Incomplete adherence to antiretroviral therapy is associated with higher levels of residual HIV-1 viremia

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Objectives: To evaluate the relationship between incomplete antiretroviral therapy (ART) adherence and levels of residual HIV-1 viremia.

Design: Medication adherence and residual viremia less than 50 copies/ml were quantified in participants of a cohort of homeless and marginally housed individuals with HIV/AIDS.

Methods: Participants had at least 6 months of virologic suppression of less than 50 copies/ml and were in the adherence monitoring cohort with monthly unannounced pill counts. Residual viremia was measured by the single-copy assay.

Results: The median average ART adherence over the prior 1 and 2 months were 94% [interquartile range (IQR) 79–100%] and 93% (IQR 82–98%), respectively. Average ART adherence over the past 2 months was significantly associated with levels of residual HIV viremia (Spearman $r = -0.25$, $P = 0.04$). One-third of participants with 100% ART adherence over the past 2 months had detectable residual viremia. On multivariate regression analysis, ART adherence over the past 2 months, but not duration of virologic suppression, $CD4^+$ T-cell count or or ART regimen, was significantly associated with levels of residual HIV viremia. Detectable residual viremia was associated with virologic failure (>50 copies/ml) on univariate Cox proportional hazard analysis (hazard ratio 2.08, $P = 0.02$). However, on multivariate analysis, only ART adherence was associated with risk of virologic failure.

Conclusion: Incomplete ART adherence is associated with higher levels of residual HIV-1 viremia, but detectable residual viremia can be present despite 100% measured ART adherence.

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Introduction

The persistence of HIV-1 in the latent reservoir is reflected in the frequent detection of residual HIV-1 viremia despite long-term antiretroviral therapy (ART) [1–3] and is likely due to a number of factors, including the long half-life of some infected cell types and homeostatic proliferation [4]. However, it remains controversial whether such residual viremia is caused exclusively by intermittent HIV-1 release from the latent reservoir or whether active viral replication also plays a role. Incomplete ART adherence is common in HIV-infected patients [5,6], but its impact on residual HIV-1 viremia is unknown. However, incomplete ART adherence may go unnoticed, as complete adherence is not always needed to maintain virologic suppression to less than 50 copies/ml [7,8]. We hypothesize that incomplete ART adherence may lead to active viral replication and higher levels of residual HIV-1 viremia, even for individuals with apparently successful virologic suppression. Such a finding may indicate another mechanism for the continual replenishment of the viral reservoir and an additional cause of persistently elevated T-cell activation and systemic inflammation seen in HIV-infected patients despite virologic suppression.

We evaluated the role of ART adherence on levels of residual HIV-1 viremia using the Research on Access to Care in the Homeless (REACH) cohort. This cohort is a prospectively evaluated group of HIV-infected adults recruited from San Francisco homeless shelters, free meal programmes and low-income single-room occupancy hotels [9–12]. Participants on three-drug combination ART were invited to participate in a rigorous adherence monitoring substudy that involved unannounced visits to the participants in the community every 3–6 weeks to count the number of antiretroviral pills in their possession. This measure is closely associated with viral suppression [13], development of drug resistance [11] and disease progression [14]. We determined the association between ART adherence and levels of residual HIV-1 viremia using both univariate and multivariate analysis. We also evaluated the association between residual viremia and ART adherence with the risk of subsequent virologic failure.

Materials and methods

Study population and adherence monitoring

The REACH cohort was a prospectively followed cohort of HIV-infected adults recruited between 1996 and 2010 from San Francisco homeless shelters, free meal programmes and low-income single-room occupancy hotels [9–12]. Participants treated with at least three antiretroviral medications were invited to participate in the REACH Adherence Monitoring Cohort during which blood draws and unannounced pill counts would be performed on a random day at the participants' usual place of residence every 3–6 weeks. The inclusion criteria for the current study included individuals who had at least 6 months of virologic suppression (<50 HIV-1 RNA copies/ml), enrollment in the REACH Adherence Monitoring Cohort for at least 3 months and had available plasma for the single-copy assay (SCA).

