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Dynamics of Viremia in Primary HIV-1 infection in Africans: Insights from Analyses of Host and Viral Correlates

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Abstract

In HIV-1 infection, plasma viral load (VL) has dual implications for pathogenesis and public health. Based on well-known patterns of HIV-1 evolution and immune escape, we hypothesized that VL is an evolving quantitative trait that depends heavily on duration of infection (DOI), demographic features, human leukocyte antigen (HLA) genotypes and viral characteristics. Prospective data from 421 African seroconverters with at least four eligible visits did show relatively steady VL beyond 3 months of untreated infection, but host and viral factors independently associated with cross-sectional and longitudinal VL often varied by analytical approaches and sliding time windows. Specifically, the effects of age, HLA-B*53 and infecting HIV-1 subtypes (A1, C and others) on VL were either sporadic or highly sensitive to time windows. These observations were strengthened by the addition of 111 seroconverters with 2–3 eligible VL results, suggesting that DOI should be a critical parameter in epidemiological and clinical studies.

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Competing Interests
The authors declare no conflict of interests.

Keywords

Africa; HIV-1; subtype; HLA; statistical models; viral load

Introduction

As an informative trait specific for HIV-1 infection, plasma viral load (VL) is well known for its strong correlation with the probability of sexual transmission (Fideli et al., 2001; Quinn et al., 2000) and rate of disease progression (Mellors et al., 1995; Mellors et al., 2007). Even in individuals with uncertain duration of infection, a relatively steady set-point VL can last for years to serve as a proxy of host-virus equilibrium (Arnaout et al., 1999; Fiebig et al., 2003). Factors independently associated with set-point VL range from viral characteristics to multiple quantitative trait loci (QTLs) in the human genome (Prentice and Tang, 2012; Yue et al., 2013), especially genes in the human major histocompatibility complex (MHC) that encode human leukocyte antigens (HLA) (Apps et al., 2013; Fellay et al., 2009; Leslie et al., 2010).

The widely accepted assumption and interpretation of set-point VL can have various problems. For example, HIV-1 immune escape can frequently tip the initial equilibrium in favor of the virus (Crawford et al., 2009; Kawashima et al., 2009; Mellors et al., 1995), which may obscure the relationship between set-point VL and HIV-1 disease progression (Mellors et al., 2007; Rodriguez et al., 2006). A second issue relates to various statistical methods that either fully embrace the concept of set-point VL (Prentice and Tang, 2012) or provide two alternative strategies, i.e., mixed model for repeated measures (Prentice et al., 2013; Shrestha et al., 2009; Tang et al., 2011) and assessment of cumulative viral load burden (Arnaout et al., 1999; Cole et al., 2010; Mugavero et al., 2011). To establish the advantages and disadvantages of these analytical approaches, we have tested a central hypothesis that cross-sectional and longitudinal VL data are evolving outcomes that depend heavily on duration of infection DOI), demographic features, HLA genotypes and viral characteristics.

Results

Characteristics of 421 HIV-1 seroconverters (SCs) in the study population

For this study, sufficient prospective data were available for 421 SCs from Zambia (Lusaka and Copper Belt, *n*=164), Uganda (Entebbe and Masaka, *n*=110), Kenya (Kilifi and Nairobi, *n*=83) and Rwanda (Kigali, *n*=64) (Table 1). In each subgroup, males outnumbered females, especially in Kenya. All volunteers were relatively young at enrollment (mean age between 25.1 and 33.6 years by site). The estimated dates of infection (EDI) ranged from May 2005 to March 2011, being highly comparable across study sites. Based on a 1.3-kb fragment of the HIV-1 *pol* gene sequences (successful in 93.4% of SCs), subtypes A1 and C accounted for 74.2% of the total, while other subtypes (B or D) and recombinant forms were infrequent, precluding further stratification (Table 1).

As reported earlier (Prentice et al., 2013; Tang et al., 2011), HIV-1 subtypes A1 and C were predominant in Rwandan and Zambian SCs, respectively. Kenyan SCs differed from others in their lower age (25.1 ± 4.4 years), high male-to-female ratio (5.9), and prevalence of multiple infecting HIV-1 subtypes (A1, C, D and others) (6.0% to 69.9% per subtype). Ugandan SCs had the greatest frequency (52.8%) of non-A1 and non-C subtypes (mostly subtype D) (Table 1).

The spectrum of VLs and cumulative viremia (VCY) in 421 SCs during early HIV-1 infection

In the 3–24 months interval after EDI, VL was measured at a total of 3,154 person-visits. Cross-sectional VLs beyond 3 months of infection ranged from below detection (<2.60 $\log_{10}$ copies/mL) to 6.6 $\log_{10}$. The mean $\log_{10}$ VL in SCs from each country was stable, with little (≤0.40 $\log_{10}$) fluctuation between visit intervals, especially adjacent intervals (≤0.30 $\log_{10}$) (Table 1). Geometric mean VLs were also quite similar between year 1 and year 2 (≤0.30 $\log_{10}$). On the other hand, country-specific variations in VLs began to emerge during later visits (P <0.001 by ANOVA) (Table 1). Reflecting a strong collinearity between region (geography) and HIV-1 subtypes, Zambian SCs consistently had higher VLs than Kenyan and Rwandan SCs for the 18–24 months VL, year 2 geometric mean VL and cumulative viremia (P <0.05 in all six comparisons).

