HIV-1 outcompetes HIV-2 in dually infected Senegalese subjects with low CD4 counts

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Abstract

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Dual infection with HIV-1 and HIV-2, which is not uncommon in West Africa, has important implications for transmission, progression, and antiretroviral therapy. Few studies have examined HIV viral dynamics in this setting. We compared HIV-1 and HIV-2 viral loads from 65 dually infected, antiretroviral therapy-naïve Senegalese subjects. Participants provided demographic information and blood, oral fluid, and cervicovaginal lavage (CVL) or semen samples for virologic and immunologic testing. Associations between HIV-1 and HIV-2 levels in plasma, PBMC, oral and genital samples were assessed using linear regression models with generalized estimating equations to account for subjects with multiple samples over time. In analyses adjusting for CD4 count, age, sex, and commercial sex work, HIV-1 RNA levels were significantly higher than HIV-2 levels in semen, CVL, and oral fluids. HIV-1 and HIV-2 PBMC viral DNA loads were similar in those with CD4 counts above 500 cells/μl. However, compared to those with high CD4 counts, subjects with CD4 counts below 500 cells/μl had higher HIV-1 and lower HIV-2 DNA levels. In plasma, subjects with CD4 counts above 500 cells/μl had mean HIV-1 plasma RNA viral loads approximately one $\log_{10}$ copies/ml higher than HIV-2, with HIV-1 levels significantly higher and HIV-2 levels showing a trend toward lower mean viral loads among subjects with CD4 counts below 500 cells/μl. Our data are consistent with the hypothesis that with decreasing CD4 counts and HIV disease progression, HIV-1 outcompetes HIV-2 in dually-infected individuals. This finding may help explain the differences in epidemiology between HIV-1, HIV-2, and HIV-dual infection.

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INTRODUCTION

Worldwide, only a small proportion of those living with HIV are infected with HIV-2 [1], which is endemic in West Africa [2-4]. Although HIV-1 and HIV-2 share a common genome structure and clinical syndrome, HIV-2 infection is characterized by significantly lower viral loads in plasma [5-10], oral fluid [4], semen [11] and female genital tracts [12-14], despite similar proviral DNA levels [4-7, 15]. Vertical [16-18], and heterosexual [19, 20] transmission rates are much lower, and individuals infected with HIV-2 experience a much slower decline in CD4+ T cell counts [19, 21-23], longer asymptomatic stage, and slower progression to AIDS [21, 24-27]. Nevertheless, without treatment, a significant proportion of those infected will progress to AIDS [28, 29].

Dual infection with both HIV-1 and HIV-2 was first confirmed in 1988 [30], but the prevalence of this phenomenon is unclear and studies of clinical progression and viral load dynamics are extremely limited. Dual infection is rare outside of West Africa, although it is not uncommon in HIV-2 endemic areas, with a number of West African HIV cohorts reporting dually reactive serologies in 1-6% of participants [8, 31-33]. However, studies of dual infection are hampered by the difficulty in diagnosing true dual infection, as opposed to dual seropositivity [34-37].

There is no consensus regarding the clinical significance of dual infection. It has been speculated that HIV-2 might have a protective effect against HIV-1 – a hypothesis supported by numerous biological mechanisms and limited epidemiological data [38-42]. However, epidemiological reports from Cote d'Ivoire, [43], The Gambia [44], and Guinea-Bissau [45, 46] have failed to support the protective hypothesis, and it is unclear what role, if any, the order of infection may play in this issue. The implications of such a protective effect for dual infection are unknown. Dual infection progresses similarly to HIV-1 [31, 47, 48], with similar mortality [23, 49] despite lower HIV-1 plasma RNA levels compared to HIV-1 singly infected subjects [8, 48]. Further, ART outcomes among HIV-2 and HIV-dually infected individuals are generally poor, often worse than HIV-1 alone [50-53], and the number of antiretroviral drugs to which HIV-2 is susceptible is limited [54]. The consequences of dual infection for viral dynamics are also poorly understood. It has been suggested that the presence of HIV-1 may lead to viral synergism that could upregulate HIV-2 [55], or that alternately, the higher replication potential of HIV-1 would allow HIV-1 to outcompete HIV-2 in dually infected individuals. Limited previous studies have demonstrated diverging trends in viral loads between CD4 strata [8, 31, 36, 48, 56], supporting this idea, however no clear consensus can be reached from the existing data.

Although dually infected subjects represent a very small proportion of HIV-infected individuals worldwide, dual infection has critical implications for transmission, disease progression, and therapeutic options. We attempt to further elucidate viral dynamics in subjects dually infected with HIV-1 and HIV-2 through cross-sectional analysis of plasma and proviral loads from these subjects.

METHODS

Study population

Between 1994 and 2007, HIV serologic testing was offered to 10,000 men and women aged 16 and older presenting to the University of Dakar Infectious Disease Clinic (Fann Hospital, Dakar), the ASBEF (Association Sénégalaise pour le Bien-Etre Familial) family planning clinic, and public health STD clinics for commercial sex workers at M'Bour and Dakar in Senegal, West Africa. This testing was conducted as a part of recruitment for four longitudinal studies with the goals of studying the natural history of cervical neoplasia in HIV-1 and HIV-2, epidemiology of HIV-1 and HIV-2 in the cervix and vagina, epidemiology of HIV-1 and HIV-2 associated oral disease, and control of HIV-1 by HIV-2 associated immune responses. Of those screened, 7586 (75.9%) were HIV negative, 1922 (19.2%) were HIV-1 positive, and 320 (3.2%) were positive for HIV-2, and 172 (1.7%) subjects were dually seropositive. Of the dually seropositive subjects identified, 109 enrolled into prospective studies, returning for follow-up visits every four to six months. All participants gave written informed consent and studies were conducted according to procedures approved by Institutional Review Boards at the University of Washington, the University of Dakar, and the Senegalese National AIDS Committee.

Collection of specimens and study procedures

Consenting participants underwent a general physical examination, completed a standardized interview including demographic factors as well as medical and sexual history, and had blood collected for HIV-1 and HIV-2 viral loads and T-lymphocyte counts at study visits. Serologic testing was conducted by microwell plate enzyme immunoassay, with confirmatory testing using rapid synthetic peptide-based membrane immunoassays, which can differentiate between HIV-1 and HIV-2, and follow-up HIV-1- and HIV-2- specific Western blot assays. Quantitative and qualitative viral load assays for HIV-1 and HIV-2 plasma, peripheral blood mononuclear cells (PBMC), 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 [4, 11, 14]. 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. A person was considered dually seropositive, but not dually infected, if serologic testing was positive for both HIV-1 and HIV-2 but one or both viruses could never be detected by NAAT.

Statistical analysis

Categorical variables were compared using Pearson's χ^2 tests or Fisher's exact tests, and continuous variables were compared by non-parametric 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 subjects, and were adjusted for CD4+ T cell count (categorized, <200, 200-500, and >500 cells/μl), age, sex, and history of commercial sex work. Viral load measurements were $\log_{10}$ transformed to account for non-normality. 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, while 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. Similarly, for the HIV-2 assay, which had twice the sensitivity of the HIV-1 assay, such samples were assigned values of 5 and 50 copies/ml, respectively. A value of 0.5 copies/μg of PBMC DNA were assigned to samples in which HIV PBMC DNA could not be detected, and a value of 1 copy/μg of PBMC DNA to samples in which PBMC DNA was detected but could not be quantified. We have previously demonstrated no qualitative difference when setting values for quantitatively negative samples over a range of values between zero and the limit of detection [9, 11]. For those subjects 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 visit dates was used where longitudinal follow-up was available; where longitudinal follow-up was not obtained, viral loads were censored. Viral load assays for HIV-1 and HIV-2 were sometimes not run in parallel, so for paired analyses, we matched HIV-1 and HIV-2 viral load data within a six-month window so long as no true matched pairs fell within the interval. Artificially matched pairs accounted for less than 10% of pairs included in these analyses. Viral loads obtained after initiation of antiretroviral therapy were excluded from analysis. Because previous studies have demonstrated a strong association between viral load and CD4 count, where CD4 data were missing, CD4 counts from the closest study visits were used. 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 subjects identified, 109 subjects enrolled in our follow-up studies and had viral load testing conducted. Of 88 subjects for whom at least one viral load test was completed for each virus, we could only confirm dual infection (as opposed to dual seropositivity) by NAAT for both HIV-1 and HIV-2, in any sample collected, for 65 subjects (60% of enrolled subjects, 74% of those with viral load results available for both viruses). Among the remaining subjects, we could detect only HIV-1 for 16 subjects, only HIV-2 for 5 subjects, and were unable to amplify either virus for 2 subjects. Subjects with multiple viral load tests run, from multiple anatomic sites and/or multiple study visits, were more likely to be identified as dually infected than those with only one set of viral load tests (results not shown). Our primary analyses were carried out within the subset of individuals for whom we could confirm dual infection by NAAT.

