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HIV-1 outcompetes HIV-2 in dually infected Senegalese individuals with low CD4⁺ cell counts

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Abstract

Objective—Dual infection with HIV-1 and HIV-2, which is not uncommon in West Africa, has implications for transmission, progression, and antiretroviral therapy (ART). Few studies have examined viral dynamics in this setting. Our objective was to directly compare HIV-1 and HIV-2 viral loads and to examine whether this relationship is associated with CD4⁺ cell count.

Study design—This is a retrospective analysis of data from observational cohort studies.

Methods—We compared HIV-1 and HIV-2 viral loads from 65 dually infected, ART-naïve Senegalese individuals. Participants provided blood, oral fluid, and cervicovaginal lavage (CVL) or semen samples for virologic and immunologic testing. We assessed relationships between HIV-1 and HIV-2 levels using linear regression with generalized estimating equations to account for multiple study visits.

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Conflicts of interest

There are no conflicts of interest.

Results—After adjusting for CD4⁺ cell count, age, sex, and commercial sex work, HIV-1 RNA levels were significantly higher than HIV-2 levels in semen, CVL, and oral fluids. Despite similar peripheral blood mononuclear cell DNA levels among individuals with CD4⁺ cell counts above 500 cells/μl, individuals with CD4⁺ cell counts below 500 cells/μl had higher HIV-1 and lower HIV-2 DNA levels. Individuals with high CD4⁺ cell counts had higher mean HIV-1 plasma RNA viral loads than HIV-2, with HIV-1 levels significantly higher and HIV-2 levels trending toward lower mean viral loads among individuals with low CD4⁺ cell counts.

Conclusion—Our data are consistent with the hypothesis that with disease progression, HIV-1 outcompetes HIV-2 in dually infected individuals. This finding helps explain differences in prevalence and outcomes between HIV-1, HIV-2, and HIV-dual infection.

Keywords

HIV dual infection; HIV viral load; HIV-1; HIV-2; Senegal

Introduction

Worldwide, 1–2 million of those living with HIV are infected with HIV-2 [1], which is endemic in West Africa [2]. Although HIV-1 and HIV-2 are both causative agents of AIDS, HIV-2 infection is characterized by lower plasma viral loads despite similar proviral DNA levels [3–5]. Vertical and heterosexual transmission rates are lower [6,7], and individuals infected with HIV-2 experience a slower decline in CD4⁺ T-cell counts, longer asymptomatic stage, and slower disease progression [8–10]. Nevertheless, without treatment, a significant proportion of those infected with HIV-2 progress to AIDS [11]. Dual infection with HIV-1 and HIV-2 was first confirmed in 1988 [12], but the difficulty in distinguishing between dual seropositivity and dual infection [13–15] has hampered efforts to estimate the prevalence of this phenomenon, and studies of clinical progression and outcomes are scarce.

Since a 1995 report suggested that HIV-2 might have a protective effect against subsequent HIV-1 infection [16], there has been significant debate surrounding the clinical significance of dual infection. Despite biological mechanisms supporting a protective effect *in vitro* [17–19], most epidemiologic studies report no difference in HIV-1 acquisition or survival (reviewed in [20]) among dually infected individuals. Two recent studies from Guinea-Bissau yielded conflicting results – one demonstrated similar survival among HIV-dually infected individuals compared to HIV-1 monoinfected [21] and one reported significantly longer time to AIDS after HIV-1 infection among those also infected with HIV-2 [22]. The reasons for this inconsistency are unclear. Limited previous studies comparing dually infected individuals to monoinfected demonstrated diverging viral loads between CD4⁺ strata [15,23–26], suggesting that HIV-1 might outcompete HIV-2 among dually infected individuals; however, some studies disagree [27], and no study compared HIV-1 to HIV-2 viral loads within dually infected individuals. Further, although we and others previously demonstrated higher RNA levels in oral and genital tracts in HIV-1 monoinfected individuals compared to HIV-2 monoinfected [28–31], viral burdens in these sites have never been examined in dually infected individuals, and may be important factors in transmission and prevalence.

Antiretroviral therapy (ART) outcomes among HIV-2 and HIV-dually infected individuals are generally poor, often worse than HIV-1 alone, and HIV-2 is susceptible to a limited number of antiretroviral drugs [32,33]. Therefore, although dually infected patients represent a very small proportion of HIV-infected individuals worldwide, dual infection has critical implications for transmission, disease progression, and ART. A greater understanding of viral dynamics among those infected with HIV-1 and HIV-2 may be useful to guide clinical management of these patients. To this end, we examined the relationship between HIV-1 and HIV-2 viral loads from dually infected subjects from Senegal, West Africa.

