral suppression has been associated with reductions in systemic inflammation and immune activation in HIV-infected individuals [5-7]. However, cART-induced viral suppression does not reduce inflammation to levels observed in HIV-uninfected persons, even in the setting of sustained viral suppression [7-10]. The state of persistent inflammation and immune activation has been linked to the development of non-AIDS adverse events including cardiovascular disease (CVD), end-stage renal disease, cognitive decline, frailty and cancer [9, 11-14]. Although the mechanism(s) behind this are not fully understood, low-level HIV viremia (viral replication below the limits of detection of most commercially available assays) may contribute to this phenomenon by inducing intermittent bursts of inflammation and immune activation [15, 16]. While the mechanism(s) of low-level viremia in chronically virally suppressed, HIV-infected individuals on cART remain poorly

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understood, variations in cART adherence could be a contributing factor [17-20]. Thus, it is feasible that suboptimal cART adherence, even among virologically suppressed individuals, could lead to persistently higher levels of inflammation and immune activation, and thereby increased risk for non-AIDS adverse events. Clear links between suboptimal adherence to cART and immune activation and/or inflammation among virologically suppressed HIV-infected individuals are lacking.

In this study we investigated whether variations in cART adherence are associated with greater levels of inflammation and immune activation among HIV-infected men, independent of plasma HIV RNA suppression.

Methods

Study design and participants

We evaluated prospectively-collected longitudinal self-reported cART adherence data from men enrolled in the Multicenter AIDS Cohort Study (MACS) between October 1998 and September 2009. The MACS is an ongoing study of HIV-1 infection among men who have sex with men at four US sites: Baltimore, MD/Washington, DC; Chicago, IL; Los Angeles, CA; and Pittsburgh, PA [21]. Briefly, participants are evaluated at 6-month intervals, and study visits include standardized interviews, physical examinations, and blood collection for concurrent laboratory analyses and storage. The MACS study protocols were approved by the local institutional review boards at each study site and informed consent was obtained from all participants before enrollment.

Our study population was restricted to HIV-infected men who reported taking cART and whose plasma HIV RNA levels were <50 copies/mL (Roche Amplicor assay) at the time of their study visit. These person-visits were further restricted to men with available biomarker measurements from a previous MACS study of inflammation and immune activation [7]. cART was defined as a regimen that contained at least 3 antiretroviral drugs including 2 nucleoside reverse-transcriptase inhibitors (NRTIs) with either an unboosted protease inhibitor (PI), a boosted PI or a non-nucleoside reverse-transcriptase

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inhibitor (NNRTI), or as an NRTI-only regimen. Due to the time period evaluated, this analysis did not include men taking integrase strand transfer inhibitors (INSTIs).

Antiretroviral adherence evaluation

Adherence to cART was measured using self-reported data collected at each study visit. Men were asked about the number of pills taken over the prior 4 days for each medication in their cART regimen and whether their cART usage in the 4 days was typical of their use since their prior study visit 6 months earlier. Two measures of adherence were calculated: a dichotomous 6-month adherence variable (based on whether their 4-day cART intake was typical since last study visit), and a categorical 4-day adherence variable (based on a percentage of the number of pills taken versus prescribed in the last 4 days). For the dichotomous 6-month adherence variable, men were classified as 100% adherent if they reported no missed doses in the past 4 days and additionally reported that this pattern was typical of the time since last study visit. Men with any other response were assigned <100% adherence. For the categorical 4-day adherence variable, a percentage of expected adherence was calculated as described previously in the MACS: (∑# of times drug taken over 4 days)/(∑# of times per day drug prescribed*4) *100 [4]. When different values of adherence were reported across drugs, the lowest adherence percentage was used. We then classified the percentage into three groups based upon data suggesting that these are clinically significant thresholds: 100%, 85-99%, and <85% [3, 4, 22-24].