Measurement of residual HIV-1 RNA

Low-level HIV-1 viral loads were measured using the SCA as previously described [15]. The SCA was performed on plasma samples previously found to have less than 50 HIV-1 RNA copies/ml from a time point matching the inclusion criteria above. The SCA was also performed on an available plasma sample collected 1–3 months before the primary time point [median 28 days, interquartile range (IQR) 28–31 days]. The change in viral loads between the two time points was correlated with the average ART adherence in the interval between the two time points. In addition, sequencing of the HIV-1 *gag* region and the SCA were performed on plasma samples with detectable viremia by a commercial assay to exclude individuals with potential inefficient HIV amplification by the SCA. The limit of detection of the SCA was determined by the amount of available plasma for the assay and standardized to the highest limit for any individual (0.8 copies/ml).

Statistical analysis

The associations between residual HIV-1 viremia and factors such as ART adherence were performed by Spearman correlation and Wilcoxon rank sum testing. Univariate and multivariable regression analysis were performed to assess predictors of residual HIV-1 viremia levels using a method (Proc LIFEREG, SAS 9.2) appropriate for analysis of censored viral load measurements [16]. Four factors were chosen *a priori* as potential predictors: ART adherence, duration of virologic suppression, $CD4^+$ cell count and ART regimen. The relationships between HIV-1 residual viremia, $CD4^+$ cell count, ART adherence and substance abuse with risk of subsequent virologic failure were evaluated by univariate and multivariable Cox proportional hazard models. Virologic failure was defined in the REACH study as plasma HIV-1 RNA more than 50 copies/ml. Sensitivity analysis was performed using an alternative virologic failure definition of HIV-1 RNA at least 200 and at least 1000 copies/ml. In the Cox proportional hazard models of virologic rebound, participants (28%) with regimen switch or who exited the study prior to virologic failure were censored. Substance abuse was defined as evidence of hazardous drinking as defined by the US Preventive Services Task Force [17] or any illicit drug use in the prior 90 days.

Results

Participant and adherence characteristics

In total, 64 participants met the inclusion criteria and had evaluable SCA results (Table 1). The median time of virologic suppression was 10.5 months (IQR 7.5–18.4 months) and the median ART adherence by unannounced pill counts was 94% (IQR 79–100%) in the past month and 93% (IQR 82–98%) over the past 2 months. The second SCA time point was 1 month before the primary time point in 81% of participants, 2 months before in 14% and 3 months prior in 5% of participants.

Relationship between residual HIV-1 viremia and antiretroviral therapy adherence

At the primary time point, 47% of participants had detectable residual HIV-1 viremia. There was no significant association between ART adherence over the past 1 month and HIV-1 viral load (Spearman $r = -0.13$, $P = 0.32$). However, average ART adherence over the past 2 months was significantly associated with levels of residual HIV viremia (Spearman $r = -0.25$, $P = 0.04$). On multivariable regression analysis, ART adherence over the past 2 months was significantly associated with residual viremia ($P = 0.004$), even after controlling for duration of virologic suppression, $CD4^+$ cell count and ART regimen.

For each participant, the viral loads were also compared between the primary time point and the second time point 1–3 months beforehand. No significant association was detected between the change in HIV-1 viral load and interval ART adherence (Spearman $r = 0.08$, $P = 0.56$).

Table 1. Characteristics of the participants at the primary time point ($N = 64$).

Characteristic	Median or % (*N*)	IQR
Age (years)	46	39–51
Male sex % (*N*)	81% (52)	
Ethnicity % (*N*)		
White	45% (29)	
Black	39% (25)	
Hispanic	8% (5)	
Others	8% (5)	
Substance abuse (%)	34% (22)	
cART % (*N*)		
NNRTI	47% (30)	
PI (boosted)	23% (15)	
PI (unboosted)	19% (12)	
Both NNRTI and PI	8% (5)	
$CD4^+$ cell count (cells/μl)	477	321–728
Duration of viral suppression (months)	10.5	7.5–18.4
ART adherence over prior 1 month	94%	79–100%
ART adherence over prior 2 months	93%	82–98%

cART, combination antiretroviral therapy; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptor inhibitor; PI, protease inhibitor.