Methods

Study population

As part of enrollment for four prospective studies, HIV serologic testing was offered to 10 000 men and women age 16 and older presenting to clinics in Senegal, West Africa, between 1994 and 2007. Clinics included the Clinique des Maladies Infectieuses Ibrahima DIOP Mar – Centre Hospitalier Universitaire (CHU) de Fann at the Universite Cheikh Anta Diop de Dakar, the Association Sénégalaise pour le Bien-Etre Familial (ASBEF) family planning clinic, and public health (Institut d'Hygiene Sociale) STD clinics for commercial sex workers at M'Bour and Dakar. Among those screened, 7586 (75.9%) were HIV negative, 1922 (19.2%) were HIV-1 positive, 320 (3.2%) were HIV-2 positive, and 172 (1.7%) individuals were dually seropositive. Of dually seropositive individuals identified, 109 enrolled into studies, returning for follow-up visits every 4–6 months. All participants provided written informed consent. Studies were conducted according to procedures approved by institutional review boards at the University of Washington (IRB00000242), and the Universite Cheikh Anta Diop de Dakar and Senegalese National AIDS Committee (IRB00002659).

Collection of specimens and study procedures

At study visits participants underwent a physical examination, completed a standardized interview including demographics, medical and sexual history, and had blood collected for viral load testing and CD4⁺ T-lymphocyte counts. Serologic testing was conducted by microwell plate HIV-1/HIV-2 enzyme immunoassay or ELISA (Genie; Sanofi Diagnostics Pasteur, Redmond, Washington, USA; ELISA; Genetic Systems, Redmond, Washington, USA; GenScreen version 2; BioRad, Hercules, California, USA), with confirmatory testing using rapid synthetic peptide-based membrane immunoassays, which can differentiate between HIV-1 and HIV-2 (Multispot, Genetic Systems; ImmunoComb BiSpot, Orgenics, Yavne, Israel), and follow-up HIV-1-specific and HIV-2-specific western blot assays (BioRad), according to manufacturer instructions [5,34,35]. HIV-1 and HIV-2 quantitative and qualitative viral load assays for plasma, peripheral blood mononuclear cells (PBMCs), cervicovaginal lavage (CVL), semen, and oral fluids were performed using polymerase chain reaction-based assays developed at Roche Molecular Systems (Pleasanton, California, USA) as described previously [29–31]. A person was considered dually infected if both HIV-1 and HIV-2 nucleic acid amplification testing (NAAT) were ever positive, from any anatomic site, either at baseline or during follow-up. If serologic testing was positive for

both HIV-1 and HIV-2 but one or both viruses could never be detected by NAAT, individuals were considered dually seropositive but not dually infected.

Statistical analysis

Categorical variables were compared using Pearson's χ^2 tests or Fisher's exact tests; continuous variables were compared by nonparametric Mann–Whitney U tests for medians or Student's t tests for means. Linear regression models were generated using generalized estimating equations, assuming an independent correlation structure, to account for repeated observations for some individuals, and were adjusted for CD4⁺ T-cell count (categorized, <200, 200–500, and >500 cells/ μ l), age, sex, and history of commercial sex work. Viral load measurements were log₁₀ transformed to account for nonnormality. In order to include samples below the limit of detection, samples in which HIV-1 RNA was not detected were assigned a value of 10 copies/ml, whereas samples in which HIV-1 RNA was detected but below the reliable limit of detection of the quantitative assay were assigned a value of 100 copies/ml. For the HIV-2 assay, which had twice the sensitivity of the HIV-1 assay, samples were assigned values of 5 and 50 copies/ml, respectively. A value of 0.5 copies/ μ g of PBMC DNA was assigned to samples in which HIV PBMC DNA could not be detected, and a value of 1 copy/ μ g of PBMC DNA to samples in which PBMC DNA was detected but could not be quantified. We previously demonstrated no qualitative difference in results when setting values for quantitatively negative samples over a range of values between zero and the limit of detection [5,29]. For those individuals with a qualitative positive PCR result but for whom no quantitative test was run, that individual's mean viral load for that site from other visits was used where longitudinal follow-up was available. Viral load assays for HIV-1 and HIV-2 were sometimes not run in parallel, so for paired correlation analyses, we matched HIV-1 and HIV-2 viral load data within a 6-month window when no true matched pairs fell within the interval. Artificially matched pairs accounted for less than 10% of pairs in these analyses. Viral loads obtained after initiation of ART were excluded. CD4⁺ cell counts were imputed from the closest study visit when missing. The level of statistical significance used for all analyses was $P < 0.05$. All analyses were carried out in Intercooled Stata version 11.2 (StataCorp, College Station, Texas, USA).