Evaluation of linear correlation between longitudinal and cross-sectional VL data

In 56 (8 × 7) pairwise comparisons of eight distinct outcomes of VL, the Pearson r values ranged from 0.51 to 0.92 before and after statistical adjustments for age, sex, country of origin, and duration of infection (P <0.0001 for all tests) (Table 2). For the seven cross-sectional VL measurements with slight variation in effective sample sizes (n = 381–419), the overall pattern persistently revealed that correlation was weak unless the tests involved cross-sectional results from two adjacent visits (e.g., 3–6 months versus 6–9 months and 9–12 months vs. 12–18 months). Even so, correlation that was strong enough to imply two mutually interchangeable outcomes (i.e., r^2 >0.80) was rare, being detected on a single occasion. In this case, the 6–9 months set-point VL showed a strong collinearity with the geometric mean VL during the 3–12 months interval regardless of statistical adjustments (r = 0.92, r^2 = 0.85, and P <0.0001). In addition, as far as the adjusted r values were concerned, country of origin as a covariate was interchangeable with HIV-1 subtype as a covariate.

Identification of independent correlates of longitudinal viremia in 421 SCs

On the basis of adjusted statistical significance (P <0.05), multivariable models identified sex, duration of infection, infecting HIV-1 subtypes, and *HLA-B* genotypes as independent predictors of longitudinal VL outcomes (Table 3). These factors, along with age, accounted for about 5% of the overall variability in the VL dataset (P <0.0001). Ranking by univariable r^2 values placed viral subtype C, HLA-B*57, and sex as the top three predictors. Alternatively, the top three predictors ranked by unadjusted VL differences (mean beta estimates) were HLA-B*57 (P <0.0001), HLA-B*18 (P = 0.018), and sex (P <0.0001) (Table 3).

However, heterogeneity in HIV-1 viremia fluctuated over time when SCs defined by host and viral factors were compared. For example, clear separation in VL was seen between HLA-B*18 positive and HLA-B*18 negative SCs throughout the 3–24 months interval, but differences between HIV-1 subtypes A1 and C were not apparent in the 3–9 months period (Figure 1). Like HLA-B*18, B*45, B*57 and female sex also had highly persistent effects on VLs during the study intervals; these relationships were readily confirmed by analyses of cumulative viremia (data not shown).

Viral and host factors associated with cross-sectional viremia: sensitivity analyses based on generalized linear regression models (GLMs)

Analyses of seven cross-sectional VL outcomes led to highly variable findings (Table 4). The number of independent predictors ranged from two (sex and HLA-B*45) for the 3–6 months VL to six (all but HIV subtype) for the 6–9 months VL. HLA-B*18 and B*45 as unfavorable factors were each detected in 4 out of 6 models, without a clear preference for

early or later intervals (Table 4). HLA-B*57 was a favorable factor in 5 out of 6 models, with an effect size exceeding 0.30 $\log_{10}$ after the first 6 months of infection. Overall, host and viral factors explained 7.2% to 14.8% of variability in the six VL cross-sectional phenotypes (P <0.0001 in each test). Of note, analyses of GM VLs had little advantage over individual VLs.

Findings based on alternative approaches

In alternative analyses, HIV-1 VL dynamics differed between eastern and southern Africa (P <0.0001) (Figure 2), which was consistent with differences seen with HIV-1 subtypes. In addition, analyses guided by LOESS curves revealed that HLA-B*53 had modest association with VL in the 3–18 months intervals (2,361 person-visit, P = 0.043) (Figure 2). VLs in the 75 subjects with HLA-B*53 had a unique pattern: a modest separation between B*53-positive and B*53-negative subjects (average $\Delta = 0.21 \pm 0.1$ $\log_{10}$) faded shortly after the 18 months mark (Figure 2). In the final round of data analyses for all 532 eligible SCs (Table 5), HLA-B*53 explained 1.1% of the overall variance in longitudinal VLs (P = 0.055 by univariable analysis), while the multivariable model confirmed its independent association with longitudinal VLs (adjusted P = 0.025).

Additional observations on HLA variants

Among the four HLA variants independently associated with longitudinal VLs (Table 5), HLA-B*18, B*53 and B*57 had similar distribution in the four African countries (Table 1). Two thirds of subjects with B*57 had HLA-B*57:03, which had its own association with reduced viremia (data not shown). On the other hand, HLA-B*18, B*53 and B*45 were each represented by a single allele (B*18:01, B*53:01 and B*45:01, respectively) regardless of study sites. Other HLA alleles relevant to Africans, including A*29, A*36, A*74, B*13, B*14, B*35, B*39, B*42, B*51, B*58:01, B*58:02, B*81, C*04, C*18, and the A*30 + C*03 combination (Apps et al., 2013; Leslie et al., 2010; McLaren et al., 2012; Tang et al., 2010), had minimal impact on VL outcomes (data not shown). Alternative analyses of structurally defined HLA-A and HLA-B supertypes were inconclusive (data not shown).