Dually infected subjects were representative of the 109 dually seropositive subjects identified, with respect to recruitment site, sex, commercial sex work, age, country of birth, religion, marital status, children, contraceptive use, education, tobacco and alcohol use, and CD4 count at screening (Table 1). Subjects in whom dual infection was confirmed were predominantly born in Senegal (88%), with the three most common ethnicities reported being Wolof (44%), Pulaar (19%) and Serere (13%). Most were recruited from the Fann Infectious Diseases clinic in Dakar (68%) and slightly more than half were female (57%); of the women enrolled, 57% reported having traded sex for money or goods. The average age was approximately 37 years. Education above primary level was relatively uncommon (17%). Cigarette and alcohol use (20% and 13%, respectively) were generally confined to commercial sex workers. The majority of subjects were Muslim (84%). Marital status was split approximately evenly between single, married (monogamous), and separated or divorced, with the rest of subjects either being in polygamous marriage or widowed. Baseline CD4 count was approximately 350 cells/μl, although dually infected women enrolled with significantly higher CD4 counts than dually infected men (mean 475 vs 192 cells/μl, P=0.0001 by Student's t test). Those individuals in whom dual infection could not be confirmed were generally similar to those in whom we could detect both viruses. The most notable difference between the groups was an average CD4 count nearly 70 cells/μl lower than those in whom we could detect both viruses, although this difference was not statistically significant (P=0.28 by Student's t test).

Samples analyzed

In all, we analyzed HIV-1 and HIV-2 viral loads from 304 and 211 (respectively) plasma, 124 and 117 PBMC, 20 and 14 oral, 19 and 18 cervicovaginal lavage, and 5 and 5 semen samples available from a total of 109 dually seropositive subjects. When restricted to dually infected subjects, we assessed 224 and 172 plasma, 115 and 109 PBMC, 19 and 12 oral, 18 and 16 CVL, and 5 and 5 semen samples from a total of 65 subjects.

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

At baseline, median plasma RNA viral loads among confirmed dually infected subjects were 4.4 and 2.3 $\log_{10}$ copies/ml for HIV-1 and HIV-2, respectively (P<0.0001 by Mann-Whitney U test) (Table 2). HIV-1 RNA was not detected in 6 (10%) specimens, and HIV-2 RNA was not detected in 23 (38%) specimens (P<0.001 by binomial detectable/undetectable Pearson's χ^2 test). Median PBMC DNA viral loads were 1.2 and 1.1 $\log_{10}$ copies/μg of PBMC DNA, respectively (P=0.24 by Mann-Whitney U test). DNA was not detected in 6 (11%) and 7 (15%) specimens, respectively (P=0.63 by Pearson's χ^2 test). Few oral fluid, cervicovaginal lavage, and semen sample viral loads were available for analysis. However, in oral fluid, HIV-1 was not detectable in 2 (14%) specimens, compared to 11 (100%) specimens for HIV-2 (P<0.001 by Fisher's exact test). HIV-1 RNA was not detected in 8 (67%) cervicovaginal lavage samples, compared to 7 (100%) for HIV-2 (P=0.25 by Fisher's exact test). Among semen samples, HIV-1 RNA was detected in all five samples, while HIV-2 RNA was not detected in 3 (60%) (P=0.17 by Fisher's exact test).

Mean HIV-1 and HIV-2 plasma RNA levels are more divergent at lower CD4 counts

Within an individual, HIV-1 and HIV-2 viral loads in plasma were inversely associated (Figure 1; Pearson R=-0.2274). In order to assess potential differences in HIV-1 and HIV-2 viral load trends among dually infected subjects, we stratified HIV-1 and HIV-2 PBMC DNA and plasma RNA levels by CD4 count (categorized as >500, 200-500, and <200 cells/μl) (Figure 2). We found that among subjects with high CD4 counts above 500 cells/μl, there was less difference between mean HIV-1 and HIV-2 plasma viral loads compared to subjects with medium and low CD4 count categories (200-500 and <200 cells/μl, respectively) (Table 3). This trend remained after controlling for age, sex, and commercial sex work.

After adjusting for covariates, among subjects with a CD4 count above 500 cells/μl, mean HIV-1 plasma viral load was 0.88 $\log_{10}$ copies/ml (95% CI 0.37 to 1.38) higher than HIV-2, with mean HIV-1 plasma viral load 2.90 $\log_{10}$ 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) $\log_{10}$ copies/ml. Among dually infected subjects with CD4 counts between 200 and 500 cells/μl, mean HIV-1 RNA viral load was greater than 2.5 $\log_{10}$ copies/ml higher than among subjects with CD4 counts greater than 500 cells/μl (β=2.79, 95% CI 1.71 to 2.86). There was some evidence to suggest that those with CD4 counts in the lowest category (<200 cells/μl) had even higher HIV-1 viral loads than those with CD4 counts in the medium category (β=2.79, 95% CI 1.71 to 2.86; β=2.83, 95% CI 1.80 to 2.86, respectively), although this difference was not statistically significant. HIV-2 viral loads did not differ significantly across CD4 categories but showed a trend towards lower RNA levels in those with more severe immune dysfunction.

HIV-1 and HIV-2 PBMC DNA levels are only similar among subjects with high CD4 counts

Similarly to plasma RNA levels, paired HIV-1 and HIV-2 PBMC DNA levels were inversely correlated (Figure 3; Pearson R=-0.3628). However, when all available viral load data were considered, we found that mean HIV-1 levels were higher, and HIV-2 levels lower, among those in lower CD4 count strata (Figure 4). In a multivariate regression analysis, after controlling for age, sex, and commercial sex work, we found no difference between HIV-1 and HIV-2 proviral DNA levels in PBMC (β=0.17, 95% CI -0.24 to 0.58) among subjects with high CD4 counts (Table 3). However, among those with medium (200-500 cells/μl) and low (<200 cells/μl) CD4 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) while mean HIV-1 DNA levels were higher by 1.22 (95% CI 0.25 to 2.18) and 1.26 (95% CI 0.38 to 2.15) $\log_{10}$ copies/μg of PBMC DNA, respectively.

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

HIV-2 RNA levels in cervicovaginal lavage 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) $\log_{10}$ copies/ml, respectively, after controlling for age, sex, history of commercial sex work, and CD4 count (Table 3). There were insufficient data from subjects with CD4 counts greater than 500 cells/μl to investigate whether the diverging trend observed in plasma and PBMC was also present locally in oral and genital secretions.

PBMC DNA levels are predictive of plasma RNA levels in subjects with high CD4 counts

Within individuals, PBMC DNA viral loads were predictive of plasma RNA levels, regardless of HIV type (Figure 5; HIV-1 Pearson R=0.6965, HIV-2 Pearson R=0.6737). Based the diverging trend of RNA and to a lesser extent, DNA viral loads, between those with CD4 count greater than 500 cells/μl and those with substantially impaired immune function (CD4<=500 cells/μl), we conducted our regression analyses, stratifying based on CD4 count (Table 4). After controlling for age, sex, and commercial sex work, among those with CD4 counts above 500 cells/μl, we found that PBMC DNA levels remained highly associated with plasma RNA levels, irrespective of HIV type. Each $\log_{10}$ increase of HIV-1 PBMC DNA copies/μg was associated with a mean 1.38 (95% CI 0.85 to 1.92) $\log_{10}$ increase in HIV-1 plasma RNA copies/ml; likewise, each $\log_{10}$ increase in HIV-2 PBMC DNA copies/μg was associated with a mean 0.98 (95% CI 0.57 to 1.40) $\log_{10}$ copies/ml increase in HIV-2 plasma RNA level. Curiously however, no such association was found in those with low CD4 counts, although HIV-1 levels trended towards a negative association (β=-0.48, 95% CI -1.14 to 0.17).

DISCUSSION

We examined HIV-1 and HIV-2 levels both systemically as well as locally in oral and genital sites in 65 confirmed dually infected, antiretroviral therapy-naïve men and women from Senegal, West Africa. We found that HIV-1 RNA levels in plasma, oral fluid, semen, and cervicovaginal lavage were significantly higher than HIV-2 levels, and that such associations were also correlated with CD4 count. In particular, we observed that HIV-1 plasma RNA levels were higher, and HIV-2 levels lower, in subjects with low CD4 counts than high. Although HIV-1 and HIV-2 PBMC DNA levels were equivalent in subjects with high CD4 counts, among those with CD4 counts below 500 cells/μl, HIV-1 DNA was, on average, present at higher levels than HIV-2.