Interestingly, 33% (4/12) of participants had detectable residual viremia despite 100% ART adherence over the past 2 months at either time points.

Detectable residual viremia and the risk of virologic failure

On univariate Cox proportional hazard modelling (Table 2), factors significantly associated with virologic failure (HIV-1 RNA >50 copies/ml) included detectable residual HIV-1 viremia (hazard ratio 2.08, $P = 0.02$), ART adherence over the past 2 months (hazard ratio 1.21 per 5% decrease, $P < 0.001$) and recent substance abuse (hazard ratio 1.83, $P = 0.049$). $CD4^+$ cell count was not significantly associated with risk of virologic failure. On multivariable analysis, only ART adherence remained significantly associated with risk of virologic failure (hazard ratio 1.21 per 5% decrease, $P < 0.001$). In a sensitivity analysis, we also determined factors associated with virologic failure as defined by a viral load at least 200 or at least 1000 copies/ml. On univariate Cox proportional hazard modelling, detectable residual HIV-1 viremia was no longer associated with time to viral rebound at either at least 200 or at least 1000 copies/ml (Table 2). On multivariable modelling, ART adherence and $CD4^+$ cell count were both associated with viral rebound at least 200 and at least 1000 copies/ml.

Discussion

In this study, we evaluated the relationship between recent ART adherence and levels of residual HIV-1 viremia in participants of the REACH cohort with at least 6 months of virologic suppression below 50 copies/ml. Residual viremia was detectable even in those with 100% unannounced pill count ART adherence. Lower ART adherence was associated with higher levels of residual HIV-1 viremia, even after taking into account duration of virologic suppression and other potential confounding factors. Finally, detectable residual HIV-1 viremia was associated with virologic rebound more than 50 copies/ml, but not after taking into account ART adherence, $CD4^+$ cell count and recent substance abuse.

Residual HIV-1 viremia can still be detected in the majority of individuals even after many years of suppressive ART [1–3]. There remains some controversy over whether this low level viremia is representative of viral release from the latent reservoir or ongoing viral replication. The results of this study suggest that both factors may contribute to residual viremia. The lack of ART adherence assessment has been a significant limitation of previous studies of residual HIV-1 viremia in ART-treated individuals. In contrast, the robust evaluation of adherence by unannounced pill counting is a major strength of this study. The finding that detectable residual viremia is present in some participants

Table 2. Cox proportional hazard models of virologic failure defined as HIV-1 RNA rebound more than 50, at least 200 or at least 1000 copies/ml.

Virologic failure definition	Factors	Univariate		Multivariable	
		HR [95% CI]	*P*	HR [95% CI]	*P*
VL >50 copies/ml	Detectable RV[a]	2.08 [1.13–3.81]	0.02	1.58 [0.85–2.93]	0.15
	$CD4^+$ cell count[b]	1.05 [0.99–1.11]	0.10	1.05 [0.99–1.12]	0.11
	ART adherence[c]	1.21 [1.12–1.32]	<0.001	1.21 [1.11–1.33]	<0.001
	Substance abuse[d]	1.83 [1.0–3.35]	0.049	1.57 [0.84–2.93]	0.16
VL ≥200 copies/ml	Detectable RV[a]	1.55 [0.74–3.22]	0.24	1.19 [0.57–2.49]	0.65
	$CD4^+$ cell count[b]	1.08 [1.0–1.16]	0.05	1.10 [1.02–1.19]	0.02
	ART adherence[c]	1.14 [1.05–1.23]	0.003	1.19 [1.07–1.31]	<0.001
	Substance abuse[d]	2.31 [1.12–4.74]	0.02	1.70 [0.82–3.56]	0.16
VL ≥1000 copies/ml	Detectable RV[a]	1.86 [0.78–4.43]	0.16	1.40 [0.58–3.41]	0.45
	$CD4^+$ cell count[b]	1.07 [0.99–1.17]	0.11	1.10 [1.00–1.21]	0.04
	ART adherence[c]	1.14 [1.03–1.25]	0.007	1.17 [1.06–1.31]	0.003
	Substance abuse[d]	1.54 [0.66–3.56]	0.32	1.07 [0.45–2.53]	0.88