Discussion

Through systematic analyses of prospective data from African seroconverters with early HIV-1 infection, our work demonstrated that cross-sectional VL outcomes change rapidly as the infection progresses. In other words, VLs separated by as little as three months can be considered as different outcomes (Table 2) with varying correlates (Table 4). For subgroups of SCs defined by host and viral factors (Table 4), three patterns of local regression curves (Figures 1–2) represent (i) steady and robust differences across visits, (ii) convergence followed by gradual divergence, and (iii) divergence followed by gradual convergence. Other patterns (e.g., wave-like and U-shaped) were less obvious, probably because the overall study interval (from 3 to 24 months after EDI) was relatively short.

In previous work based on 269 SCs (homosexual men in the U.S.) with semi-annual follow-up visits, VL trajectories (slopes) during the first three years after seroconversion correlated with AIDS-free time, while VLs in the three years immediately before AIDS diagnosis had no predictive values (Lyles et al., 1999). When stratified by host genotypes, SCs from the same study showed different patterns of VL dynamics in early infection as well (Tang et al., 2002a). While our new findings here are largely consistent with the reports from the Multicenter AIDS Cohort Study, three major advantages in study design and analytical approaches here can help strengthen the search for underlying mechanisms. First, frequent testing before and during seroconversion (at monthly to quarterly visits) has improved the precision in assigning EDI and DOI. Second, follow-up visits after seroconversion are

frequent enough to facilitate the comparison of early virologic outcomes in sliding time windows (Table 4). Third, host and viral genotypes can be analyzed jointly in multivariable models. The two major HIV-1 subtypes, A1 and C, are widespread in sub-Saharan Africa (Osmanov et al., 2002; Tebit and Arts, 2011), with some evidence for disparity in evolutionary and pathophysiologic attributes when compared with other subtypes (Baeten et al., 2007; Kaleebu et al., 2001; Kiwanuka et al., 2008; Vasan et al., 2006). VL differences between HIV-1 subtypes A1 and C infection (Table 3 and Figure 1) are generally consistent with the contrasting rates of heterosexual HIV-1 transmission in Rwanda and Zambia where the respective subtypes predominate (Dunkle et al., 2008; Fideli et al., 2001).

Recent assessment of disease progression (time to severe immunodeficiency) has revealed the role of African HIV-1 subtypes in pathogenesis (Amornkul et al., 2013). However, given the strong collinearity between geography and HIV-1 subtypes (Table 1), a more definitive elucidation of host and viral factors in HIV-1 pathogenesis in Africa may require the assembly of a genetically homogeneous cohort with diverse HIV-1 subtypes and adequate follow-up. Such study is no longer feasible as new treatment guidelines recommended by the World Health Organization are expected to transform the test-and-treat concept into a global practice (Cohen et al., 2011; Montaner et al., 2010; Mugavero et al., 2012).

Among the three *HLA-B* variants independently and persistently associated with VL heterogeneity, HLA-B*57 is a well-known favorable factor, while HLA-B*18 and HLA-B*45 are consistently unfavorable in multiple studies (Apps et al., 2013; Lazaryan et al., 2011; Leslie et al., 2010; McLaren et al., 2012; Tang et al., 2010). Generalizable findings about these *HLA-B* variants, now in the context of sub-Saharan African populations, should benefit future epidemiologic and experimental studies, especially in the context of HIV-1 adaptation at the population level (Kawashima et al., 2009). HLA-B*18 has also been reported as partially protective against mother-to-child HIV-1 transmission in Kenyan infants (Farquhar et al., 2004), suggesting again that mechanisms for immune control of established HIV-1 infection can be quite distinct from those mediating acquisition of infection (Gao et al., 2010; Song et al., 2011; Tang et al., 2008).

HLA allelic products contribute to immune control of viral infection through both innate and adaptive immune pathways (Carrington, Martin, and van Bergen, 2008; Merino et al., 2013; Merino et al., 2012; Stewart et al., 2005). Alleles with early influences on HIV-1 infection tend to impose a strong selection pressure for viral immune escape mutations a phenomenon that has been repeatedly examined in individuals with HLA-B*57 and related alleles (Bansal et al., 2007; Crawford et al., 2009; Leslie et al., 2004; Novitsky et al., 2010; Wang et al., 2009). The unfavorable effect of HLA-B*18 on VL started early and remained stable (Figure 1), which may translate to a durable impact on HIV-1 pathogenesis. In settings where treatment priority is necessary, timely interventions directed to subjects carrying unfavorable HLA profile may maximize the benefits by preventing pathogenesis as well as transmission.

Our work also indicates that modest and relatively transient effects attributable to host factors like HLA-B*53 can easily escape detection when VLs in the first few months of infection are missed (Figure 2). As a common allele in Africans and African Americans, its relevance to HIV-1 infection has been highlighted in at least two recent studies (Apps et al., 2013; Lazaryan et al., 2011). Although VLs were only slightly elevated in Africans subjects with HLA-B*53 during the first 18 months of infection (Figure 2), the potential impact on HIV-1 reservoir and transmission rate may deserve some investigation.