Although we and others have previously demonstrated that HIV-2 levels in these sites are lower than HIV-1 in cohorts of singly-infected subjects, to our knowledge this is the first study to examine such correlations specifically among dually infected subjects, and is unique both in the breadth of data available and the size of the study population. The majority of studies of HIV viral dynamics have focused solely on HIV-1 and/or HIV-2 single infections, treating dual seropositivity as an exclusion criterion. We are only aware of six studies [8, 31, 36, 48, 55, 56] that have compared viral dynamics of dually infected subjects to singly infected subjects, of which five have noted CD4-dependent differences in viral dynamics between dual and single infection. The sixth study, by Andersson and colleagues, reported lower HIV-2 plasma RNA levels among dually infected subjects compared to singly infected subjects, but found no correlation between either HIV-1 and HIV-2 plasma RNA levels or plasma RNA levels and CD4 count [8]. Our study provides further evidence demonstrating that HIV-2 in the setting of HIV-1 coinfection behaves differently than infection with HIV-2 alone and that such differences are likely CD4 count-associated.

It has long been known that that HIV-2 proviral DNA levels are inversely correlated with CD4 counts among singly infected subjects, and that proviral DNA levels of both viruses are similar at each stage of infection [5-7, 10, 15]. However, in 1998, Dieng-Sarr and colleagues published findings that they were less frequently able to detect HIV-2 provirus among hospitalized dually seropositive subjects compared to asymptomatic dually seropositive subjects, despite equivalent detection of HIV-1 provirus [36]. This suggested for the first time that HIV-2 viral loads might be associated with CD4 count. In a follow-up study comparing HIV-1/2 dually

infected subjects to HIV-2 singly infected subjects in Senegal, Dieng-Sarr *et al.* confirmed this CD4-dependent relationship on HIV-2 proviral DNA levels. Among those with high CD4 counts (above 400 cells/μl), they reported lower HIV-2 DNA levels among dually seropositive subjects than among those infected with HIV-2 alone. At low CD4 counts, single infection was associated with high HIV-2 DNA levels compared to typically undetectable levels in dual infection [36, 55]. These studies made no attempt to compare quantitative HIV-1 DNA levels to HIV-2 levels among the first group of dually seropositive subjects, nor did they compare HIV-1 levels in dually versus singly infected subjects. We confirm their finding of lower HIV-2 DNA levels among dually infected subjects with low CD4 counts compared to those with high CD4 counts. To our knowledge, however, ours is the first study to suggest that HIV-1 and HIV-2 DNA levels are similar among those with high CD4 counts but are quite divergent at lower CD4 counts.

Our study demonstrates higher mean HIV-1 plasma RNA levels, and a corresponding trend toward lower mean HIV-2 levels, associated with lower CD4 count. These findings provide the first direct support for the hypothesis that HIV-1 outcompetes HIV-2 over the course of dual infection. As observed in proviral DNA levels, several studies in HIV-2 mono-infection have demonstrated that plasma viral load and CD4 count are inversely related [5-10]. We have previously demonstrated that equal plasma viral loads predict a similar rate of CD4 count decline in HIV-1 and HIV-2 single infections, but HIV-2 plasma levels are typically much lower than HIV-1 [9]. Again, however, studies comparing single infection to dual infection provide evidence for differing viral dynamics in dually infected subjects. In 2000, Nkengasong *et al.* reported that HIV-1 and HIV-2 plasma viral loads were inversely correlated in dually infected sex workers from Cote d'Ivoire [31]. In a follow-up study, they found that after adjusting for CD4 count, sex, and age, HIV-2 plasma viral loads were higher among dually infected subjects with high CD4 counts than HIV-2 singly infected subjects, but among subjects with CD4 counts below 200 cells/μl, dually infected subjects had lower HIV-2 viral loads than singly infected subjects [56]. In a cohort of commercial sex workers from The Gambia, Alabi *et al*. described similar HIV-1 levels in HIV-1 singly infected subjects compared to those dually infected over all CD4 strata, but reported that among dually infected subjects with CD4 percentage below 14%, HIV-2 RNA viral loads were lower than in singly infected subjects in the same strata [48]. In those with the highest CD4 percentage, (over 28%), HIV-2 plasma viral loads were higher among dually infected subjects than HIV-2 singly infected. None of these three studies directly compared HIV-1 to HIV-2 plasma RNA levels among dually infected subjects; however, these

important studies provided some of the first evidence suggesting that viral dynamics in dual infection might be CD4 count-dependent.

Although we were unable to show a statistically significant lower HIV-2 viral load among those with lower CD4 count, our data trended in that direction. Because HIV-2 plasma RNA viral loads are frequently below the limit of detection irrespective of CD4 count, our viral load distribution is strongly left-truncated. Considering the high frequency of undetectable viral loads particularly among those with low CD4 counts, it is possible that this trend toward lower viral loads among more immunocompromised subjects is stronger than we can detect. If an ultra-sensitive assay, such as that used to quantify persistent HIV-1 replication in patients on suppressive therapy, existed for HIV-2, we might be able to provide stronger evidence supporting this CD4 count-dependent decrease in HIV-2 levels. It is noteworthy that the mean CD4 counts in those 44 subjects in whom we could not detect both viruses tended to be lower than the mean among those in whom we did detect HIV-1 and HIV-2. Since we were unable to detect HIV-2 in 18 of the 23 (78%) dually-seropositive subjects with viral load testing against both viruses completed, these findings may be related – that is, HIV-2 levels may have been below the limit of detection due to very low CD4 counts.

Neither this study nor any previous study of viral loads in HIV dual infection have attempted to carry out a longitudinal analysis to investigate whether this association between HIV levels and CD4 count is present over time. Although we have longitudinal follow-up on some of our subjects out to approximately nine years, these individuals are rare – for the majority, we have only one to two years of follow-up data, which are insufficient to allow for determination of temporal trends. Further, based on what we know of the natural histories of HIV-1 and HIV-2, regular follow-up off ART for seven to ten years would likely be needed to observe disease progression sufficient to allow for these comparisons. Since antiretroviral therapy has been widely available to infected individuals meeting criteria since the early 2000s, this type of prospective study is no longer feasible or ethical.

Our examination of local viral dynamics in oral and genital secretions was limited by small numbers of viral loads available, the majority of which were from subjects with CD4 counts below 500 cells/μl. Although we lack the power to investigate this role, we propose that this may be a critical area for understanding horizontal transmission in HIV-1/2 dually infected subjects. We have previously demonstrated in singly-infected subjects that HIV-2 levels in semen are

significantly lower than HIV-1 levels, and that semen shedding is correlated with plasma viral load, but not CD4 count, irrespective of virus type [11]. Similarly, we and others have demonstrated that HIV-2 RNA and/or DNA is less frequently present at detectable levels in cervicovaginal lavage samples than HIV-1, and that CVL shedding is associated with plasma viral load but not CD4 count [12, 14]. We have also demonstrated this link between plasma viral load and local shedding of HIV-2 in the oral cavity [4]. However, it remains unclear whether this relationship translates to dual infection, and whether CD4 count plays an independent role in oral and/or genital HIV-1 and HIV-2 levels.

HIV-1 and HIV-2 are distinct, yet similar viruses, and studies of dual infection including this work, are plagued by the difficulty of accurate diagnosis. We enrolled 109 dually seropositive individuals in our study, but only reported results on those we were confident were truly dually infected based on nucleic acid amplification for both viruses. However, lingering questions remain. Of our 109 dually seropositive subjects enrolled, 21 subjects were excluded due to the lack of viral load testing for either HIV-1 or HIV-2. Of the 88 subjects for whom at least one viral load assay was conducted for each virus, we could detect both viruses in only 65. Five of the remaining 23 subjects had undetectable HIV-1, 16 had undetectable HIV-2, and we could not detect either virus from the final two subjects. 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 our nucleic acid tests, resulting in false negative tests. Lack of assay specificity, or cross-reactivity in the serologic tests, may have resulted in false positive tests. In serological tests, HIV-1 and HIV-2 are known to be cross-reactive [57]. With serology and virus culture results being concordant approximately 90% of the time [58], presence of HIV-1 and HIV-2 specific antibodies is probably more sensitive (but less specific) for detecting dual infection than PCR positivity at a single time point [59]. By contrast, previous studies indicate concordance between serology and nucleic acid detection ranges from 30-70% [3131, 34-37, 60, 61]. Further, the HIV-1 nucleic acid test is slightly cross-reactive with HIV-2, and particularly for subjects with high HIV-2 viral loads, may falsely detect HIV-1. These findings point to the need for more sensitive and specific algorithms for identifying dual infection**.** The challenge of accurately diagnosing HIV-dually infected subjects means that we may be including subjects who are not truly dually infected, or excluding some who are, but for whom our diagnostics are not sufficient for accurate virus detection. It is worth noting however, that we obtained similar results and reach similar conclusions when we carried out our analyses using

the full set of 109 dually seropositive subjects, setting viral loads for a given virus type in those with unconfirmed infection to the lower limit of detection.