Results

Characteristics of the study population

Of 172 HIV-1/2 dually seropositive individuals identified, 109 individuals enrolled in follow-up studies. Eighty-eight individuals had at least one viral load test completed for each virus; of these we could only confirm dual infection by NAAT for both HIV-1 and HIV-2 for 65 individuals (74%). Among the remaining individuals, we could detect only HIV-1 for 16 individuals, only HIV-2 for five, and were unable to amplify either virus for two individuals. Individuals with multiple viral load tests run, from multiple anatomic sites and/or study visits, were more likely to be identified as dually infected (not shown). Primary analyses were carried out within the subset of individuals for whom we could confirm dual infection by NAAT.

Dually infected individuals were generally representative of the 109 dually seropositive individuals identified (Table 1). Individuals in whom dual infection was confirmed were predominantly born in Senegal (88%). Most were recruited from Clinique des Maladies Infectieuses Ibrahima DIOP Mar – CHU de Fann in Dakar (68%) and slightly more than half were female (57%); of the women enrolled, 57% reported having traded sex for money or goods. Cigarette and alcohol use (20 and 13%, respectively) were generally confined to commercial sex workers. The average age was approximately 37 years. Baseline CD4⁺ cell count was 355 cells/μl, although women enrolled with significantly higher CD4⁺ cell counts than men (mean 475 vs. 192 cells/μl, $P = 0.0001$). Those individuals in whom dual infection could not be confirmed were generally similar to those in whom we could detect both viruses, although those for whom we could not detect both viruses had an average CD4⁺ cell count nearly 70 cells/μl lower than those in whom we could detect both viruses ($P = 0.28$).

Samples analyzed

We analyzed viral loads from 224 HIV-1 and 172 HIV-2 plasma, 115 and 109 PBMC, 19 and 12 oral, 18 and 16 CVL, and five and five semen samples, respectively, from a total of 65 dually infected individuals.

Baseline HIV-1 and HIV-2 levels differ in plasma, oral fluid, cervicovaginal lavage, and semen

At baseline, median plasma RNA viral loads among dually infected individuals were 4.4 and 2.3 log₁₀ copies/ml for HIV-1 and HIV-2, respectively ($P < 0.0001$ by Mann–Whitney U test) (Table 2). HIV-1 plasma RNA was not detected in six (10%) specimens, and HIV-2 plasma RNA was not detected in 23 (38%) specimens ($P < 0.001$ by binomial Pearson's χ^2 test). Median PBMC DNA viral loads were 1.2 and 1.1 log₁₀ copies/μg of PBMC DNA ($P = 0.24$); HIV-1 or HIV-2 PBMC DNA was not detected in six (11%) and seven (15%) specimens, respectively ($P = 0.63$). Viral loads from oral fluid, CVL, and semen samples were available for a subset of individuals. In oral fluid, HIV-1 RNA was not detectable in two (14%) specimens, compared to all 11 specimens for HIV-2 ($P < 0.001$ by Fisher's exact test). HIV-1 RNA was not detected in eight (67%) CVL samples, compared to seven (100%) for HIV-2 ($P = 0.25$), and among semen samples, HIV-1 RNA was detected in all five samples, whereas HIV-2 RNA was not detected in three (60%) ($P = 0.17$).

HIV-1 and HIV-2 plasma RNA levels are divergent at lower CD4⁺ cell counts

HIV-1 and HIV-2 log₁₀ transformed viral loads in plasma were inversely associated in unadjusted analysis considering only paired data (Fig. 1a; Pearson $R = -0.2274$, $P = 0.004$), as well as in a regression analysis of viral type on all viral load data ($\beta = -1.90$, 95% CI -2.20 to -1.61). When stratifying HIV-1 and HIV-2 plasma RNA levels by CD4⁺ cell count at the same visit, lower CD4⁺ cell count appeared associated with higher HIV-1 viral load, but decreased HIV-2 viral load (Fig. 1b).