Conclusions

Analyses of cross-sectional data often failed to identify host and viral factors (e.g., HLA-B*53 and HIV-1 subtypes) due to time-varying associations with VLs, even in early infection when complications by immune escape, co-infection and other forms of comorbidities should be minimal. For correlates (e.g, HLA-B*57) that can be readily identified using randomly chosen phenotypes, the magnitude of associations can vary from one interval to another. These observations continue to support our notion that DOI is an important parameter when cross-sectional VL results are assessed in clinical research (Prentice et al., 2013; Prentice and Tang, 2012; Tang et al., 2010). As early diagnosis of HIV-1 infection remains costly and difficult (Kozak et al., 2013), few studies can actually assess the timing of VL measurements in prevalent HIV-1 infection. One reasonable compromise is to down-play findings based solely on random sampling of cross-sectional data. Alternatively, immunologic and virologic techniques suitable for inferring DOI in seroprevalent infection (Cousins et al., 2013) may provide valuable information about DOI.

Materials and Methods

Study population

Recent HIV-1 seroconverters (SCs) were enrolled from Kenya, Rwanda, Uganda, and Zambia (Table 1) under a uniform study protocol developed and implemented by the International AIDS Vaccine Initiative (IAVI) (Amornkul et al., 2013). The procedures for written informed consent and research activities were approved by institutional review boards at all collaborating clinical research centers, with further compliance to human experimentation guidelines set forth by the United States Department of Health and Human Services.

Follow-up strategies before and after HIV-1 infection

Identification of SCs relied on frequent (monthly to quarterly) testing of HIV-1 seronegative subjects at high risk of HIV-1 infection through heterosexual (common) and homosexual (occasional) exposure, with the vast majority being partners of HIV-1 discordant couples and/or individuals diagnosed with sexually transmitted infections. As described in detail elsewhere (Amornkul et al., 2013; Karita et al., 2007; Prentice et al., 2013), the estimated dates of HIV-1 infection (EDI) were defined as one of the following: (i) the midpoint between the last seronegative and first positive HIV-1 antibody tests, (ii) two weeks before the first positive test for HIV-1 p24 antigen in plasma, (iii) 10 days before the first positive test for plasma viral load (VL) while being negative for both p24 and rapid HIV-1 antibody tests, and (iv) event date for the only high-risk exposure. Following confirmation of HIV-1 infection (detection of VL), clinical visits were scheduled monthly for the first three months after EDI and quarterly for the 3–24 months interval. Initiation of antiretroviral therapy (ART) followed national guidelines (Ngongo et al., 2012), and all visits and VL measurements after ART initiation were excluded. In all, 421 SCs (Table 1) were selected based on: (i) availability of more than 50 SCs from a single country, with biological specimens for DNA extraction and HLA class I genotyping, (ii) at least four time points of VL in the early chronic phase (3–24 months) of infection, with no gap greater than one year between two consecutive VL measurements, and (iii) no ART during the eligible study intervals. An additional 111 SCs with only 2–3 eligible VL measurements were available for secondary analyses. The remaining SCs excluded from analyses here included those enrolled from South Africa (n = 26) and a small group (n = 46) with limited follow-up (no more than a single VL for eligible visits).

HIV-1 viral load (VL) as a quantitative trait

Plasma VL (RNA copies/mL) was measured at a central location (Clinical Laboratory Services, Johannesburg, South Africa) using the Amplicor Monitor v1.5 assay (Roche Applied Science, Indianapolis, IN) and following good clinical laboratory practices (Amornkul et al., 2013). With a focus on visits beyond acute-phase (the first 3 months after EDI), eligible VLs in the 3–24 months intervals were first treated as the longitudinal outcome and then divided into cross-sectional outcomes corresponding to five visit intervals: 3.1 to 6.0 months, 6.1 to 9.0 months, 9.1 to 12.0 months, 12.1 to 18.0 months and 18.1 to 24.0 months (rounded to the nearest integer for tables and figures). Repeated measures in the 3.1–12.0 and 12.1–24.0 months intervals also allowed the calculation of geometric mean (GM) VLs. In addition, cumulative viremia as a time-varying measurement of VL in the 3.1–24.0 months after EDI was expressed as the number of virus copies per mL of plasma multiplied by time of follow-up (years) (Cole et al., 2010; Mugavero et al., 2011). A trapezoidal rule was used to approximate the integral representing the area under the curve for each participant's longitudinal VL. Within each segment (time between two VL sampling dates), VL burden was the mean of the two VL measurements multiplied by the time interval between the sampling dates. Summation of the individual segments (3–24 months after EDI) gave rise to the viremia copy-year (VCY) outcome. For $\log_{10}$ transformation, all VLs below the lower limit of detection (LLD = 400 RNA copies/mL) were assumed to be 1.30 (half of $\log_{10}400$). In alternative analyses, setting VL below LLD to 2.30 $\log_{10}$ (200 copies/mL) did not change statistical models (data not shown).

Viral sequencing and human leukocyte antigen (HLA) class I genotyping

Methods for HIV-1 *pol* gene sequencing and HLA class I genotyping have been described elsewhere (Price et al., 2011; Tang et al., 2011). Viruses were grouped into subtypes (mostly A1, C and D) and recombinant forms (rare) (Table 1). Allelic variants at three HLA class I loci (*HLA-A*, *HLA-B* and *HLA-C*) were fully resolved to the 4-digit level. Data analyses focused on three prominent variants, HLA-B*18 (unfavorable), B*45 (unfavorable), and -B*57 (favorable), based on their confirmed effects on VL in native Africans and African-Americans (Apps et al., 2013; Leslie et al., 2010; Tang et al., 2010).