It is critical to note that our conclusions are based on average viral load trends and are likely not applicable to all dually infected subjects. However, our results provide evidence to support the theory that, on average, HIV-1 outcompetes HIV-2 at lower CD4 counts. This may help to explain previous findings that dually infected individuals experience disease progression at a similar rate as those singly infected with HIV-1. Despite early hopes that HIV-2 infection might be protective against HIV-1 infection or progression, within the context of viral loads in dually infected subjects, we were unable to find evidence for such an effect. However, we have no way to determine the order of infection of the subjects in our cohort – some were likely infected with HIV-1 first, others with HIV-2 first, and some with HIV-1 and HIV-2 concurrently. Thus, the influence of HIV-1 levels on HIV-2 (or vice versa) may depend on the order of infection, the state of the host immune system at the time of second infection, or perhaps other unidentified factors. It is unlikely that this question will ever be adequately answered in the era of highly active antiretroviral therapy; regardless, clinical and therapeutic outcomes of HIV dual infection remain largely unknown and warrant further study.

Table 1: Characteristics of HIV-1/2 dually seropositive subjects.

Characteristic	All dually seropositive subjects enrolled (n=109)	NAAT confirmed dually infected subjects (n=65)	Not NAAT confirmed dually infected subjects (n=44)
Recruitment Site			
Mbour STD Clinic	7 (6)	5 (8)	2 (5)
ASBEF Family Planning Clinic	1 (1)	1 (2)	0 (0)
IHS STD Clinic	25 (23)	15 (23)	10 (23)
Fann Infectious Disease Clinic	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)
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 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.
[a] Enrollees in one study were not asked about history of commercial sex work. For these subjects, clinic type (STD vs other) was used as a proxy for history of commercial sex work.
[b] Age of one dually seropositive subject was unknown.
[c] Ethnicity, education, and marital status were unknown for <10% of subjects.
[d] Religion was unknown for 10-15% of subjects.
[e] CD4 counts were missing for 11% of enrolled subjects, 8% of confirmed dually infected subjects, and 18% of dually seropositive subjects 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* [b]
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) [a]	4.4 (3.7-5.1)	2.3 (0.7-3.0)	**<0.0001**
PBMC (DNA copies/μg)	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/μg) [a]	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) [a]	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)	
Median (IQR) (log_{10} copies/ml) [a]	1.0 (1.0-3.2)	0.7 (0.7-0.7)	**0.0002**
Semen (RNA copies/ml)	n=5	n=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) [a]	4.1 (2.6-4.6)	0.7 (0.7-3.5)	0.07

PBMC, peripheral blood mononuclear cells; CVL, cervicovaginal lavage.
[a] Medians and interquartile ranges including values assigned to samples below the limit of detection (see Statistical Methods).
[b] *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.

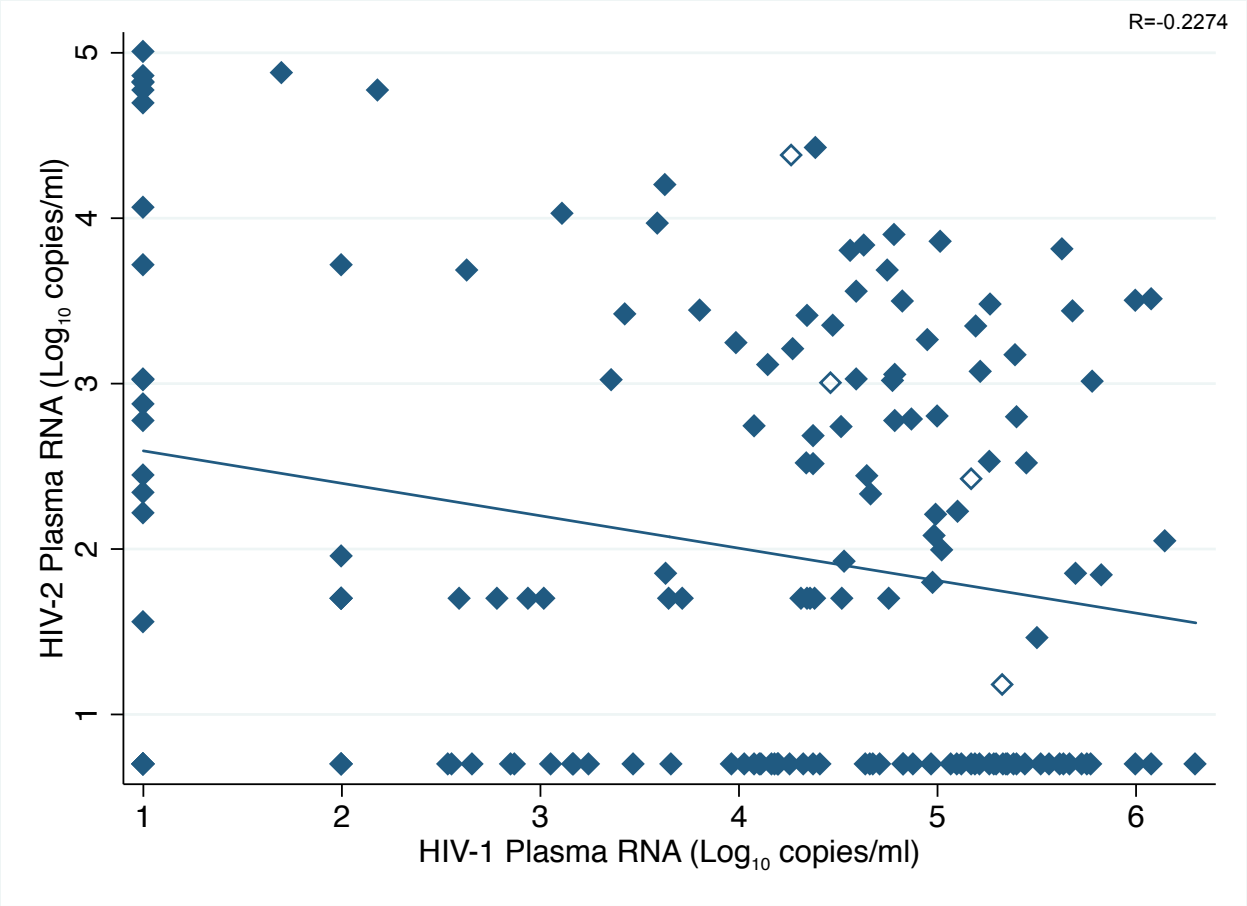

Figure 1: Correlation of HIV-1 and HIV-2 plasma RNA levels in HIV-1/2 dually infected subjects. Data points represent HIV-1/ HIV-2 pairs in dual HIV-1/2 infection. Filled markers indicate that both viral loads are from the same study visit, open markers indicate that one level is from a subsequent visit within six months. The linear regression line is also shown.