Biomarkers of inflammation and immune activation

Serologic markers of inflammation and immune activation were quantified using the MesoScale Discovery (MSD, Gaithersburg, Maryland, USA) and Luminex (Luminex, Austin, Texas, USA) platforms, as previously described [7]. Serum levels of interleukin (IL)-1β, IL-2, IL-6, IL-10, IL-12p70, tumor necrosis alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon (IFN)- were measured using the Human Pro Inflammatory 9-Plex Ultra-Sensitive Kit (MSD). Chemokine (C-C motif) ligand (CCL)2, CCL4, CCL11, CCL13, CCL17, chemokine (C-X-C motif)

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ligand 10 (CXCL10)and IL-8 were measured using the Human Chemokine 7-Plex Ultra-Sensitive Kit (MSD). Soluble (s)CD14, sCD27, soluble glycoprotein 130 (sgp130), soluble IL-2 receptor-α (sIL-2Rα), soluble IL-6 receptor (sIL-6R), soluble TNF receptor 2 (sTNF-R2), B-cell activating factor (BAFF), and CXCL13 were measured on the Luminex platform. To minimize variability, all samples from a participant were tested on one plate. Levels of C-reactive protein (CRP) were measured using a high-sensitivity immunonephelometric assay performed through a clinical reference laboratory (Quest Diagnostics, Madison, New Jersey, USA).

Statistical analysis and covariate definitions

We included possible confounding covariates based on examination of covariate-adherence and covariate-biomarker relationships, and describe only those included in the final analysis. Age was treated as continuous. Race was defined by self-report and was dichotomized as white/nonwhite. Infection with hepatitis C virus (HCV) was defined by the presence of detectable plasma HCV RNA. Tobacco smoking at time of visit was based on self-report and treated as dichotomous (yes/no). Depressive symptoms were defined as a Center for Epidemiologic Studies Depression score ≥16. Diabetes mellitus was defined as an HbA1c level ≥6.5%, a fasting glucose level ≥126 mg/dL or the use of anti-diabetic medications. Anemia was defined as a hemoglobin concentration below the 5th percentile of the general population. Hypertension was defined as either systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or the use of antihypertensive medications. Absolute CD4^{+} T lymphocyte counts (cells/μL) were determined by flow cytometry and classified as >500, 351-500, 201-350, or ≤200.

Biomarker concentrations exhibited heterogeneous distributions that were not always lognormal. We therefore modeled these concentrations as generalized gamma, a flexible three-parameter distribution encompassing the lognormal, Weibull, and exponential distributions [25], to avoid imposing strong distributional assumptions on the data. In multivariate models, covariates modified the location (β) parameter of each biomarker distribution. With scale (σ) and shape (λ) parameters held constant, the effect of a covariate on the β parameter may be interpreted as a constant percentage shift in a biomarker

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distribution across percentiles of that distribution. Biomarker values that were below the lower limit of detection (LLD) for a given assay were handled by modeling the inverse of concentrations and thus employing standard methods for right-censored survival data.

We adjusted models for multiple measurements per individual. Because we tested relationships between adherence and 24 different biomarker concentrations, we adjusted for multiple tests by controlling the false discovery rate at 5% using the Benjamini-Hochberg procedure [26]. Analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, North Carolina, USA) and Stata 13 (StataCorp LP, College Station, Texas, USA).

Sensitivity analyses

We performed supplementary analyses to examine the robustness of our findings to alternate assumptions. We tested whether statin use may have confounded any associations between adherence and biomarker concentrations. We also tested whether estimated effects of lower adherence on biomarker concentrations differed by type of cART regimen, restricting regimens to either ritonavir-boosted PIs or NNRTIs, and by time on therapy. We also strengthened our definition of HIV suppressed visits by restricting the person-visits to those from men with: a) undetectable plasma HIV RNA , b) no prior visit with detectable HIV RNA (while on cART), and c) no time gaps >1 year without an HIV RNA measurement, all within the previous 5 years.