CI, confidence interval; HR, hazard ratio; RV, residual viremia; VL, viral load.
[a]Detectable residual viremia by the single-copy assay.
[b]Per 50 $CD4^+$ cells/μl decrease.
[c]Average past 2 months, per 5% decrease in adherence.
[d]Any drug use in the past 3 months or hazardous alcohol use in the past month.

despite 100% recent measured ART adherence is consistent with the ongoing release of HIV-1 from the latent reservoir and support several previously reported lines of evidence showing sequence homology between plasma virus and latent virus from resting $CD4^+$ cells [18], minimal HIV evolution in those with detectable residual viremia [19,20] and the lack of effect of ART intensification on residual viremia or the HIV reservoir [21–25].

However, it is possible that in a subset of patients, viral replication also contributes to residual viremia as evidenced by ongoing viral evolution [26,27], changes in the viral reservoir after some treatment intensification studies [28–31] or an association of residual viremia with risk of subsequent virologic failure [32,33]. Our finding that incomplete ART adherence was associated with residual viremia levels supports this conclusion and provides a potential explanation for some of the divergent results that have been reported. In addition, a recent study [34] also demonstrated that incomplete ART adherence is associated with increased levels of cell-associated HIV-1 RNA. Together, these findings suggest that in the setting of incomplete ART adherence, a component of the plasma residual viremia may represent new rounds of HIV replication. Such replication may provide another mechanism for the persistence of HIV, as intermittent reseeding of the viral reservoir may be occurring due to incomplete adherence, even in individuals with apparently successful ART suppression by commercial viral load assays. The stronger association detected between residual viremia and average ART adherence over the past 2 months compared with average adherence over the past 1 month could suggest that average ART adherence over the past 2 months is a more accurate reflection of recent adherence, the presence of a delay in the effects of incomplete adherence and/or a greater effect of prolonged incomplete adherence.

We also found that detectable HIV-1 residual viremia was significantly associated with viral rebound more than 50 copies/ml, but not viral rebound at least 200 and at least 1000 copies/ml. Only ART adherence was consistently associated with viral rebound at all viral load thresholds on multivariate analysis. These results suggest that detectable residual viremia may precede detectable viral load more than 50 copies/ml and is likely mediated by incomplete ART adherence. Given the lack of significant association with viral rebound at least 200 copies/ml, its clinical significance remains unclear. These results provide a potential mechanism underlying the findings of several previous studies showing that the detection of viral load less than 50 copies/ml was associated with viral rebound more than 50 copies/ml [32,33].

Limitations of this study include the variety of antiretroviral regimens in this real-world cohort of HIV-infected patients and the use of older antiretroviral medications in a subset of participants. However, the association of residual viremia levels with ART adherence was apparent even after controlling for ART regimen. This study was also limited by the relatively high proportion of individuals with undetectable residual viremia, which can complicate the statistical analysis. In addition to the Spearman correlation analysis, we performed a multivariate regression modelling appropriate for fitting censored values and the results were consistent. Finally, therapeutic drug levels and tissue sampling were not available for this study and it is possible that the detectable residual viremia could also be due to incomplete absorption or inadequate penetration of drug into some HIV-infected compartments.

Although continuous virologic suppression can occur despite moderate ART nonadherence [7,8], we found

that such incomplete adherence can be associated with higher levels of residual HIV-1 viremia, which could reflect new rounds of HIV replication. However, detectable residual viremia can also be present despite 100% measured ART adherence. These results provide additional insight on the mechanisms underlying HIV persistence and suggest a benefit of routine medication adherence counselling to counter the effects of pill fatigue in patients on long-term ART with apparently successful virologic suppression.

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Conflicts of interest

This study was funded in part by a research grant from Bristol-Myers Squibb to J.Z.L. D.R.K. has served as a consultant to and/or has received research grant support from Abbott, Avexa, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, Glaxo-SmithKline, Merck, Oncolys, Pfizer, Roche, Tobira, Vertex, ViroStatistics and ViiV Healthcare. No other potential conflicts of interest relevant to this article were reported.

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