In regression analyses stratified by CD4⁺ cell count, among individuals with a CD4⁺ cell count above 500 cells/μl, we found less difference between mean HIV-1 and HIV-2 plasma viral loads compared to individuals in medium and low CD4⁺ cell count categories (Table 3). After adjusting for age, sex, and history of commercial sex work, among individuals with

a CD4⁺ cell count above 500 cells/ μ l, mean HIV-1 plasma viral load was 0.88 log₁₀ copies/ml (95% CI 0.37 to 1.38) higher than HIV-2, with mean HIV-1 plasma viral load 2.90 log₁₀ copies/ml (95% CI 1.91 to 3.88), and mean HIV-2 plasma viral load 2.02 (95% CI 1.21 to 2.73) log₁₀ copies/ml. Among individuals with CD4⁺ cell counts between 200 and 500 cells/ μ l, mean HIV-1 RNA viral load was greater than 2.5 log₁₀ copies/ml higher than among subjects with CD4⁺ cell counts greater than 500 cells/ μ l (β = 2.79, 95% CI 1.71 to 2.86). HIV-2 viral loads did not differ significantly across CD4⁺ categories but showed a trend toward lower RNA levels at lower CD4⁺ cell counts.

HIV-1 and HIV-2 peripheral blood mononuclear cell DNA levels are only similar among individuals with high CD4⁺ cell counts

Overall, PBMC-associated DNA from both viruses was detected less frequently than in a previous study in monoinfected subjects [5]. Mean CD4⁺ cell count among those with detectable HIV-1 DNA was lower than among those with undetectable HIV-1 DNA viral loads (316.7 cells/ μ l vs. 564.5, P = 0.038), although we observed no difference in mean CD4⁺ cell count among those with detectable vs. undetectable HIV-2 (398.7 vs. 333.9, P = 0.331).

Paired log₁₀-transformed HIV-1 and HIV-2 PBMC DNA levels were inversely correlated (Fig. 1c; Pearson R = -0.3628, P = 0.0003). This association was confirmed by regression analysis of all viral load data (β = -0.34, 95% CI -0.57 to -0.11). Although comparable at high CD4⁺ cell counts, mean HIV-1 DNA levels were higher, and HIV-2 DNA levels lower, among those in lower CD4⁺ cell count strata (Fig. 1d). Multivariate regression analysis, after controlling for age, sex, and commercial sex work, revealed no difference between HIV-1 and HIV-2 DNA levels in PBMC (β = 0.17, 95% CI -0.24 to 0.58) among subjects with high CD4⁺ cell counts (Table 3). However, among those with medium (200–500 cells/ μ l) and low (<200 cells/ μ l) CD4⁺ cell counts, mean HIV-2 DNA viral load was below the assay limit of detection (β = -0.80, 95% CI -1.40 to -0.20, and β = -0.75, 95% CI -1.30 to -0.21, respectively), whereas mean HIV-1 DNA levels were higher than among those with high CD4⁺ cell counts by 1.22 (95% CI 0.25 to 2.18) and 1.26 (95% CI 0.38 to 2.15) log₁₀ copies/ μ g of PBMC DNA, respectively.

HIV-1 RNA levels are higher than HIV-2 levels in oral and genital secretions

HIV-2 RNA levels in CVL samples, semen, and oral fluid were lower than HIV-1 by 1.37 (95% CI 0.83 to 1.91), 2.05 (95% CI 0.44 to 3.66), and 1.93 (95% CI 1.56 to 2.30) log₁₀ copies/ml, respectively, after controlling for age, sex, history of commercial sex work, and CD4⁺ cell count (Table 3). Data were insufficient from individuals with CD4⁺ cell counts greater than 500 cells/ μ l to investigate whether the diverging trend observed in plasma and PBMC was also present in oral and genital secretions.

Peripheral blood mononuclear cell DNA levels are predictive of plasma RNA levels in individuals with high CD4⁺ cell counts

Within individuals, PBMC DNA viral loads were predictive of plasma RNA levels, regardless of HIV type (Fig. 2; HIV-1 Pearson R = 0.6965, HIV-2 Pearson R = 0.6737). In multivariate analysis, among those with CD4⁺ cell counts above 500 cells/ μ l, PBMC DNA

levels remained highly associated with plasma RNA levels, irrespective of HIV type (Supplemental Digital Content, Table 1, <http://links.lww.com/QAD/A360>). Each log₁₀ increase of HIV-1 PBMC DNA copies/μg was associated with a mean 1.38 (95% CI 0.85 to 1.92) log₁₀ increase in HIV-1 plasma RNA copies/ml; likewise, each log₁₀ increase in HIV-2 PBMC DNA copies/μg was associated with a mean 0.98 (95% CI 0.57 to 1.40) log₁₀ copies/ml increase in HIV-2 plasma RNA level. No association was found in those with low CD4⁺ cell counts, although HIV-1 levels trended toward a negative association ($\beta = -0.48$, 95% CI -1.14 to 0.17).