Results

Study Population

We analyzed data derived from 912 men who contributed a total of 2816 person-visits from 1998-2009 (median 2006, interquartile range [IQR]: 2003, 2008) at which HIV viral suppression was documented. Participant demographics and person-visit characteristics are shown in **Table 1**. Each participant contributed a median of 3 visits (IQR: 2, 4), and had accrued a median of 5.4 years of cART use (IQR: 2.9, 8.0) at the time of each visit. The median age across visits was 48.4 years (IQR: 42.6,

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54.0), and the median CD4$^+$ T-lymphocyte count was 584 cells/μL (IQR: 425, 775). Imperfect (<100%) and 100% 6-month adherence were reported in 362 (13%) and 2454 (87%) person-visits, respectively. Based on the 4-day adherence only, 100% adherence was reported in 2491 person-visits (88%), 85-99% adherence was reported in 112 person-visits (4%), and <85% adherence was reported in 213 person-visits (8%). Discordant adherence across ART drug types was reported in 180 (6%) person-visits (lowest level utilized). Distributions of biomarker concentrations are reported in Supplementary Table 1. Eight biomarkers had ≥1% of measurements below the LLD at the time of study visit. The proportions of LLD measurements in these biomarkers were: IL-1β (43%), IFN-γ (42%), GM-CSF (38%), IL-2 (28%), IL-12p70 (13%), CRP (4%), IL-10 (2%), and IL-6 (1%).

Relationships between cART adherence and biomarker concentrations

Models estimating biomarker concentrations as a function of 6-month adherence were adjusted for age, race, HCV infection, smoking, depressive symptoms, diabetes mellitus, anemia, hypertension, and CD4$^+$ cell count. Imperfect adherence was associated with higher concentrations of 21 out of the 24 biomarkers, and significantly associated with higher concentrations of CRP (21%, p=0.006), IFN-γ (15%, p=0.008), IL-2 (14%, p=0.022), IL-6 (12%, p=0.014), TNF-α (11%, p<0.001), and IL-10 (11%, p=0.023) relative to 100% adherence (**Figure 1**, Supplementary Table 2). After adjustment for multiple comparisons, <100% adherence was significantly associated with higher concentrations of TNF-α.

The estimated effects on biomarker concentrations associated with the categorical 4-day adherence from the multivariate models are displayed in **Figure 2** and Supplementary Table 3. Adherence between 85% and 99% was not significantly associated with concentrations of any biomarker relative to 100% adherence, with the exception of TNF-α (10% increase, p=0.019). By contrast, adherence below 85% was significantly associated with higher concentrations of six biomarkers relative to 100% adherence (same biomarkers found in the 6-month analysis): CRP (22%, p=0.019), IL-2 (20%, p=0.011), IFN-γ (17%, p=0.012), IL-6 (16%, p=0.010), IL-10 (13%, p=0.035), and TNF-α (10%,

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p=0.001). As in the 6-month analysis, the estimate for TNF-α remained significant after adjusting for multiple comparisons.

Sensitivity analyses

We additionally adjusted models for statin use, which reduced the person-visits by 256 (due to missing data). However, our findings were nearly identical and inferences remained unchanged (data not shown). We also restricted the study population to persistently virologically suppressed person-visits; among this group (n=1279 person-visits), associations between suboptimal 6-month adherence and the previously-identified biomarkers were similar, if not stronger (Supplementary Figure 1, Supplementary Table 4): TNF-α concentrations were 15% higher in person-visits reporting <100% adherence (p<0.001), which remained significant after adjustment for multiple comparisons. Additionally, imperfect 6-month adherence was significantly associated higher concentrations of IL-2 (26%, p=0.007), CRP (25%, p=0.03), IFN-γ (22%, p=0.008) and IL-6 (18%, p=0.017), plus 3 additional biomarkers, sCD27 (7%, p=0.032), CXCL13 (6%, p=0.027), and sIL-2Rα (6%, p=0.031). Furthermore, we restricted the person-visits to those in which participants were receiving cART that included either a ritonavir-boosted PI and/or an NNRTI-based (n=2283), and evaluated whether the type of cART regimen altered the effect of lower adherence upon biomarker levels (Supplementary Table 5). On the multiplicative scale, there was evidence for a significant interaction between cART regimen and non-adherence for only IFN-γ and CCL4. In both cases, lower adherence among men taking NNRTI-based cART was associated with greater increases in biomarker concentrations compared to men taking a ritonavir-boosted PI regimen (IFN-γ: 30% higher, p=0.032; CCL4: 22% higher, p=0.009). Finally, the addition of time on therapy to our model did not affect the point estimates for adherence, and inferences remained identical (data not shown).

