Predictors of Residual Viremia in Patients on Long-term Suppressive Antiretroviral Therapy

Lu Zheng[1], **Ronald J. Bosch**[1], **Ellen S. Chan**[1], **Sarah Read**[2], **Mary Kearney**[3], **David M. Margolis**[4], **John W. Mellors**[5], **Joseph J. Eron**[4], and **Rajesh T. Gandhi**[6] **on behalf of the AIDS Clinical Trials Group (ACTG) A5244 team**

[1]Harvard School of Public Health, Boston, Massachusetts, USA

[2]National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

[3]National Cancer Institute, Frederick, Maryland, USA

[4]University of North Carolina, Chapel Hill, North Carolina, USA

[5]University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[6]Massachusetts General Hospital and Ragon Institute, Boston, Massachusetts, USA

Abstract

Background—HIV-1-infected individuals with plasma RNA <50 copies/mL on antiretroviral therapy (ART) may have residual, low-level viremia detectable by PCR assays which can detect a single copy of viral RNA (single-copy assay, SCA). The clinical predictors of residual viremia in patients on long-term suppressive ART are incompletely understood.

Methods—We evaluated factors associated with residual viremia in patients on suppressive ART who underwent screening for a raltegravir intensification trial (ACTG A5244). The screened population was HIV-1-infected adults receiving ART for 12 months with pre-ART HIV-1 RNA >100,000 copies/mL and on-therapy RNA levels below detection limits of commercial assays for 6 months.

Results—Of 103 patients eligible for analysis, the median age was 46 years and the median duration of viral suppression was 4.8 years. Sixty-two percent had detectable viremia (>0.2 copies/mL) by SCA (median 0.2 copies/mL; quartile [Q] 1, Q3 [<0.2, 1.8]). Younger patients had lower HIV-1 RNA levels than older individuals (r=0.27, p=0.005). Patients with virologic suppression on ART for 2 years or less had higher residual viremia than those with suppression for more than 2 years (median 2.3 vs. 0.2 copies/mL, p=0.016).

Conclusions—Among HIV-1-infected patients with pre-ART HIV-1 RNA >100,000 copies/mL, residual viremia was detectable in the majority (62%) despite many years of suppressive ART. Higher level viremia was associated with older age and less than 2 years of virologic suppression on ART. These findings should help in selection of candidates for clinical trials of interventions designed to eliminate residual viremia.

Keywords

HIV-1; Single-copy assay; residual viremia

Introduction

Antiretroviral therapy (ART) prevents HIV-1-related complications by blocking viral replication [1]. Current antiretroviral regimens are highly efficacious in suppressing viral replication and reducing morbidity and mortality [2–3]. However, cure of HIV-1 infection is not currently achievable because of long-lived viral reservoirs that persist despite ART. To design effective strategies to eradicate HIV-1, a more detailed understanding of persistent viral reservoirs is needed. Using PCR assays that can detect a single copy of HIV-1 RNA (single-copy assay, SCA), low-level viremia is detectable in many patients whose plasma HIV-1 RNA is suppressed on ART to below the detection limits of standard commercial assays [4–5]. This low-level viremia may represent ongoing replication or release of virus from long-lived cellular reservoirs, such as resting memory CD4 cells [6–7], and likely other, as yet undefined, sources [8]. Identifying clinical characteristics associated with the level of residual viremia may provide further insight into the host and virologic mechanisms involved in HIV-1 persistence.

AIDS Clinical Trial Group (ACTG) study A5244 was a randomized controlled trial of raltegravir intensification in patients receiving suppressive ART. The primary outcome of this trial, as previously reported [9], is that raltegravir intensification did not reduce residual viremia (plasma HIV-1 RNA) measured by SCA. Using data from patients who screened for enrollment into A5244, we now report the findings of analyses to identify predictors of residual viremia in HIV-1-infected individuals on suppressive ART.

Methods

Study Population

The A5244 trial design and study population have been previously described (NCT#00515827) [9]. The main inclusion criteria to enter A5244 were: 1) HIV-1 infected adults receiving ART for 12 months with 2 nucleoside reverse transcriptase inhibitors (NRTI) and a ritonavir-boosted protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI); 2) plasma HIV-1 RNA levels below detection limits for 6 months using commercial assays; 3) CD4 cell count 200/mm^3; 4) pre-ART HIV-1 RNA >100,000 copies/mL; and 5) detectable HIV-1 RNA by SCA. All screened patients who had SCA results, whether or not viremia was detected, and who met eligibility criteria 1–4 above, were included in the current analyses. Data were cross-sectional and collected at the patients' screening visit. All patients provided written informed consent for the collection of samples and subsequent analyses.