Statistical Analysis

Using software packages in SAS, version 9.2 (SAS Institute, Cary, NC), SCs were stratified by country of origin and tabulated for demographic data, laboratory findings (viral subtypes and VL) (Table 1) and distribution of major HLA variants of interest. Methods used for comparing baseline characteristics included (i) analysis of variance (ANOVA), (ii) *t*-test for quantitative variables with a normal distribution, and (iii) χ^2 and Fisher exact tests for categorical variables. The extent of collinearity among eight VL outcomes was determined by Pearson's correlation coefficients (r), and paired data with $r \geq 0.90$ ($r^2 \geq 0.80$) were considered as mutually interchangeable (Craney and Surles, 2002). Subsequent association analyses, including mixed models, generalized linear models (GLMs) and local regression (LOESS) curves, aimed at identifying independent correlates of VL and assessing their relative effects on VL over time during the 3–24 months follow-up period. Based on evidence from earlier studies (Prentice et al., 2013; Tang et al., 2010), age, sex, duration of infection (DOI, measured monthly or quarterly), infecting viral subtypes and common HLA variants were tested as independent cofactors in univariable and multivariable models. The performance of individual models was gauged by their overall R^2 values (corresponding to variability explained by factors in the model), while the performance of individual factors was ranked first by the regression beta (mean difference) and then by the R^2 values from univariable models. Statistical significance was accepted at the level of $P \leq 0.05$, but age was kept in all multivariable models regardless of statistical significance (for consistency with

earlier work). Major findings from these models were subjected to three sets of sensitivity analyses: (i) replacing HIV-1 subtype with country of origin as the two covariates were highly collinear (Tang et al., 2011); (ii) including regional groupings (eastern versus southern Africa) as a covariate (Prentice et al., 2013); and (iii) adding the results from 101 eligible SCs with limited (2–3) VL data points to the overall model for repeated VL measurements.

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Research Highlights

- In recent HIV-1 seroconverters, plasma viral load (VL) is a rapidly evolving outcome;
- Host and viral factors show time-varying associations with longitudinal or cross-sectional VL;
- Three types of VL trajectories are readily detected in analyses of longitudinal VL.

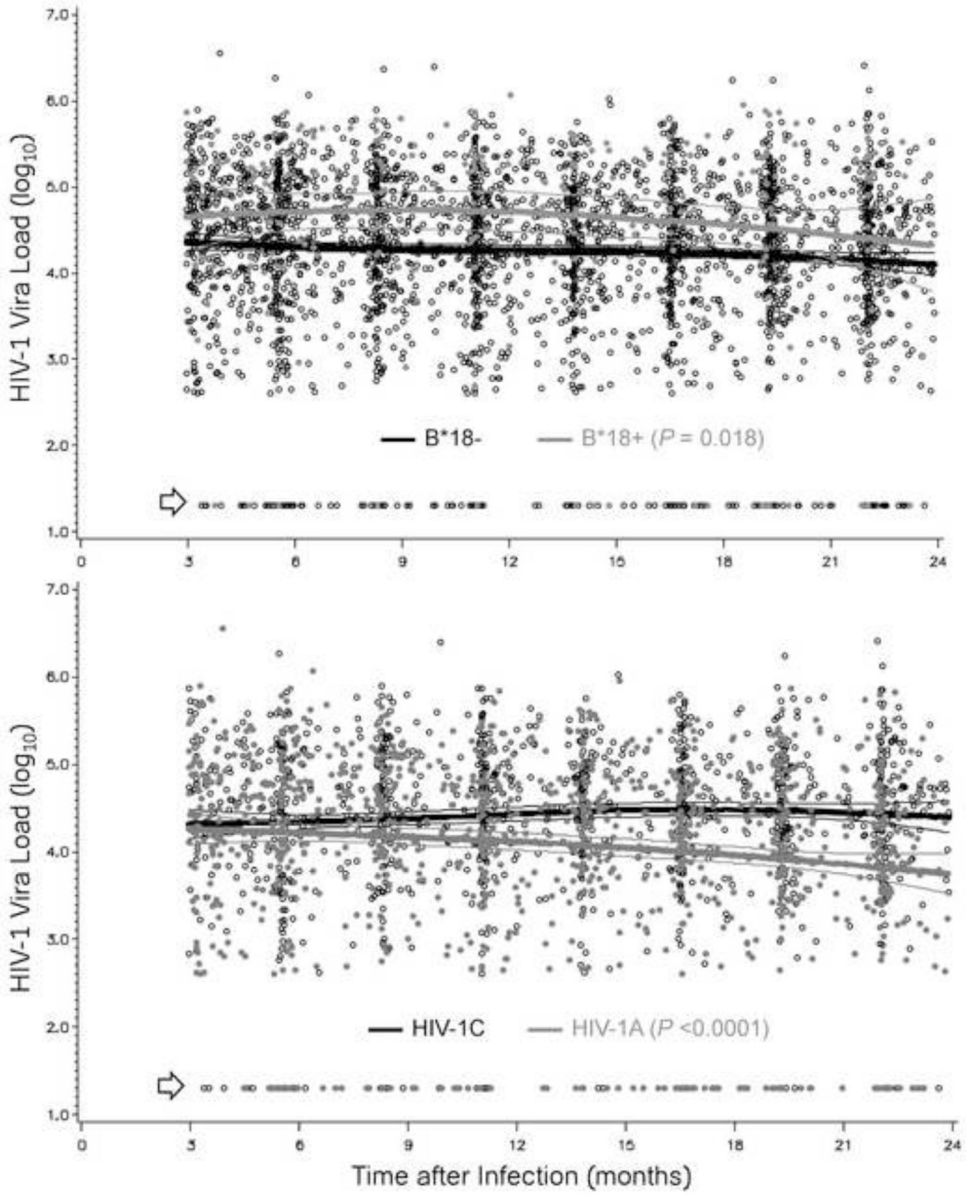

Figure 1. Contrasting dynamics of HIV-1 viremia in African seroconverters defined by host and viral genotypes