Discussion

We examined HIV-1 and HIV-2 levels both systemically (plasma and PBMC) and locally in oral and genital sites in 65 confirmed dually infected, ART-naïve men and women from Senegal. We found significantly higher HIV-1 RNA levels in plasma, oral fluid, semen, and CVL lavage than HIV-2 levels; the magnitude of the difference in plasma increased at lower CD4⁺ cell counts. Although HIV-1 and HIV-2 PBMC DNA levels were similar in individuals with high CD4⁺ cell counts, among those with CD4⁺ cell counts below 500 cells/μl, HIV-1 DNA was, on average, present at higher levels than HIV-2.

Most studies of HIV viral dynamics have focused solely on HIV-1 and/or HIV-2 monoinfections, treating dual seropositivity as an exclusion criterion. These studies showed that both HIV-1 and HIV-2 plasma RNA and PBMC DNA levels are inversely correlated with CD4⁺ cell counts among monoinfected individuals, and that proviral DNA levels of both viruses are similar at each stage of infection [3–5,27]. However, in 1998, Sarr *et al.* [15] were less frequently able to detect HIV-2 provirus among hospitalized dually seropositive individuals compared to asymptomatic dually seropositive individuals, despite equivalent detection of HIV-1 provirus, suggesting for the first time that HIV-2 viral loads might be associated with CD4⁺ cell count. This hypothesis has been supported in PBMCs [23] and plasma [24–26] when comparing dually infected individuals to HIV-2 monoinfected. However, Alabi *et al.* [26] reported similar HIV-1 plasma RNA levels across CD4⁺ strata when comparing HIV dual infection to HIV-1 monoinfection, and a study by Andersson *et al.* [27] found no correlation between either HIV-1 and HIV-2 plasma RNA levels or plasma RNA levels and CD4⁺ cell count.

Previous studies showing that HIV-1 outcompetes HIV-2 compared HIV viral loads from monoinfected to dually infected individuals; to our knowledge, ours is the first to directly compare HIV-1 levels to HIV-2 within dually infected individuals. Our results reveal that HIV-1 and HIV-2 DNA levels are similar among those with high CD4⁺ cell counts but are quite divergent at lower CD4⁺ cell counts, and demonstrate higher HIV-1 plasma RNA levels, and a corresponding trend toward lower HIV-2 levels, associated with lower CD4⁺ cell count. Considering the high frequency of undetectable HIV-2 viral loads among those with low CD4⁺ cell counts, it is possible that this trend toward lower HIV-2 viral loads at low CD4⁺ cell counts is stronger than we can detect. These results, although they are cross-sectional rather than longitudinal, directly support the hypothesis that HIV-1 generally outcompetes HIV-2 over the course of dual infection. Importantly, these findings may not represent the experience of all dually infected individuals and are based on data from

seroprevalent cases – the influence of HIV-1 levels on HIV-2 (or vice versa) may depend on the order of infection, or perhaps other unidentified host immune or viral factors.

We demonstrate for the first time that among HIV-1/2 dually infected individuals, HIV-1 viral loads are significantly higher than HIV-2 in oral and genital fluids. Viral load dynamics in the genital tract have critical implications for understanding transmission, and to our knowledge, no studies have ever examined nonblood viral burden among dually infected individuals. Although we and others have previously shown in monoinfected individuals that HIV-2 levels in semen, oral fluids, and CVL are significantly lower than HIV-1 levels, and that shedding is correlated with plasma viral load but not CD4⁺ cell count, irrespective of virus type [28–31], whether this relationship could be generalized to dual infection was unclear. Therefore, although our examination of local viral dynamics in oral and genital tracts is limited by small numbers of specimens with available viral load data and we are unable to assess the association of CD4⁺ cell count with viral burden in these settings, these data offer a unique insight into virus type-specific infectiousness in this population.