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Discussion

In this study, we identified a positive association between suboptimal cART adherence and higher levels of inflammation among HIV RNA-suppressed, HIV-infected men receiving cART. We initially found that <100% adherence was associated with higher levels of TNF-α, IFN-γ, CRP, IL-2, IL-6, and IL-10. Further analysis revealed that these associations were largely driven by adherence levels below 85%. These associations remained significant after adjusting for various potential confounding factors that can be associated with increased inflammation and, for TNF-α, even after adjusting for multiple comparisons. In addition, these findings were unchanged after controlling for statin use, which can exert an anti-inflammatory effect [27], and after restricting the analysis to persistently HIV RNA-suppressed individuals. To our knowledge, this is the first report in which suboptimal cART adherence has been associated with heightened levels of inflammation and immune activation despite suppressed HIV viremia using standard clinical assays.

Our findings suggest that cART adherence variations could have significant biological consequences despite apparent HIV suppression, since persistent inflammation and immune activation are associated with increased morbidity and mortality among HIV-infected persons [9, 10, 28]. Although the mechanism behind this association remains unclear, residual and/or intermittent (*i.e.*, unmeasured) viral replication below the threshold of detection of conventional assays is a likely explanation that should be evaluated. Recent data from another cohort have shown that suboptimal cART adherence is associated with residual plasma viremia (quantified by ultrasensitive HIV RNA single-copy assays), despite apparent virologic suppression and regardless of cART regimen type [18]. Residual viremia has been associated with residual inflammation, increased intestinal microbial translocation, and increased cardiovascular morbidity [29, 30]. Thus, it is plausible that suboptimal cART adherence could lead to episodes of low-level HIV replication and consequent enhanced inflammation and immune activation among HIV-infected individuals who appear to remain virologically suppressed on cART. Although our data alone cannot prove this mechanism, a recent study in a small group of HIV virologic "elite" controllers (HIV

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VL <40 copies/mL), where cART initiation decreased immune activation, is consistent with this hypothesis [31].

To date, HIV viral suppression (by clinically-available assays) has been used as the primary surrogate clinical marker of cART adherence, and maintenance of undetectable HIV RNA has been presumed to indicate a level of cART adherence that is "sufficient" to avoid the adverse effects of viral replication, even if an individual is not 100% adherent [23]. Various studies have demonstrated that the level of cART adherence required to maintain virologic suppression decreases with longer duration of cART-induced virologic control [32, 33]. In this context, the association between suboptimal adherence and increased inflammation could have unique clinical significance, since declining cART adherence could contribute to levels of residual inflammation observed among HIV-infected individuals despite long-standing apparent viral suppression. It could also explain why reductions in inflammation to levels observed among HIV-uninfected persons have proven difficult to achieve among cART-treated HIV-infected persons [34, 35]. Thus, our data support routine consideration of levels of cART adherence in current and future studies evaluating HIV-associated chronic inflammation.

The biomarkers associated with cART adherence in this study reflect a wide range of inflammatory and clinical pathways that could be heightened in the non-adherent population. For example, higher IFN-□ might indicate an ongoing endogenous anti-HIV responses (perhaps driven by persistent HIV replication), while higher TNF-□ levels suggest activation of innate and adaptive immunity [36]. Similarly, elevated IL-6 and CRP levels suggest responses to an ongoing stimulus (*e.g.* residual HIV viremia) that could result in endothelial inflammation and atherosclerosis [37]. Collectively, these inflammatory and immune activation responses could contribute to the high rate of non-infectious and non-traditionally-HIV-associated clinical outcomes observed in excess (compared to HIV-uninfected persons) among HIV-infected individuals who appear to remain virologically suppressed.