Single Copy Assay Methods

Plasma samples from patients were tested with a single copy HIV-1 RNA assay which can detect one RNA molecule per RT-PCR reaction [10]. Controls containing 0 copies and plasma from HIV-1 uninfected individuals were consistently negative. Detectable SCA is defined as HIV-1 RNA above the lower limit of quantification. In this assay, the quantification limit is determined by the volume of plasma assayed. For example, if a single copy of HIV-1 RNA is detected in an 8 mL plasma sample, the quantity of HIV-1 RNA is 0.2 copies/ml (note, 54.5% of the plasma sample is assayed for HIV-1 RNA and the remainder is used for controls). The volume of plasma assayed ranged from 4–10 mL; as

previously described [9], 97% of patients had 7 mL of plasma assayed and 75% had 10 mL of plasma assayed.

Statistical Methods

Associations between screening SCA results and specific variables from screening visits were assessed using rank-based (Spearman) correlations. Continuous variables were dichotomized at the median (age and pre-treatment HIV-1 RNA), a commonly used threshold (screening CD4 cell count vs. > 500 cells/mm^3), or a threshold based on prior findings (years of virologic suppression vs. > 2 years was chosen because the HIV-1 RNA decay rate slows after 2–3 years of therapy [5])--and were compared using Wilcoxon rank-sum test. Analysis of a particular factor after adjustment for other factors was done using stratified Wilcoxon rank-sum tests. Binary outcomes were compared using Fisher's exact test. Undetectable screening SCA measurements were assigned an HIV-1 RNA value of ½ the detection limit when analyzed as a continuous variable. All statistical tests were two-sided, exploratory without adjusting for multiple comparisons, and at nominal 0.05 level of significance.

Results

One hundred and twenty-five patients screened for the A5244 study and had a SCA result; 22 of these patients were excluded from this analysis because they failed to meet A5244 entry criteria. The reasons for exclusion were no documented pre-ART HIV-1 RNA measurement or pre-ART HIV-1 RNA 100,000 copies/mL (n=12), missing documentation of 6 months virologic suppression (n=4), CD4 cell count <200/mm^3 or no CD4 cell count (n=4), and ineligible ART regimen (n=2). We examined predictors of screening plasma HIV-1 RNA level in the remaining 103 patients.

The median age of the 103 individuals was 46 years and 89% were male. Sixty percent were white non-Hispanic, 25% were black non-Hispanic, and 13% were Hispanic. Sixty-five percent of the patients were on an NNRTI-containing regimen and 35% were on a PI-containing regimen. The median (quartile [Q] 1, Q3) screening CD4 cell count was 582 (474, 795)/mm^3 and the median duration on suppressive ART (time since the first HIV-1 RNA below detection limit by commercial assay) was 4.8 (3.1, 6.4) years (N=99, 4 patients did not provide this information). Forty-two (41%) patients had a pre-ART HIV-1 RNA between 100,000–249,999 copies/mL, 23 (22%) were between 250,000 and 499,999 copies/mL, 13 (13%) were between 500,000 – 749,999 copies/mL, 16 (16%) were 750,000 copies/mL, and 9 (9%) were greater than the upper limits of quantification by assays with different maximum cutoffs (4 with >500,000 copies/mL and 5 with >100,000 copies/mL). The median pre-ART HIV-1 RNA among the 94 patients with quantified HIV-1 RNA was 5.5 (5.2, 5.8) log$_{10}$ copies/mL.

Sixty-four (62%) of the 103 patients had a detectable HIV-1 RNA level by SCA (>0.2 copies/mL), and 39 (38%) patients had an undetectable level. The median HIV-1 RNA by SCA was 0.2 (<0.2, 1.8) copies/mL. Among the 64 patients with a detectable HIV-1 RNA, 28 (44%) had <1 copy/mL and 36 (56%) had 1 copy/mL ranging up to 16.4 copies/mL [Figure 1]. Among the 39 patients with an undetectable SCA, 38 patients had 8 mL of plasma tested and, therefore, had < 0.2 copies/mL; 1 patient had 4 mL of plasma tested and, therefore, had < 0.4 copies/mL.