Prospective viral load measurements are compared between HLA-B*18-positive and HLA-B*18-negative subjects (top panel, 3,154 person-visits) and between two major HIV-1 subtypes, HIV-1C and HIV-1A (bottom panel, 2,306 person-visits). Thick and thin lines correspond to the expected mean (average) value and 95% confidence intervals for each stratum (see Table 3 for summary statistics based on mixed models). Arrows point to plasma viral load measurements that are <400 RNA copies/ml (transformed to 1.30 $\log_{10}$).

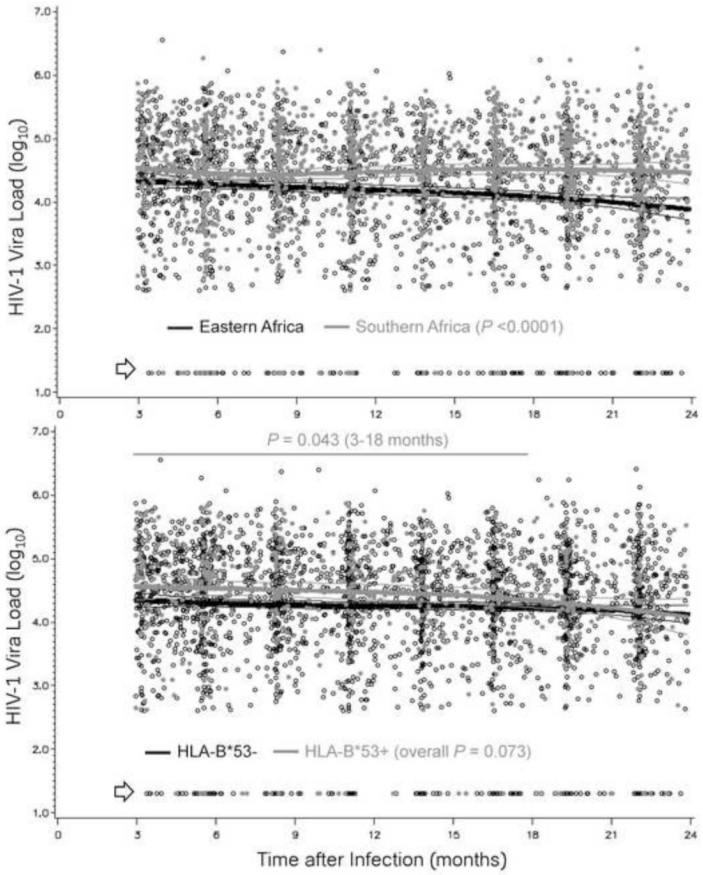

Figure 2. Viremia in HIV-1 seroconverters stratified by geography and HLA-B*53

Prospective viral load measurements (3,154 person-visits) are compared between Eastern Africa (Kenya, Rwandan and Uganda) and Southern Africa (Zambia) (top panel) and between HLA-B*53-positive and negative subgroups (bottom panel). Thick and thin lines correspond to the expected average value and 95% confidence intervals for each stratum (overall unadjusted $P = 0.056$). Arrow points to plasma viral load measurements that are <400 RNA copies/ml (transformed to 1.30 $\log_{10}$).

Table 1

Characteristics of 421 HIV-1 seroconverters enrolled from four African countries.