Studies of dual infection are hampered by the difficulty of accurate diagnosis, and more sensitive and specific algorithms for identifying true dual infection are needed. Two key issues complicate the detection of HIV dual infection: lack of serologic assay specificity and host virus control. Host virus control results in viral loads below the limit of nucleic acid test detection and false-negative tests, and is commonly reported in HIV-2 monoinfection, as well as HIV-1/2 dual infection. The best assays detect dual PCR positivity at a single time-point in only ~70–85% of cases [13–15,36,37]. Further, although our HIV-2 viral load test was HIV-2-specific, early versions of the Roche Amplicor HIV-1 assay were slightly cross-reactive with HIV-2. Lack of assay specificity, especially cross-reactivity in the serologic test detection, may result in false-positive tests and subsequent subject misclassification [38]. Although HIV is typically more frequently detectable from PBMC DNA than plasma RNA, rates of undetectable viral loads for both viruses among dually infected individuals were higher than we have reported in monoinfected cohorts [5]. We cannot rule out technical issues that may have impacted our ability to detect virus in these samples; however, the correlation between HIV-1 PBMC DNA detection and CD4⁺ cell count results suggests a possible biological explanation, further complicating detection of dual infection. Due to the higher sensitivity of serologic tests compared to nucleic acid testing, both the WHO [39] and CDC [40] recommend serologic testing for diagnosis of HIV-1, HIV-2, and HIV-dual infection, and dual seropositivity is thought to be highly suggestive of dual infection. We only reported results for dually seropositive individuals in whom we could confirm dual infection based on nucleic acid amplification at any time-point and in any anatomic site. Although the potential for misclassification cannot be ignored, by using these criteria we established a more stringent definition of dual infection than relying solely on serologic testing, yet maximize the inclusion of individuals who may not have had detectable circulating nucleic acid at baseline. Of note, we obtained similar results and reached similar conclusions when we carried out our analyses using the full set of 109 dually seropositive individuals, setting viral loads for a given virus type in those with unconfirmed infection to the lower limit of detection (not shown).

Our results provide substantial evidence to support the theory that, on average, HIV-1 outcompetes HIV-2 at lower CD4⁺ cell counts, offer insight into localized viral dynamics in oral and genital tract fluids and the natural history of dual infection, and provide possible explanations of the differences in prevalence between HIV-1, HIV-2, and HIV-dual infection. In the era of highly active ART, it is unlikely that this relationship will ever be completely understood; regardless, clinical and therapeutic outcomes of HIV dual infection remain largely unknown and warrant further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. HIV-1 and HIV-2 plasma RNA levels among HIV-1/2 dually infected individuals
Correlation between HIV-1 and HIV-2 is shown in panels a and c, respectively. Data points represent paired HIV-1 and HIV-2 viral loads. Filled markers indicate that both viral loads are from the same study visit, open markers indicate that one viral load is from a subsequent visit within 6 months. The linear regression line is also shown. Plasma RNA and PBMC DNA levels, stratified by CD4⁺ cell count, are shown in panels b and d, respectively. PMBC, peripheral blood mononuclear cell.

Fig. 2. Correlation of HIV-1 and HIV-2 plasma RNA with peripheral blood mononuclear cell DNA levels in HIV-1/2 dually infected individuals

Data points represent HIV-1 (black diamonds) or HIV-2 (gray circles) plasma RNA/PBMC DNA pairs in dual HIV-1/2 infection. Filled markers indicate that plasma RNA and PBMC DNA levels are from the same study visit, open markers indicate that one level is from a subsequent visit within 6 months. Linear regression lines for the relationship between DNA and RNA are also shown, solid lines for individuals with $CD4^+$ cell counts greater than or equal to 500 cells/ μl or dashed lines for $CD4^+$ cell counts less than 500 cells/ μl . PMBC, peripheral blood mononuclear cell.

Table 1

Characteristics of HIV-1/2 dually seropositive subjects.

Characteristic	All dually seropositive individuals enrolled (n = 109)	NAAT confirmed dually infected individuals (n = 65)	Not NAAT confirmed dually infected individuals (n = 44)
Recruitment site			
Institut d'Hygiene Sociale – Mbour	7 (6)	5 (8)	2 (5)
ASBEF Family Planning Clinic	1 (1)	1 (2)	0 (0)
Institut d'Hygiene Sociale – Dakar	25 (23)	15 (23)	10 (23)
Clinique des Maladies Infectieuses CHU - Fann	76 (70)	44 (68)	32 (73)
Sex, female	62 (57)	37 (57)	25 (57)
Commercial sex worker ^a	34 (31)	21 (32)	13 (30)
Age (years) ^b			
20–29	23 (21)	14 (22)	8 (19)
30–39	37 (34)	25 (38)	11 (26)
40–49	38 (35)	22 (34)	16 (38)
50	11 (10)	4 (6)	7 (17)
Mean ± SD	37.7±8.9	36.6±8.2	39.3±9.6
Born in Senegal	96 (91)	56 (88)	40 (95)
Ethnicity ^c			
Wolof	50 (47)	28 (44)	22 (51)
Pulaar	21 (20)	12 (19)	9 (21)
Serere	12 (11)	8 (13)	4 (9)
Sarakhole	2 (2)	2 (3)	0 (0)
Mandjack	1 (1)	0 (0)	1 (2)
Diola	5 (5)	2 (3)	3 (7)
Other	16 (15)	12 (19)	4 (9)
Religion ^d			
Muslim	86 (88)	46 (84)	40 (93)
Christian	11 (11)	8 (15)	3 (7)
Other	1 (1)	1 (2)	0 (0)