Of particular interest in our study was the finding that increases in inflammatory biomarkers persisted after we restricted our analysis to individuals with long-term HIV suppression. This suggests that the negative effects of suboptimal adherence on residual inflammation and immune activation include

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a patient population generally presumed to have the highest level of cART adherence, and supports the premise that incomplete adherence, although sufficient to achieve and sustain viral suppression by conventional assays, may have significant detrimental consequences not previously identified. Whether adherence intensification in virologically suppressed persons on cART could translate into a decrease in chronic inflammation remains unclear, but warrants future study.

The strengths of our study include its prospective nature in addition to the large sample size and the comprehensive inflammatory biomarker profile obtained. Among its main limitations is that self-report may overestimate cART adherence [38, 39]; however, any such misclassification may have attenuated effect estimates relative to those that could be obtained if better measures of adherence were available. In addition, we did not include individuals taking INSTIs in this analysis. Recent reports have demonstrated that INSTIs could have a greater effect on residual inflammation in comparison to NNRTIs due to their potency and more forgiving pharmacokinetics [6]. Thus, the evaluation of these findings in individuals on chronic INSTI-based cART is needed. Furthermore, our study did not evaluate whether low levels of cART adherence were associated with the development of non-AIDS clinical outcomes, which is worthy of further investigation. Lastly, residual potential confounding associated with low adherence and high levels of inflammation, such as a poor overall health status, concomitant risk factors or lifestyle (*i.e.*, exercise, diet), could not be evaluated systematically in this population.

In summary, we demonstrated that suboptimal cART adherence is associated with enhanced inflammation and immune activation despite apparent HIV virologic suppression. Our findings set the framework to better understand the biological consequences of cART adherence variations and has identified adherence as a target for future interventions aimed at further reducing residual chronic inflammation and immune activation in HIV-infected individuals.

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Potential Conflicts of Interest

T.T.B has served as a consultant for Gilead Sciences, Merck, BMS, EMD-Serono, and Theratechnologies. K.M.E has received research grant support from Gilead Sciences and has served as a consultant for Theratechnologies. F.J.P has served as a speaker and consultant for Gilead Sciences,

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Figure Legends

Figure 1. Percent shifts in distribution of biomarker concentrations associated with <100% 6-month cART adherence, compared to 100% adherence.

Biomarker data were analyzed at person-visits where HIV-infected men reported taking cART and had plasma HIV RNA levels <50 copies/mL. Generalized gamma models were adjusted for age, race, hepatitis C virus infection, smoking, depressive symptoms, diabetes mellitus, anemia, hypertension, and CD4+ T-lymphocyte cell count. Error bars represent 95% confidence intervals. Orange filled squares indicate hazard ratios that are statistically significant at $P<0.05$. Red filled square indicates hazard ratio that is statistically significant after adjusting for multiple tests using the Benjamini-Hochberg procedure to control the false discovery rate at 5% [26]. Abbreviations: BAFF, B-cell activating factor; CCL, chemokine C-C motif ligand; CXCL chemokine C-X-C motif ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon γ; IL, interleukin; sCD14, soluble CD14; sCD27, soluble CD27; sgp130, soluble glycoprotein 130; sIL-2Rα, soluble IL-2 receptor-α; sIL-6R, soluble IL-6 receptor; sTNF-R2, soluble tumor necrosis factor 2; TNF-α, tumor necrosis factor α; CRP, C-reactive protein.

Figure 2. Percent shifts in distribution of biomarker concentrations associated with 85-99% and <85% 4-day cART adherence, compared to 100% adherence.