Characteristics[a] and HLA variants of major interest	**Kenya**	**Rwanda**	**Uganda**	**Zambia**
Number of seroconverters with sufficient data	83	64	110	164
Sex ratio (M/F)	5.9 (71/12)	1.4 (37/27)	1.4 (65/45)	1.3 (92/72)
Age: mean ± SD (yr)	25.1 ± 4.4	31.8 ± 8.9	31.9 ± 8.3	33.6 ± 8.0
Age ≥ 40 yr: no. (%)	0 (0.0)	11 (16.9)	22 (20.0)	34 (20.7)
Estimated dates of infection				
Earliest	Nov. 2005	May 2005	Sep. 2005	June 2005
Latest	Feb. 2011	Jan. 2011	Sep. 2010	Mar. 2011
Person-visits with eligible viral load (VL) data (3–24 months)	617	486	862	1,189
HIV-1 subtype:[b] no. (%)				
A1	58 (69.9)	50 (78.1)	41 (37.3)	0 (0.0)
C	8 (9.6)	7 (10.9)	5 (4.6)	144 (87.8)
D	8 (9.6)	1 (1.5)	52 (47.3)	0 (0.0)
Others (B and recombinants)	5 (6.0)	3 (4.7)	6 (5.5)	2 (1.2)
Unknown (no viral sequencing)	4 (4.8)	3 (4.7)	6 (5.5)	18 (11.0)
Cross-sectional viral load (VL): mean ± SD (in $\log_{10}$)				
3–6 months	4.2 ± 1.0	4.1 ± 1.1	4.4 ± 1.0	4.5 ± 1.0
6–9 months	4.4 ± 0.9	4.1 ± 1.0	4.3 ± 1.1	4.4 ± 0.9
9–12 months	4.1 ± 1.0[c]	4.1 ± 1.0[c]	4.3 ± 1.0	4.5 ± 0.9
12–18 months	4.2 ± 0.8	4.1 ± 1.0[c]	4.2 ± 1.2	4.5 ± 0.8
18–24 months	4.0 ± 1.1[c]	4.0 ± 1.1[c]	4.1 ± 1.2[c]	4.6 ± 0.8
Repeated VL measurements (in $\log_{10}$)				
Year 1 geometric mean (3–12 months): mean ± SD	4.3 ± 0.9	4.1 ± 1.0	4.3 ± 1.0	4.4 ± 0.9
Number of VLs taken within year 1: median (IQR)	3 (3–4)	3 (3–5)	3 (3–4)	3 (3–4)
Year 2 geometric mean (13–24 months): mean ± SD	4.1 ± 0.9[c]	3.9 ± 1.1[c]	4.2 ± 1.0	4.5 ± 0.7
Number of VLs taken within year 2: median (IQR)	4 (4–4)	4 (4–4)	4 (4–4)	4 (3–4)

Characteristics[a] and HLA variants of major interest	Kenya	Rwanda	Uganda	Zambia
Viremia copy years (VCY) (3–24 months): mean ± SD	4.7 ± 0.7[c]	4.6 ± 0.8[c]	4.7 ± 0.9	5.0 ± 0.6
Number of VLs used for calculating VCY: median (IQR)	7 (7–8)	7 (7–8)	7 (7–9)	7 (6–8)
HLA-B*18 (B*18:01)	5 (6.0)	5 (7.8)	10 (9.1)	11 (6.7)
HLA-B*45 (B*45:01)	18 (21.7)	4 (6.3)	15 (13.6)	26 (15.9)
HLA-B*53 (B*53:01)	14 (16.9)	10 (15.6)	22 (20.0)	29 (17.7)
HLA-B*57 (mostly B*57:03)	8 (9.6)	6 (9.4)	10 (9.1)	17 (10.4)

[a] Non-standard abbreviations: IQR, interquartile range (25th to 75% percentile); SD, standard deviation of the mean.

[b] The minor HIV-1 subtypes (B, D, and others) are combined for analysis. Unknown subtypes were assigned based on probabilities (see text).

[c] Statistically significant differences when compared with data from Zambians.

Table 2

Relationships of cross-sectional viral load (VL) and cumulative viremia (VCY) in early HIV-1 infection, as defined by Pearson correlation coefficients (*r*).[a]

	Cross-sectional (set-point) VL at five intervals					Repeated measures		VCY
VL measurements (no. of subjects)	**3–6 mo**	**6–9 mo**	**9–12 mo**	**12–18 mo**	**18–24 mo**	**3–12 mo**	**13–24 mo**	**3–24 mo**
3–6 months (381)	1.00	0.76	0.69	0.65	0.52	0.88	0.62	0.72
6–9 months (394)	0.74	1.00	0.77	0.73	0.58	**0.92**[b]	0.70	0.80
9–12 months (404)	0.68	0.76	1.00	0.75	0.61	0.88	0.75	0.82
12–18 month (416)	0.63	0.71	0.74	1.00	0.69	0.79	0.86	0.86
18–24 Month (408)	0.51	0.57	0.61	0.68	1.00	0.64	0.90	0.78
3–12 months, geometric mean (419)	0.88	**0.92**[b]	0.88	0.77	0.63	1.00	0.77	0.87
13–24 months, geometric mean (418)	0.61	0.70	0.74	0.85	0.89	0.76	1.00	0.90
3–24 months, VCY (421)[b]	0.71	0.79	0.82	0.85	0.77	0.87	0.89	1.00

[a] With $\log_{10}$-transformation before analysis, the *r* values below the diagonal are for the raw data, while *r* values above the diagonal are adjusted for age, sex, country of origin, and duration of infection (wherever applicable) ($P < 0.001$ for all pairwise tests).

[b] The R^2 values exceed 0.80 in a single test only (shown in **bold**).

Table 3

Analyses of longitudinal viral load (VL) data (repeated measurements) in early HIV-1 infection (the 3–24 months interval after infection).

Predictors (independent variables)	Effects by univariable models[b]			Multivariable model[c]	
	Δ ± SE	***P***	R^2	**Δ ± SE**	***P***
Age >40 years	−0.08 ± 0.11	0.494	0.000	−0.14 ± 0.11	0.191
Female sex	<u>−0.36 ± 0.08</u>	<0.0001	<u>0.038</u>	−0.37 ± 0.08	<0.0001
HIV-1 subtype C[a]	0.24 ± 0.08	0.004	<u>0.028</u>	0.38 ± 0.09	<0.0001
Other HIV-1 subtypes[a]	0.13 ± 0.10	0.227	0.002	0.34 ± 0.11	0.002
Duration of infection (quarterly)	−0.02 ± 0.00	0.009	0.007	−0.02 ± 0.00	0.008
HLA-B*18	<u>0.37 ± 0.16</u>	0.018	0.012	0.37 ± 0.15	0.013
HLA-B*45	0.25 ± 0.12	0.028	0.008	0.25 ± 0.11	0.021
HLA-B*57	<u>−0.53 ± 0.14</u>	0.0001	<u>0.016</u>	−0.47 ± 0.13	0.0003
Overall summary statistics	NA			r^2 =0.049, P <0.0001	

[a] HIV-1 subtype A is the reference group.