Characteristic	All dually seropositive individuals enrolled (n = 109)	NAAT confirmed dually infected individuals (n = 65)	Not NAAT confirmed dually infected individuals (n = 44)
Education ^c			
None	56 (52)	32 (50)	24 (56)
Primary	37 (35)	21 (33)	16 (37)
Secondary	12 (11)	9 (14)	3 (7)
University	2 (2)	2 (3)	0 (0)
Marital status ^c			
Single	26 (25)	16 (25)	10 (23)
Monogamous	27 (25)	15 (24)	12 (28)
Polygamous	13 (12)	8 (13)	5 (12)
Separated/divorced	29 (27)	17 (27)	12 (28)
Widowed	11 (10)	7 (11)	4 (9)
Smoker	26 (24)	13 (20)	13 (30)
Alcohol user	14 (13)	8 (13)	6 (14)
CD4 ⁺ cell count (cells/μl) at screening ^e			
<200	42 (43)	22 (36)	20 (56)
200–350	18 (19)	13 (21)	5 (14)
350–500	12 (12)	10 (16)	2 (6)
500	25 (26)	16 (26)	9 (25)
Mean±SD	330 ±290	355 ±288	288 ±293

Data are number (%) except where otherwise indicated. NAAT, nucleic acid amplification testing; SD, standard deviation.

^aEnrollees in one study were not asked about history of commercial sex work. For these subjects, clinic type (IHS vs. other) was used as a proxy for history of commercial sex work.

^bAge of one dually seropositive subject was missing.

^cEthnicity, education, and marital status were missing for <10% of individuals.

^dReligion was missing for 10–15% of individuals.

^eCD4⁺ cell counts were missing for 11% of enrolled individuals, 8% of confirmed dually infected individuals, and 18% of dually seropositive individuals without confirmation of dual infection.

Table 2

Cross-sectional detection and quantification of HIV-1 and HIV-2 RNA and DNA among HIV-1/2 dually infected subjects ($n = 65$).

HIV RNA and DNA	HIV-1	HIV-2	P^a
Plasma (RNA copies/ml)	$n = 60$	$n = 60$	
RNA not detected	6 (10)	23 (38)	<0.001
<1000	5 (8)	19 (32)	
1000–9999	7 (12)	14 (23)	
10000–99999	25 (42)	4 (7)	
100000+	17 (28)	0 (0)	
Median (IQR) ($\log_{10}$ copies/ml) ^b	4.4 (3.7–5.1)	2.3 (0.7–3.0)	<0.0001
PBMC (DNA copies/ μ g)	$n = 53$	$n = 48$	
DNA not detected	6 (11)	7 (15)	0.63
<10	17 (32)	16 (33)	
10–99	23 (43)	19 (40)	
100–999	7 (13)	6 (13)	
1000+	0 (0)	0 (0)	
Median (IQR) ($\log_{10}$ copies/ μ g) ^b	1.2 (0.6–1.8)	1.1 (0.0–1.5)	0.21
Oral fluid (RNA copies/ml)	$n = 14$	$n = 11$	
RNA not detected	2 (14)	11 (100)	<0.001
<1000	9 (64)	0 (0)	
1000–9999	3 (21)	0 (0)	
10000–99999	0 (0)	0 (0)	
100000+	0 (0)	0 (0)	
Median (IQR) ($\log_{10}$ copies/ml) ^b	2.5 (2.0–3.0)	0.7 (0.7–0.70)	<0.0001
CVL (RNA copies/ml)	$n = 12$	$n = 7$	
RNA not detected	8 (67)	7 (100)	0.25
<1000	0 (0)	0 (0)	
1000–9999	4 (33)	0 (0)	
10000–99999	0 (0)	0 (0)	
100000+	0 (0)	0 (0)	

HIV RNA and DNA	HIV-1	HIV-2	<i>P</i> ^a
Median (IQR) (log ₁₀ copies/ml) ^b	1.0 (1.0–3.2)	0.7 (0.7–0.7)	0.0002
Semen (RNA copies/ml)	<i>n</i> = 5	<i>n</i> = 5	
RNA not detected	0 (0)	3 (60)	0.17
<1000	2 (40)	0 (0)	
1000–9999	0 (0)	2 (40)	
10000–99999	2 (40)	0 (0)	
100000+	1 (20)	0 (0)	
Median (IQR) (log ₁₀ copies/ml) ^b	4.1 (2.6–4.6)	0.7 (0.7–3.5)	0.07

CVL, cervicovaginal lavage; IQR, interquartile range; PBMC, peripheral blood mononuclear cells.