Biomarker data were analyzed at person-visits where HIV-infected men reported taking cART and had plasma HIV RNA levels <50 copies/mL. Generalized gamma models were adjusted for age, race, hepatitis C virus infection, smoking, depressive symptoms, diabetes mellitus, anemia, hypertension, and CD4+ T-lymphocyte cell count. Error bars represent 95% confidence intervals. Squares indicate <85% 4-

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day adherence; triangles indicate 85-99% 4-day adherence. Orange filled symbols indicate hazard ratios that are statistically significant at $P<0.05$. Red filled symbol indicates hazard ratio that is statistically significant after adjusting for multiple tests using the Benjamini-Hochberg procedure to control the false discovery rate at 5% [26]. Abbreviations: BAFF, B-cell activating factor; CCL, chemokine C-C motif ligand; CXCL chemokine C-X-C motif ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon γ; IL, interleukin; sCD14, soluble CD14; sCD27, soluble CD27; sgp130, soluble glycoprotein 130; sIL-2Rα, soluble IL-2 receptor-α; sIL-6R, soluble IL-6 receptor; sTNF-R2, soluble tumor necrosis factor 2; TNF-α, tumor necrosis factor α; CRP, C-reactive protein.

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Table 1. Characteristics of study population and person-visits

Characteristic	n	%
Unique MACS participants (n=912)		
White, non-Hispanic	532	58
Black, non-Hispanic	221	24
Hispanic	143	16
Other race	16	2
Person-visits (n=2816)		
cART at visit		
PI-based, boosted	965	34
PI-based, not boosted	437	16
NNRTI-based without PI	1318	47
NRTI/other cART without PI	96	3
Other factors at visit		
Hepatitis C infection	215	8
Hepatitis B infection	127	5
Depressive symptoms[a]	684	24
Tobacco smoking	790	28
Obese[b]	310	11
Diabetes mellitus[c]	304	11
Anemia[d]	362	13
Hypertension[e]	623	22
Statin use	826	29
$CD4^+$ T lymphocyte count at visit		
>500 cells/μL	1767	63
351-500 cells/μL	619	22
201-350 cells/μL	319	11

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≤200 cells/μL	111	4
100% 6-month cART adherence at visit	2454	87
4-day cART adherence at visit		
100%	2491	88
85-99%	112	4
<85%	213	8

Abbreviations: cART, combination anti-retroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

[a]Depressive symptoms defined as Center for Epidemiologic Studies – Depression score ≥16.

[b]Obese defined as body mass index >30 kg/m^2.

[c]Diabetes mellitus defined as hemoglobin A1C ≥6.5%, fasting glucose ≥126 mg/dL of use of anti-diabetic medications.

[d]Anemia defined as hemoglobin < 5th percentile of the general population.

[e]Hypertension defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of antihypertensive medications.

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FIGURE 1

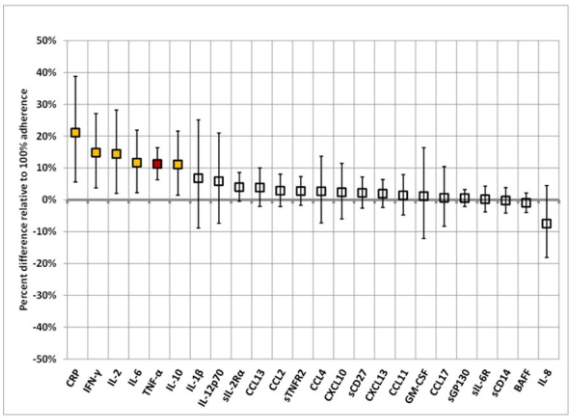

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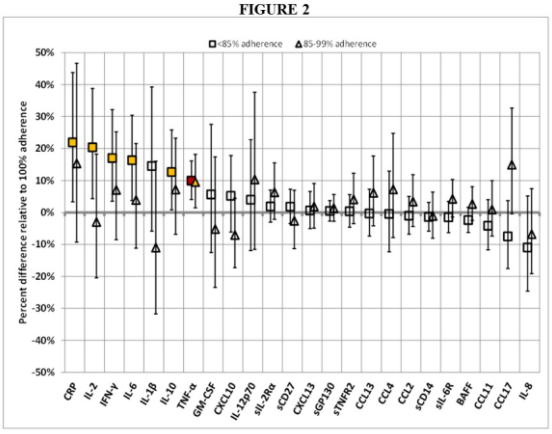
FIGURE 2



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