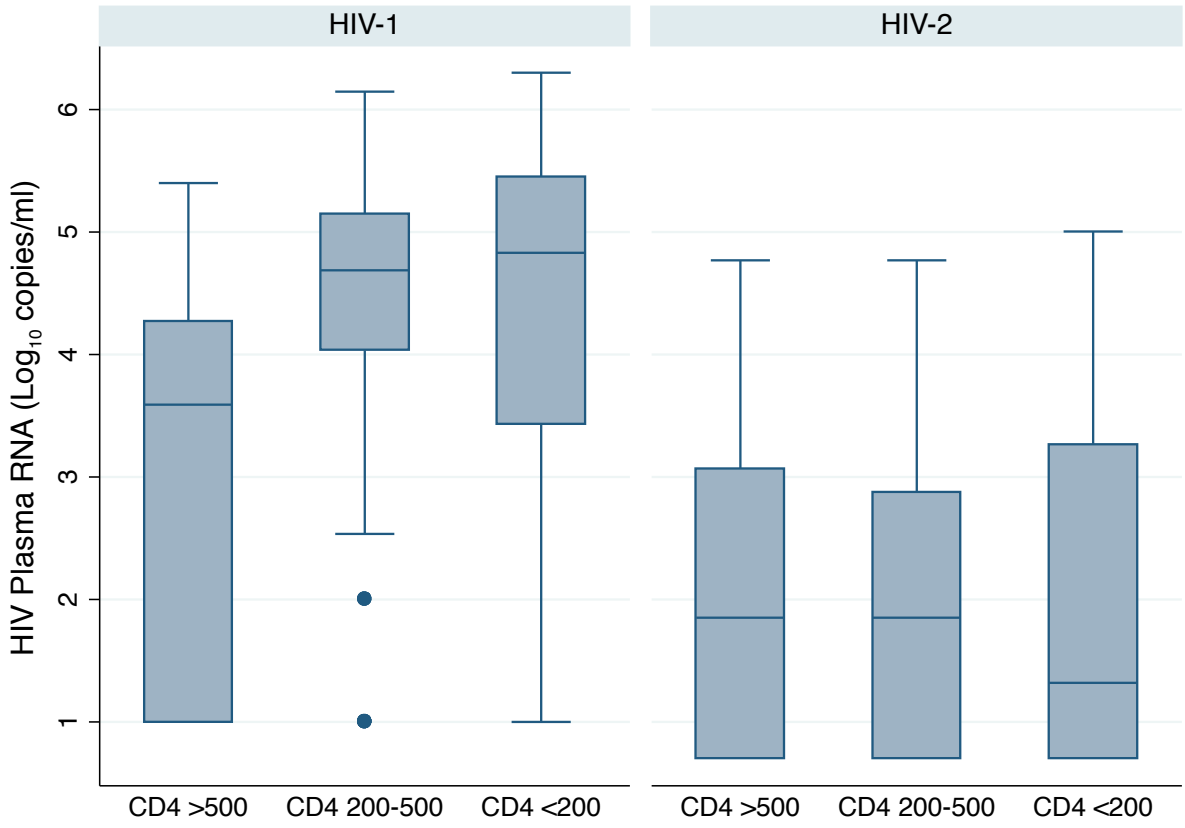

Figure 2: Comparison of HIV-1 and HIV-2 plasma RNA levels, stratified by HIV type and CD4 count, in HIV-1/2 dually infected subjects.

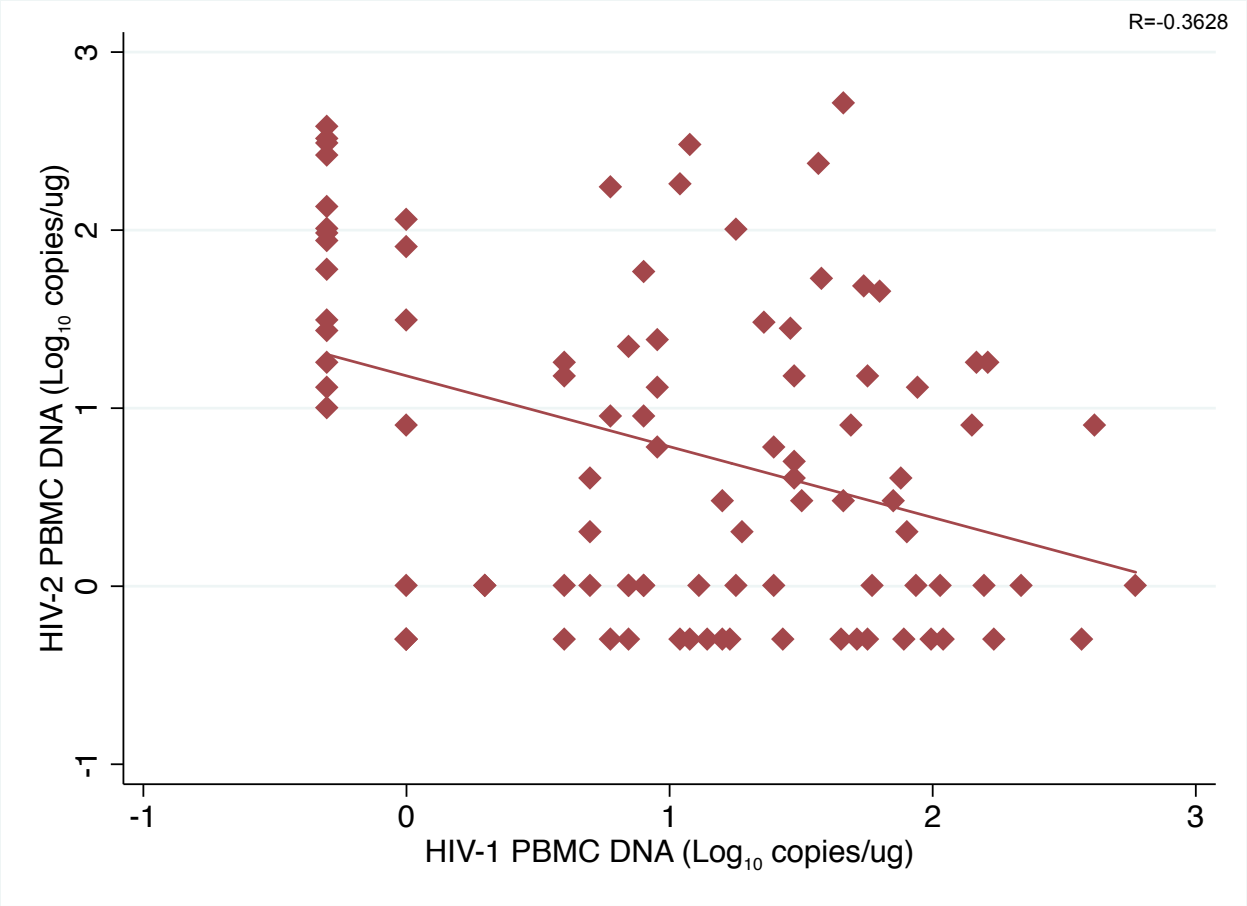

Figure 3: Correlation of HIV-1 and HIV-2 PBMC DNA levels in HIV-1/2 dually infected subjects. Data points represent HIV-1/ HIV-2 pairs in dual HIV-1/2 infection. Filled markers indicate that both viral loads are from the same study visit, open markers indicate that one level is from a subsequent visit within six months. The linear regression line is also shown.

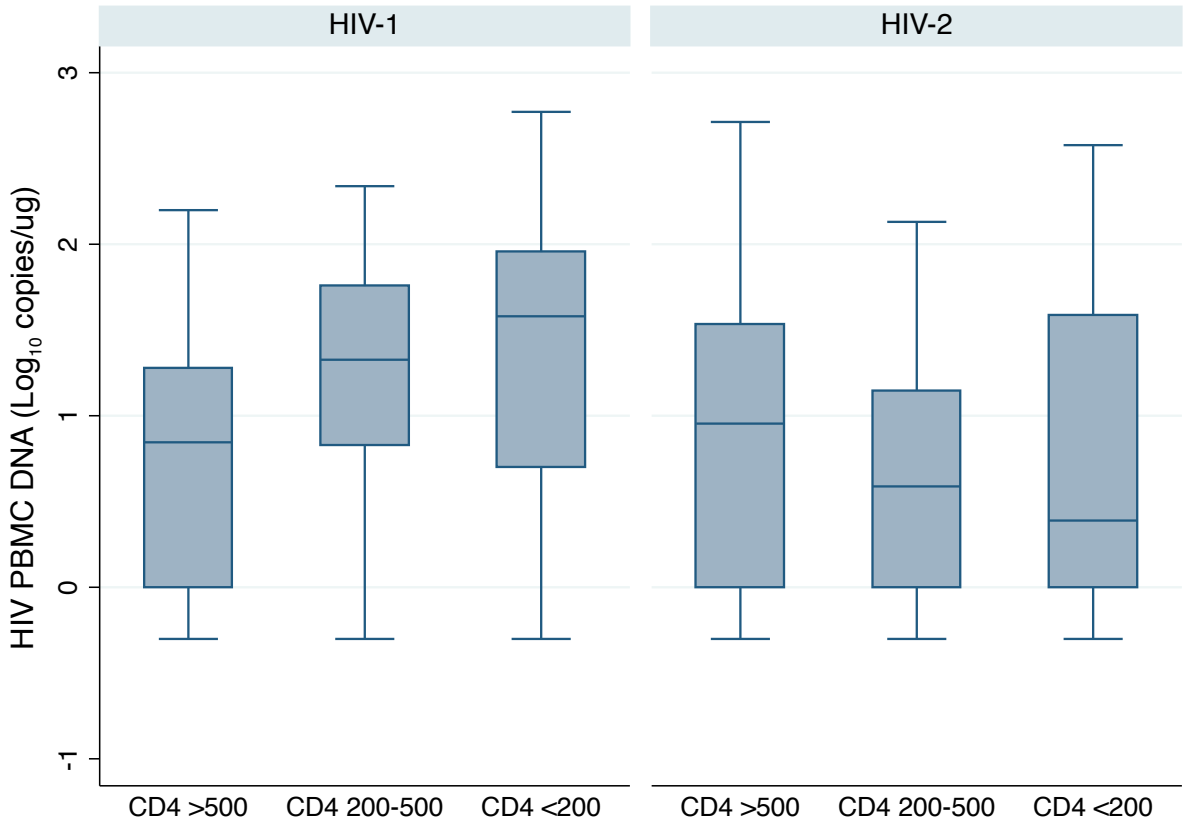

Figure 4: Comparison of HIV-1 and HIV-2 plasma PBMC DNA levels, stratified by HIV type and CD4 count, in HIV-1/2 dually infected subjects.