Younger patients had lower HIV-1 RNA values than older individuals (r =0.27, p=0.005, Spearman rank correlation). A smaller proportion of patients 46 years of age had a detectable HIV-1 RNA than those >46 years of age (27/55 [49%] vs. 37/48 [77%], p=0.004). The median HIV-1 RNA in patients 46 years of age was 0.20 copies/mL whereas the

median in those >46 years was 0.75 copies/mL (p=0.004) (Figure 2A). The association between age and HIV-1 RNA level remained significant (p=0.033) after adjustment for screening CD4 cell count, duration of virologic suppression and pre-ART HIV-1 RNA level.

In addition, among patients who had 2 years of virologic suppression, 75% (12/16) had a detectable screening SCA HIV-1 RNA, whereas among those with more than two years of virologic suppression, 58% (48/83) had a detectable HIV-1 RNA (p=0.27). Patients with 2 years of virologic suppression had significantly higher HIV-1 RNA levels than those who had suppressed for more than two years (median 2.3 vs. 0.2 copies/mL, p=0.016) (Figure 2B); this association remained significant after adjusting for age (p=0.015), screening CD4 cell count (p=0.021), and pre-ART HIV-1 RNA level (p=0.016). When duration of virologic suppression was considered as a continuous covariate, no association was apparent (r=−0.04, p=0.70).

We found no association between HIV-1 RNA level by SCA and the following factors: sex, race, background regimen (PI vs. NNRTI), or screening CD4 cell count (all p 0.20). Pre-ART HIV-1 RNA was not associated with HIV-1 RNA by SCA (p=0.58), but all patients in the analysis had a pre-ART HIV-1 RNA >100,000 copies/mL.

A sensitivity analysis in which data from all patients for whom screening SCA results were available regardless of inclusion/exclusion criteria (n=125) gave similar results. Moreover, the precision of the SCA HIV-1 RNA values below 1 copy/mL did not substantially affect our conclusions: if HIV-1 RNA values below 1 copy/mL were all assigned a value of 0.5 copies/mL, our conclusions did not change.

Discussion

In this study of 103 HIV-1-infected patients with a median duration of virologic suppression of almost 5 years, 62% had detectable HIV-1 RNA in plasma by the single-copy assay. However, a substantial minority of patients had levels below the limit of detection and the overall median HIV-1 RNA level was quite low (0.2 copies/mL) despite all patients having pre-ART plasma HIV-1 RNA >100,000 copies/mL. On average, plasma HIV-1 RNA was reduced by 500,000-fold from pre-ART levels. This finding underscores the effectiveness of current ART regimens in suppressing viral replication. Whether similar viral suppression is achieved in other anatomical sites is unresolved [11–13]. In addition, a critical unanswered question is whether the level of residual viremia is related to the size of latent or productive HIV-1 reservoirs.

In our analysis of predictors of residual viremia, we found that SCA values were higher in those who had 2 years or less of suppressive therapy compared with those who had been suppressed for >2 years. This finding is consistent with known phases of HIV-1 RNA decay after ART initiation: following the rapid first and second phases, there is a slower third phase of decay, which has a half-life of 39 weeks, followed by a fourth phase in which there is essentially no further decrease in HIV-1 RNA level [5]. Studies that seek to assess the effect of an intervention on residual viremia should focus on patients who have had virologic suppression for at least >2 years. Trials that include patients with shorter durations of suppression may observe third phase declines in HIV-1 RNA that are unrelated to the intervention; if there is not a control group, such declines could be misinterpreted.

The association of higher level residual viremia with older age is more difficult to explain and was not observed in one previous study [4]. Older individuals may have higher levels of immune activation [14] that might drive release of HIV-1 from the latent T cell or tissue reservoirs. Although we did not observe an association between age and T cell activation in the randomized portion of the A5244 study [9], the sample size in the randomized study was

smaller (n = 53) and may have not been adequate to detect a modest relationship. Alternatively, older age may be associated with a less effective immune response to HIV-1 and, therefore, less efficient suppression or clearance of cells that are producing HIV-1. The relations among age, immune activation and function, and HIV-1 reservoirs should be studied further.

We did not find an association between pre-ART HIV-1 RNA level and residual viremia, although a clear limitation of our study is that all patients in the analysis had a pre-ART HIV-1 RNA >100,000 copies/mL. This inclusion criterion was chosen for A5244 because residual viremia is more likely to be detected in individuals with higher pre-treatment HIV-1 RNA levels [4]. The range of our pre-ART HIV-1 RNA levels was only 10-fold and having a broader range of pre-ART RNA levels and a larger sample size may reveal an association between pre-ART HIV-1 RNA and residual viremia level as was reported in another study [4]. Another limitation of our study is that, even though it is one of the largest populations investigated to date with the single-copy assay, the moderate sample size does not allow us to detect factors that have a small effect on residual viremia. We also do not have information on adherence or drug levels prior to or at the time of screening nor do we have data on HIV-1 RNA levels in non-blood compartments.