[b] Top three predictors ranked by the effect size are <u>underlined</u>.

[c] Based on simultaneous evaluation of all factors as shown. For consistency with reported approach (Tang et al., 2002b), age is kept as a covariate in all multivariable models (here and Table 3) regardless of statistical significance.

Table 4

Sensitivity analyses using cross-sectional viral load (VL) results in early HIV-1 infection (multivariable models)[a].

Predictors (independent variables)	Individual and joint effects on cross-sectional VLs[d]					Geometric mean[d]
	3–6 months	6–9 months	9–12 months[e]	12–18 months	18–24 months	13–24 months
Age >40 years	−0.12 ± 0.1	−0.29 ± 0.1*	−0.21 ± 0.1	−0.18 ± 0.1	−0.12 ± 0.1	−0.13 ± 0.1
Female gender	−0.38 ± 0.1***	−0.50 ± 0.1***	−0.32 ± 0.1**	−0.50 ± 0.1***	−0.32 ± 0.1**	−0.36 ± 0.1***
HIV-1 subtype C[b]	0.22 ± 0.1	0.20 ± 0.1	0.27 ± 0.1*	0.38 ± 0.1***	0.66 ± 0.1***	0.51 ± 0.1***
Other HIV-1 subtypes[b]	0.26 ± 0.1	0.22 ± 0.1	0.31 ± 0.1*	0.33 ± 0.1**	0.39 ± 0.1**	0.40 ± 0.1***
Duration of infection (monthly)	−0.10 ± 0.1	−0.11 ± 0.1*	0.06 ± 0.1	0.04 ± 0.1	−0.05 ± 0.0	NA
HLA-B*18	0.27 ± 0.2	0.54 ± 0.2**	0.48 ± 0.2**	0.48 ± 0.2**	0.25 ± 0.2	0.40 ± 0.2*
HLA-B*45	0.30 ± 0.1*	0.32 ± 0.1*	0.21 ± 0.1	0.11 ± 0.1	0.38 ± 0.1**	0.22 ± 0.1
HLA-B*57	−0.20 ± 0.2	−0.41 ± 0.2*	−0.58 ± 0.2***	−0.57 ± 0.1***	−0.46 ± 0.2**	−0.51 ± 0.1***
Overall R^2 for each model[c]	0.072	0.129	0.104	0.152	0.137	0.151

[a] Individual predictors are defined in Table 3.

[b] HIV-1 subtype A serves as the reference group.

[c] Overall P <0.0001 for all tests.

[d] The cross-sectional outcomes are defined in Table 1. Summary statistics correspond to mean difference (beta estimate) ± standard error of the mean. Statistical significance is shown at three levels (* for P <0.05; ** for <0.01, and *** for <0.001).

[e] Estimates here are virtually identical to those based on the analysis of 3–12 months geometric mean VLs (overall r^2 = 0.108).

Table 5

Sensitivity analyses using longitudinal viral load (VL) data from 532 seroconverters who have at least two VL data points during the 3–24 months interval after infection.

Predictors (independent variables)	Effects by univariable models[b]			Multivariable model[c]	
	Δ ± SE	*P*	R^2	Δ ± SE	*P*
Age >40 years	−0.05 ± 0.10	0.611	0.000	−0.11 ± 0.08	0.237
Female sex	<u>−0.35 ± 0.08</u>	<0.0001	<u>0.025</u>	−0.38 ± 0.10	<0.0001
HIV-1 subtype C[a]	0.26 ± 0.08	0.001	<u>0.020</u>	0.37 ± 0.08	<0.0001
Other HIV-1 subtypes[a]	0.10 ± 0.10	0.320	0.001	0.29 ± 0.10	0.003
Duration of infection (monthly)	−0.01 ± 0.00	0.001	0.007	−0.01 ± 0.00	0.001
HLA-B*18	<u>0.33 ± 0.15</u>	0.025	0.007	0.36 ± 0.14	0.001
HLA-B*45	0.28 ± 0.10	0.005	<u>0.013</u>	0.27 ± 0.10	0.006
HLA-B*53	0.18 ± 0.10	0.055	0.011	0.20 ± 0.09	0.025
HLA-B*57	<u>−0.50 ± 0.13</u>	<0.0001	0.012	−0.46 ± 0.12	<0.001
Overall summary statistics	NA			R^2 = 0.041, *P* <0.0001	

[a] HIV-1 subtype A is the reference group.

[b] Top three predictors ranked by the effect size are underlined.

[c] Based on simultaneous evaluation of all factors as shown. For consistency with reported approach (Tang et al., 2002b), age is kept as a covariate in all multivariable models (here and Table 3) regardless of statistical significance.