Table 3: Immuno-virologic and demographic correlates of HIV-1 and HIV-2 viral loads among HIV-1/2 dually infected subjects (n=65).

		Univariate			Adjusted		
	Variable	α	β	(95% CI)	α	β	(95% CI)
Plasma RNA	HIV type x CD4 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)**
	Age						
	16 years	**2.27**		**(1.77 to 2.77)**		(ref)	
	Change/year		**0.04**	**(0.02 to 0.06)**		**0.04**	**(0.02 to 0.07)**
	Sex						
	Male	**3.30**		**(3.01 to 3.58)**		(ref)	
	Female		-0.36	(-0.71 to 0.00)		0.17	(-0.24 to 0.58)
	Commercial sex work						
	No	**2.31**		**(3.00 to 3.42)**		(ref)	
	Yes		**-0.40**	**(-0.76 to -0.04)**		-0.09	(-0.46 to 0.28)
PBMC DNA	HIV type x CD4 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)**
	Age						
	16 years	**0.76**		**(0.41 to 1.10)**		(ref)	
	Change/year		0.01	(-0.01 to 0.02)		0.00	(-0.01 to 0.02)
	Sex						
	Male	**1.05**		**(0.85 to 1.25)**		(ref)	
	Female		-0.19	(-0.43 to 0.06)		-0.08	(-0.40 to 0.25)
	Commercial sex work						
	No	**0.97**		**(0.82 to 1.13)**		(ref)	
	Yes		-0.12	(-0.36 to 0.12)		0.02	(-0.30 to 0.34)
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/μl)						
	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)
	Age						
	16 years	0.77		(-0.32 to 1.86)		(ref)	
	Change/year		**0.05**	**(0.00 to 0.10)**		**0.04**	**(0.02 to 0.07)**
	Sex						
	Male	**1.98**		**(1.57 to 2.39)**		(ref)	
	Female		-0.77	(-1.79 to 0.26)		-0.05	(-0.58 to 0.49)
	Commercial sex work						
	No	**1.90**		**(1.51 to 2.29)**		(ref)	
	Yes		-1.20	(-3.34 to 0.94)		0.09	(-0.94 to 1.12)

Table 3 continued:

	Variable	Univariate A	Univariate β	Univariate (95% CI)	Adjusted α	Adjusted β	Adjusted (95% CI)
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/μl)						
	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)
	Age						
	16 years	**2.60**		**(0.96 to 4.24)**		(ref)	
	Change/year		-0.07	(-0.17 to 0.03)		**-0.08**	**(-0.16 to 0.00)**
	Commercial sex work						
	No	**1.62**		**(1.17 to 2.08)**		(ref)	
	Yes		-0.40	(-1.09 to 0.28)		-0.20	(-0.74 to 0.33)
Semen RNA	HIV type						
	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/μl)						
	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)
	Age						
	16 years	2.94		(-0.86 to 6.73)		(ref)	
	Change/year		0.00	(-0.14 to 0.14)		0.03	(-0.10 to 0.16)

PBMC, peripheral blood mononuclear cells; CVL, cervicovaginal lavage; CI, confidence interval.
Values are expressed in $\log_{10}$ copies/ml (RNA) or $\log_{10}$ copies/μg of PBMC DNA (DNA).
Values in bold are statistically significant.
[a] From multivariate linear regression using generalized estimating equations, adjusting for all factors in the table.

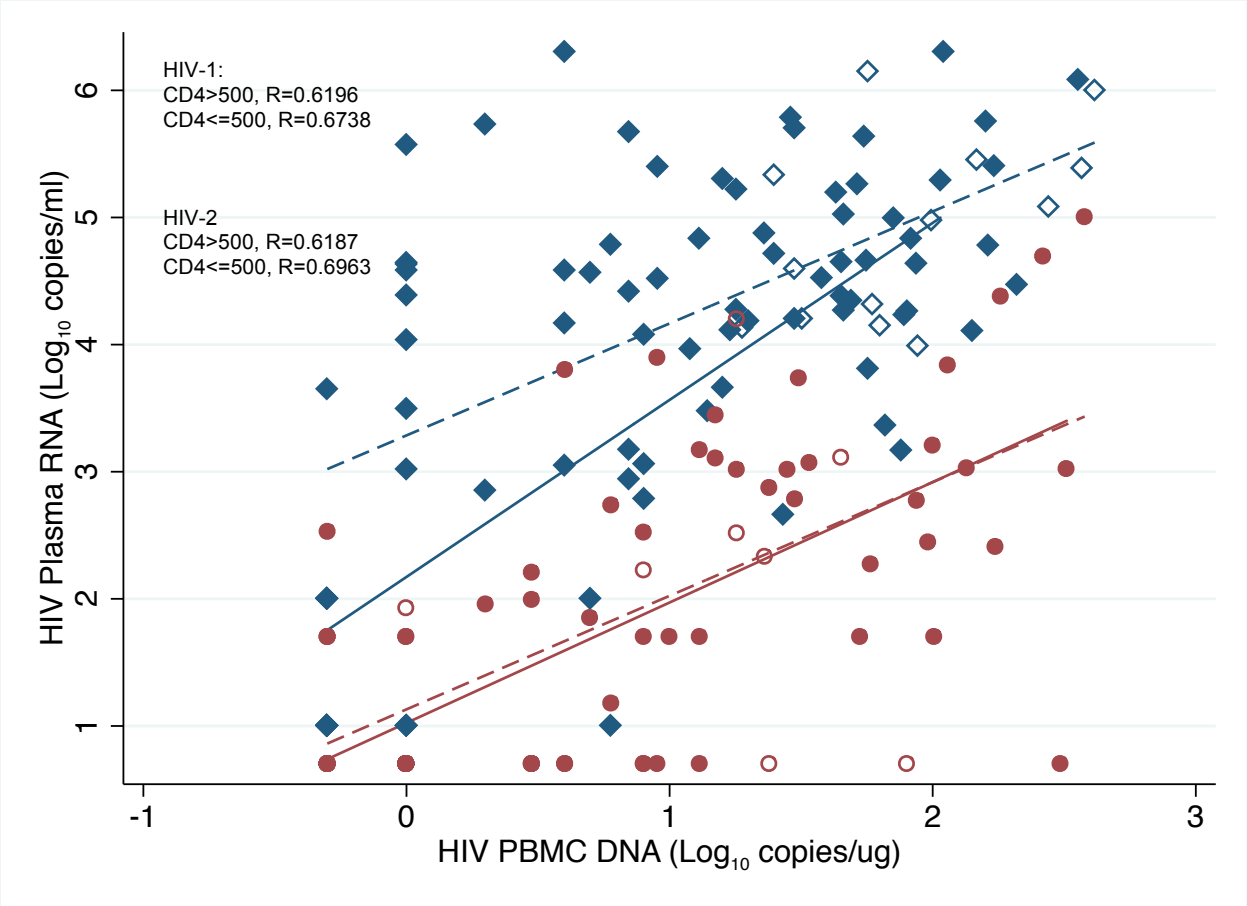

Figure 5: Correlation of HIV-1 and HIV-2 Plasma RNA with PBMC DNA levels in HIV-1/2 dually infected subjects. Data points represent HIV-1 (blue diamonds) or HIV-2 (maroon circles) plasma RNA/PBMC DNA pairs in dual HIV-1/2 infection. Filled markers indicate that plasma RNA and PBMC DNA levels are from the same visit, open markers indicate that one level is from a subsequent visit within six months. Linear regression lines for the relationship between DNA and RNA are also shown, solid lines for subjects with CD4 counts greater than or equal to 500 cells/μl or dashed lines for CD4 counts less than 500 cells/μl.

Table 4: Association between PBMC DNA and plasma RNA viral loads in HIV-1 and HIV-2 among HIV-1/2 dually infected subjects (n=65).