In summary, we find that residual viremia persists in most HIV-1 infected patients on long-term suppressive ART and that the level of this residual viremia is higher in older patients and those who have had   2 years of virologic suppression. Studies of interventions to eliminate residual viremia should focus on patients who have reached a steady-state level of residual viremia, which requires at least 2 years of suppressive ART. A better understanding of the relations between residual viremia, age, immune activation, immune function, and tissue reservoirs in patients on long-term suppressive therapy is needed to develop effective strategies to reduce or eliminate long-lived HIV-1 reservoirs— a high priority for HIV-1 research.

Acknowledgments

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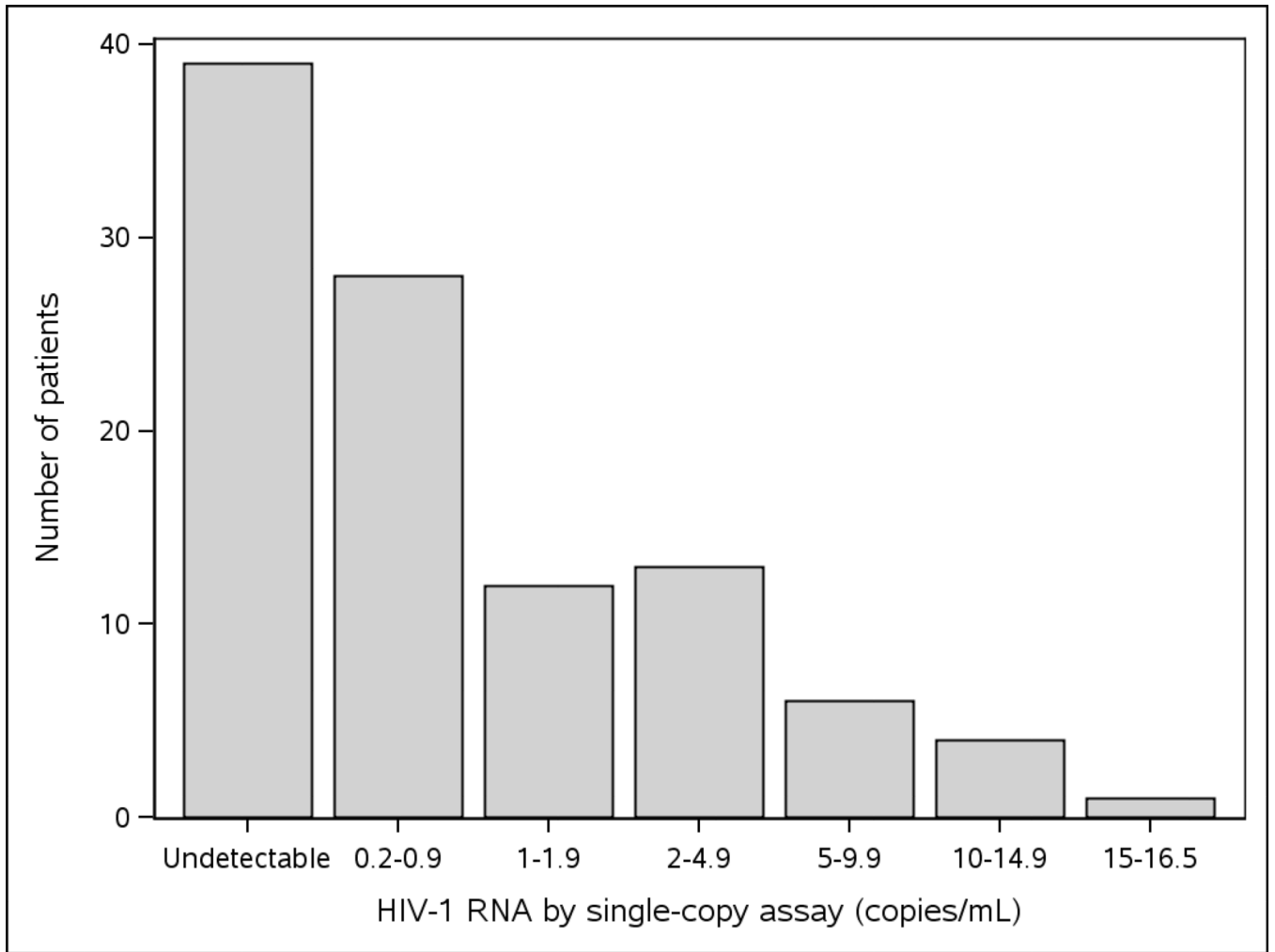