^a *P* calculated by chi square test or Fisher’s exact test to compare detectable vs. undetectable, and by Mann–Whitney *U* test to compare medians.

^b Medians and interquartile ranges including values assigned to samples below the limit of detection (see Statistical methods).

Table 3
Immunovirologic correlates of HIV-1 and HIV-2 viral loads among HIV-1/2 dually infected individuals (*n* = 65).

Variable	Univariate			Adjusted ^a		
	α	β	(95% CI)	α	β	(95% CI)
Plasma RNA	HIV type $\times$ CD4 ⁺ cell count					
	HIV-1, above 500	3.78	(3.00 to 4.56)	2.90		(1.91 to 3.88)
	HIV-1, 200–500		2.84 (1.74 to 3.94)		2.79	(1.71 to 3.86)
	HIV-1, below 200		2.97 (1.92 to 4.02)		2.83	(1.80 to 3.86)
	HIV-2, above 500		-0.84 (-1.37 to -0.32)		-0.88	(-1.38 to -0.37)
	HIV-2, 200–500		-1.49 (-2.21 to -0.77)		-1.47	(-2.18 to -0.77)
	HIV-2, below 200		-1.54 (-2.24 to -0.85)		-1.51	(-2.18 to -0.83)
PBMC DNA	HIV type $\times$ CD4 ⁺ cell count					
	HIV-1, above 500	0.58	(-0.06 to 1.22)	0.59		(-0.20 to 1.38)
	HIV-1, 200–500		1.23 (0.29 to 2.18)		1.22	(0.25 to 2.18)
	HIV-1, below 200		1.33 (0.49 to 2.17)		1.26	(0.38 to 2.15)
	HIV-2, above 500		0.17 (-0.23 to 0.58)		0.17	(-0.24 to 0.58)
	HIV-2, 200–500		-0.80 (-1.39 to -0.20)		-0.80	(-1.40 to -0.20)
	HIV-2, below 200		-0.77 (-1.31 to -0.23)		-0.75	(-1.30 to -0.21)
Oral fluid RNA	HIV type					
	HIV-1	4.48	(3.82 to 5.14)	3.13		(1.89 to 4.37)
	HIV-2		-1.89 (-2.34 to -1.44)		-1.93	(-2.30 to -1.56)
	CD4 ⁺ cell count (cells/ μ l)					(ref)
	100	1.77	(1.30 to 2.24)			(ref)
	Change/100 cells		0.10 (-0.12 to 0.32)		-0.09	(-0.19 to 0.00)
CVL RNA	HIV type					
	HIV-1	3.25	(2.38 to 4.12)	5.02		(3.42 to 6.61)
	HIV-2		-1.23 (-1.79 to -0.67)		-1.37	(-1.91 to -0.83)
	CD4 ⁺ cell count (cells/ μ l)					(ref)
	100	1.52	(1.11 to 1.92)			(ref)
	Change/100 cells		-0.03 (-0.16 to 0.10)		-0.10	(-0.20 to 0.00)
Semen RNA	HIV type					

Variable	Univariate			Adjusted ^a		
	α	β	(95% CI)	α	β	(95% CI)
HIV-1	5.96		(3.34 to 8.58)	5.43		(1.35 to 9.50)
HIV-2		-2.05	(-3.70 to -0.39)		-2.05	(-3.66 to -0.44)
CD4 ⁺ cell count (cells/ μ l)						
100	3.05		(1.84 to 4.25)		(ref)	
Change/100 cells		-0.10	(-0.50 to 0.30)		-0.15	(-0.54 to 0.23)

PBMC, peripheral blood mononuclear cells; CVL, cervicovaginal lavage; CI, confidence interval. Values are expressed in log10copies/ml (RNA) or log10copies/ μ g of PBMC DNA (DNA). Values in bold are statistically significant.

^aFrom multivariate linear regression using generalized estimating equations, adjusting for all factors in the table, as well as age, sex, and history of commercial sex work.