		Univariate			Adjusted [a]		
	Variable	α	β	95% CI	α	β	95% CI
HIV-1	CD4 count (cells/μl) x PBMC DNA viral load (log10 copies/μg)						
	Above 500, zero log10	**2.22**		**(1.70 to 2.75)**	**1.89**		**(0.81 to 2.96)**
	Above 500, per log10		**1.35**	**(0.81 to 1.90)**		**1.38**	**(0.85 to 1.92)**
	500 and below, zero log10		**1.05**	**(0.32 to 1.77)**		**0.81**	**(0.02 to 1.61)**
	500 and below, per log10		-0.44	(01.09 to 0.21)		-0.48	(-1.14 to 0.17)
	Sex						
	Male					(ref)	
	Female					0.34	(-0.34 to 1.04)
	Age						
	16 years					(ref)	
	Change per year					0.03	(-0.01 to 0.06)
	Commercial Sex Work						
	No					(ref)	
	Yes					-0.58	(-1.27 to 0.10)
HIV-2	CD4 count (cells/μl) x PBMC DNA viral load (log10 copies/μg)						
	Above 500, zero log10	**0.99**		**(0.45 to 1.54)**	**1.27**		**(0.37 to 2.16)**
	Above 500, per log10		**0.87**	**(0.47 to 1.28)**		**0.98**	**(0.57 to 1.40)**
	500 and below, zero log10		0.11	(-0.51 to 0.74)		-0.04	(-0.67 to 0.59)
	500 and below, per log10		0.14	(-0.36 to 0.64)		-0.02	(-0.47 to 0.52)
	Sex						
	Male					(ref)	
	Female					0.17	(-0.40 to 0.74)
	Age						
	16 years					(ref)	
	Change per year					0.00	(-0.03 to 0.03)
	Commercial Sex Work						
	No					(ref)	
	Yes					**-0.74**	**(-1.25 to -0.24)**

PBMC, peripheral blood mononuclear cells; CI, confidence interval.
Values are expressed in $\log_{10}$ copies/ml (RNA) or $\log_{10}$ copies/μg of PBMC DNA (DNA).
Values in bold text are statistically significant.
[a] From multivariate linear regression using generalized estimating equations, adjusting for all factors in the table.

REFERENCES

1. Arien KK, Abraha A, Quinones-Mateu ME, Kestens L, Vanham G, Arts EJ. The replicative fitness of primary human immunodeficiency virus type 1 (HIV-1) group M, HIV-1 group O, and HIV-2 isolates. *J Virol* 2005,**79**:8979-8990.
2. Fleming AF. Seroepidemiology of human immunodeficiency viruses in Africa. *Biomed Pharmacother* 1988,**42**:309-320.
3. Horsburgh CR, Jr., Holmberg SD. The global distribution of human immunodeficiency virus type 2 (HIV-2) infection. *Transfusion* 1988,**28**:192-195.
4. Pavlinac PB, Hawes SE, Gottlieb GS, Gaye A, N'Diaye C F, Critchlow CW*, et al.* HIV shedding in the oral cavity: an assessment of HIV type, immunovirologic, demographic and oral factors. *Sex Transm Infect* 2012,**88**:45-50.
5. MacNeil A, Dieng-Sarr A, Sankale JL, Meloni ST, Mboup S, Kanki P. Direct evidence of lower viral replication rates in vivo in human immunodeficiency virus type 2 (HIV-2) infection than in HIV-1 infection. *J Virol* 2007,**81**:5325-5330.
6. Simon F, Matheron S, Tamalet C, Loussert-Ajaka I, Bartczak S, Pepin JM*, et al.* Cellular and plasma viral load in patients infected with HIV-2. *AIDS* 1993,**7**:1411-1417.
7. Popper SJ, Dieng-Sarr A, Gueye-Ndiaye A, Mboup S, Essex ME, Kanki PJ. Low plasma human immunodeficiency virus type 2 viral load is independent of proviral load: low virus production in vivo. *J Virol* 2000,**74**:1554-1557.
8. Andersson S, Norrgren H, da Silva Z, Biague A, Bamba S, Kwok S*, et al.* Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. *Arch Intern Med* 2000,**160**:3286-3293.
9. Gottlieb GS, Sow PS, Hawes SE, Ndoye I, Redman M, Coll-Seck AM*, et al.* Equal plasma viral loads predict a similar rate of CD4+ T cell decline in human immunodeficiency virus (HIV) type 1- and HIV-2-infected individuals from Senegal, West Africa. *J Infect Dis* 2002,**185**:905-914.
10. Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R*, et al.* Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. *J Hum Virol* 1998,**1**:457-468.
11. Gottlieb GS, Hawes SE, Agne HD, Stern JE, Critchlow CW, Kiviat NB*, et al.* Lower levels of HIV RNA in semen in HIV-2 compared with HIV-1 infection: implications for differences in transmission. *AIDS* 2006,**20**:895-900.
12. Ghys PD, Fransen K, Diallo MO, Ettiegne-Traore V, Coulibaly IM, Yeboue KM*, et al.* The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. *AIDS* 1997,**11**:F85-93.
13. Critchlow CW, Kiviat NB. Detection of human immunodeficiency virus type 1 and type 2 in the female genital tract: implications for the understanding of virus transmission. *Obstet Gynecol Surv* 1997,**52**:315-324.
14. Hawes SE, Sow PS, Stern JE, Critchlow CW, Gottlieb GS, Kiviat NB. Lower levels of HIV-2 than HIV-1 in the female genital tract: correlates and longitudinal assessment of viral shedding. *AIDS* 2008,**22**:2517-2525.
15. Berry N, Ariyoshi K, Jobe O, Ngum PT, Corrah T, Wilkins A*, et al.* HIV type 2 proviral load measured by quantitative polymerase chain reaction correlates with CD4+ lymphopenia in HIV type 2-infected individuals. *AIDS Res Hum Retroviruses* 1994,**10**:1031-1037.
16. Comparison of vertical human immunodeficiency virus type 2 and human immunodeficiency virus type 1 transmission in the French prospective cohort. The HIV

Infection in Newborns French Collaborative Study Group. *Pediatr Infect Dis J* 1994,**13**:502-506.
17. Adjorlolo-Johnson G, De Cock KM, Ekpini E, Vetter KM, Sibailly T, Brattegaard K*, et al.* Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. *JAMA* 1994,**272**:462-466.
18. Matheron S, Courpotin C, Simon F, Di Maria H, Balloul H, Bartzack S*, et al.* Vertical transmission of HIV-2. *Lancet* 1990,**335**:1103-1104.
19. Kanki PJ, Travers KU, S MB, Hsieh CC, Marlink RG, Gueye NA*, et al.* Slower heterosexual spread of HIV-2 than HIV-1. *Lancet* 1994,**343**:943-946.
20. De Cock KM, Adjorlolo G, Ekpini E, Sibailly T, Kouadio J, Maran M*, et al.* Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic. *JAMA* 1993,**270**:2083-2086.
21. Whittle H, Morris J, Todd J, Corrah T, Sabally S, Bangali J*, et al.* HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS* 1994,**8**:1617-1620.
22. Jaffar S, Wilkins A, Ngom PT, Sabally S, Corrah T, Bangali JE*, et al.* Rate of decline of percentage CD4+ cells is faster in HIV-1 than in HIV-2 infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997,**16**:327-332.
23. Schim van der Loeff MF, Jaffar S, Aveika AA, Sabally S, Corrah T, Harding E*, et al.* Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS* 2002,**16**:1775-1783.
24. Marlink RG, Ricard D, M'Boup S, Kanki PJ, Romet-Lemonne JL, N'Doye I*, et al.* Clinical, hematologic, and immunologic cross-sectional evaluation of individuals exposed to human immunodeficiency virus type-2 (HIV-2). *AIDS Res Hum Retroviruses* 1988,**4**:137-148.
25. Marlink R. Lessons from the second AIDS virus, HIV-2. *AIDS* 1996,**10**:689-699.
26. Norrgren H, da Silva Z, Biague A, Andersson S, Biberfeld G. Clinical progression in early and late stages of disease in a cohort of individuals infected with human immunodeficiency virus-2 in Guinea-Bissau. *Scand J Infect Dis* 2003,**35**:265-272.
27. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T*, et al.* Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* 1994,**265**:1587-1590.
28. Matheron S, Pueyo S, Damond F, Simon F, Lepretre A, Campa P*, et al.* Factors associated with clinical progression in HIV-2 infected-patients: the French ANRS cohort. *AIDS* 2003,**17**:2593-2601.
29. Odehouri K, De Cock KM, Krebs JW, Moreau J, Rayfield M, McCormick JB*, et al.* HIV-1 and HIV-2 infection associated with AIDS in Abidjan, Cote d'Ivoire. *AIDS* 1989,**3**:509-512.
30. Evans LA, Moreau J, Odehouri K, Seto D, Thomson-Honnebier G, Legg H*, et al.* Simultaneous isolation of HIV-1 and HIV-2 from an AIDS patient. *Lancet* 1988,**2**:1389-1391.
31. Nkengasong JN, Kestens L, Ghys PD, Koblavi-Deme S, Otten RA, Bile C*, et al.* Dual infection with human immunodeficiency virus type 1 and type 2: impact on HIV type 1 viral load and immune activation markers in HIV-seropositive female sex workers in Abidjan, Ivory Coast. *AIDS Res Hum Retroviruses* 2000,**16**:1371-1378.
32. Chang LW, Osei-Kwasi M, Boakye D, Aidoo S, Hagy A, Curran JW*, et al.* HIV-1 and HIV-2 seroprevalence and risk factors among hospital outpatients in the Eastern Region of Ghana, West Africa. *J Acquir Immune Defic Syndr* 2002,**29**:511-516.
33. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B*, et al.* Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *AIDS* 2008,**22**:1195-1202.