Figure 1.
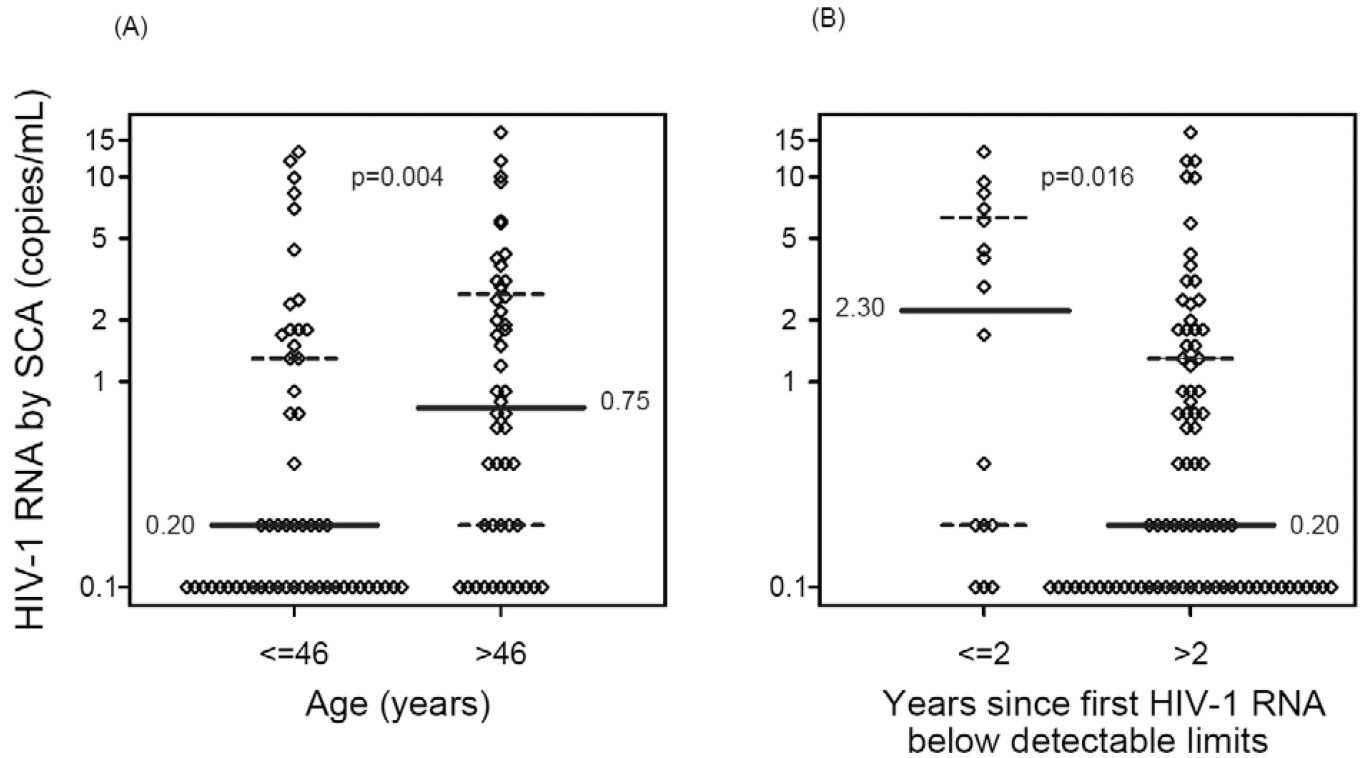Frequency of screening HIV-1 RNA by single-copy assay

(A)

(B)

Figure 2. Baseline predictors of HIV-1 RNA by single-copy assay (SCA)
(A) Age (46, > 46 years) versus HIV-1 RNA by SCA (median age of the study population
for this analysis was 46 years). (B) Years (2 vs. >2) since first HIV-1 RNA below
detectable limits by commercial assays versus HIV-1 RNA by SCA. When the SCA
measurement was below the detection limit, a value of 1/2 of the detection limit was
imputed. The solid horizontal line indicates the median SCA value; the dashed lines indicate
25th and 75th percentile. (The 25th percentile was 0.1 copies/mL for the group with age 46
years old and the group with >2 years since first HIV-1 RNA below detectable limits by
commercial assays).