34. Leonard G, Chaput A, Courgnaud V, Sangare A, Denis F, Brechot C. Characterization of dual HIV-1 and HIV-2 serological profiles by polymerase chain reaction. *AIDS* 1993,**7**:1185-1189.
35. Walther-Jallow L, Andersson S, da Silva Z, Biberfeld G. High concordance between polymerase chain reaction and antibody testing of specimens from individuals dually infected with HIV types 1 and 2 in Guinea-Bissau, West Africa. *AIDS Res Hum Retroviruses* 1999,**15**:957-962.
36. Dieng-Sarr A, Hamel DJ, Thior I, Kokkotou E, Sankale JL, Marlink RG*, et al.* HIV-1 and HIV-2 dual infection: lack of HIV-2 provirus correlates with low CD4+ lymphocyte counts. *AIDS* 1998,**12**:131-137.
37. Ishikawa K, Fransen K, Ariyoshi K, Nkengasong JN, Janssens W, Heyndrickx L*, et al.* Improved detection of HIV-2 proviral DNA in dually seroreactive individuals by PCR. *AIDS* 1998,**12**:1419-1425.
38. Travers K, Mboup S, Marlink R, Gueye-Nidaye A, Siby T, Thior I*, et al.* Natural protection against HIV-1 infection provided by HIV-2. *Science* 1995,**268**:1612-1615.
39. Browning CM, Cagnon L, Good PD, Rossi J, Engelke DR, Markovitz DM. Potent inhibition of human immunodeficiency virus type 1 (HIV-1) gene expression and virus production by an HIV-2 tat activation-response RNA decoy. *J Virol* 1999,**73**:5191-5195.
40. Rappaport J, Arya SK, Richardson MW, Baier-Bitterlich G, Klotman PE. Inhibition of HIV-1 expression by HIV-2. *J Mol Med (Berl)* 1995,**73**:583-589.
41. Dern K, Rubsamen-Waigmann H, Unger RE. Inhibition of HIV type 1 replication by simultaneous infection of peripheral blood lymphocytes with human immunodeficiency virus types 1 and 2. *AIDS Res Hum Retroviruses* 2001,**17**:295-309.
42. von Dalnok GK, Kleinschmidt A, Neumann M, Leib-Moesch C, Erfle V, Brack-Werner R. Productive expression state confers resistance of human immunodeficiency virus (HIV)-2-infected lymphoma cells against superinfection by HIV-1. *Arch Virol* 1993,**131**:419-429.
43. Wiktor SZ, Nkengasong JN, Ekpini ER, Adjorlolo-Johnson GT, Ghys PD, Brattegaard K*, et al.* Lack of protection against HIV-1 infection among women with HIV-2 infection. *AIDS* 1999,**13**:695-699.
44. Ariyoshi K, Schim van der Loeff MF, Sabally S, Cham F, Corrah T, Whittle H. Does HIV-2 infection provide cross-protection against HIV-1 infection? *AIDS* 1997,**11**:1053-1054.
45. Aaby P, Poulsen AG, Larsen O, Christiansen CB, Jensen H, Melbye M*, et al.* Does HIV-2 protect against HIV-1 infection? *AIDS* 1997,**11**:939-940.
46. Schim van der Loeff MF, Aaby P, Aryioshi K, Vincent T, Awasana AA, Da Costa C*, et al.* HIV-2 does not protect against HIV-1 infection in a rural community in Guinea-Bissau. *AIDS* 2001,**15**:2303-2310.
47. Chiara M, Rony Z, Homa M, Bhanumati V, Ladomirska J, Manzi M*, et al.* Characteristics, immunological response & treatment outcomes of HIV-2 compared with HIV-1 & dual infections (HIV 1/2) in Mumbai. *Indian J Med Res* 2010,**132**:683-689.
48. Alabi AS, Jaffar S, Ariyoshi K, Blanchard T, Schim van der Loeff M, Awasana AA*, et al.* Plasma viral load, CD4 cell percentage, HLA and survival of HIV-1, HIV-2, and dually infected Gambian patients. *AIDS* 2003,**17**:1513-1520.
49. Holmgren B, da Silva Z, Vastrup P, Larsen O, Andersson S, Ravn H*, et al.* Mortality associated with HIV-1, HIV-2, and HTLV-I single and dual infections in a middle-aged and older population in Guinea-Bissau. *Retrovirology* 2007,**4**:85.
50. Drylewicz J, Eholie S, Maiga M, Zannou DM, Sow PS, Ekouevi DK*, et al.* First-year lymphocyte T CD4+ response to antiretroviral therapy according to the HIV type in the IeDEA West Africa collaboration. *AIDS* 2010,**24**:1043-1050.
51. Harries K, Zachariah R, Manzi M, Firmenich P, Mathela R, Drabo J*, et al.* Baseline characteristics, response to and outcome of antiretroviral therapy among patients with

HIV-1, HIV-2 and dual infection in Burkina Faso. *Trans R Soc Trop Med Hyg* 2010,**104**:154-161.

52. Rodes B, Holguin A, Soriano V, Dourana M, Mansinho K, Antunes F*, et al.* Emergence of drug resistance mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy. *J Clin Microbiol* 2000,**38**:1370-1374.
53. Adje-Toure CA, Cheingsong R, Garcia-Lerma JG, Eholie S, Borget MY, Bouchez JM*, et al.* Antiretroviral therapy in HIV-2-infected patients: changes in plasma viral load, CD4+ cell counts, and drug resistance profiles of patients treated in Abidjan, Cote d'Ivoire. *AIDS* 2003,**17 Suppl 3**:S49-54.
54. Peterson K, Jallow S, Rowland-Jones SL, de Silva TI. Antiretroviral Therapy for HIV-2 Infection: Recommendations for Management in Low-Resource Settings. *AIDS Res Treat* 2011,**2011**:463704.
55. Dieng-Sarr A, Popper S, Thior I, Hamel DJ, Sankale JL, Siby T*, et al.* Relation between HIV-2 proviral load and CD4+ lymphocyte count differs in monotypic and dual HIV infections. *J Hum Virol* 1999,**2**:45-51.
56. Koblavi-Deme S, Kestens L, Hanson D, Otten RA, Borget MY, Bile C*, et al.* Differences in HIV-2 plasma viral load and immune activation in HIV-1 and HIV-2 dually infected persons and those infected with HIV-2 only in Abidjan, Cote D'Ivoire. *AIDS* 2004,**18**:413-419.
57. Peeters M, Gershy-Damet GM, Fransen K, Koffi K, Coulibaly M, Delaporte E*, et al.* Virological and polymerase chain reaction studies of HIV-1/HIV-2 dual infection in Cote d'Ivoire. *Lancet* 1992,**340**:339-340.
58. George JR, Rayfield MA, Phillips S, Heyward WL, Krebs JW, Odehouri K*, et al.* Efficacies of US Food and Drug Administration-licensed HIV-1-screening enzyme immunoassays for detecting antibodies to HIV-2. *AIDS* 1990,**4**:321-326.
59. Curlin ME, Gottlieb GS, Hawes SE, Sow PS, Ndoye I, Critchlow CW*, et al.* No evidence for recombination between HIV type 1 and HIV type 2 within the envelope region in dually seropositive individuals from Senegal. *AIDS Res Hum Retroviruses* 2004,**20**:958-963.
60. Gottlieb GS, Sow PS, Hawes SE, Ndoye I, Coll-Seck AM, Curlin ME*, et al.* Molecular epidemiology of dual HIV-1/HIV-2 seropositive adults from Senegal, West Africa. *AIDS Res Hum Retroviruses* 2003,**19**:575-584.
61. Rouet F, Ekouevi DK, Inwoley A, Chaix ML, Burgard M, Bequet L*, et al.* Field evaluation of a rapid human immunodeficiency virus (HIV) serial serologic testing algorithm for diagnosis and differentiation of HIV type 1 (HIV-1), HIV-2, and dual HIV-1-HIV-2 infections in West African pregnant women. *J Clin Microbiol* 2004,**42**:4147-4153.