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Differences in proviral DNA load between HIV-1-infected and HIV-2-infected patients

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Gueudin *et al.* [1] recently reported statistically significant lower levels of ‘proviral’ HIV-2 DNA in peripheral blood mononuclear cells (PBMC) of HIV-2-infected individuals compared with that found in HIV-1-infected individuals. They call into question numerous previous studies (including their own) that have found similar levels of HIV-1 and HIV-2 DNA in PBMC despite remarkably lower levels of plasma HIV-2 RNA than HIV-1 RNA in infected individuals [2-12]. They base their findings on a novel real-time PCR assay that uses a ‘coplasmid’ standard that contains both long-terminal repeat (LTR) targets for HIV-1 and HIV-2 primers and probes. In a stratified analysis by CD4 cell counts in 40 HIV-1-infected and 42 HIV-2-infected antiretroviral naïve patients, they reported significantly higher levels of HIV-1 DNA compared with HIV-2 DNA in patients with CD4 cell counts greater than 500 cells/ μ l, between 500 and 300 cells/ μ l, and a nonsignificant trend for those lower than 300 cells/ μ l. Similar to previous studies, they found a significant increase in DNA levels for both HIV-1 and HIV-2 as CD4 cell counts decreased.

Unfortunately (and unlike some previous studies that found similar statistical differences in unadjusted, but not in adjusted analyses), they failed to adjust for CD4 cell count, and other potential factors (age, duration of infection, sex, etc.) affecting HIV PBMC DNA levels in multivariate analyses. Thus, it is possible that their observation is biased by different distributions of CD4 cell counts (or other confounders) between the HIV-1 and HIV-2 patients (with HIV-1 patients tending to have more rapid disease and lower CD4 cell counts in each strata). Also, despite differing levels of detection sensitivity for HIV-1 (10 copies/ μ g) and HIV-2 (20 copies/ μ g) in their new assay, and differing rates of detection of HIV-1 (37/40, 93%) and HIV-2 (31/42, 74%) in infected patients, they report that they set samples below the detection limit for both HIV-1 and HIV-2 at 10 copies/ μ g. It would have been reasonable to do a sensitivity analysis to determine how that ‘arbitrary’ cutoff might affect their results.

In addition, their assay actually measures total cellular HIV DNA (integrated, unintegrated, one and two LTR circles) and is not specific for ‘proviral’ (integrated) HIV: different levels

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of one and two LTR circles between HIV-1 and HIV-2 could, for example, confound measurements. A recent study by MacNeil *et al.* [12] reported that the total amount and percentage of integrated proviral HIV-1 and HIV-2 DNA are quite similar and not statistically different, and that lower levels of HIV-2 mRNA, and not proviral load levels, may help explain the markedly lower plasma HIV-2 RNA viral loads that have been universally observed.

Finally, Gueudin *et al.* [1] report that the HIV-2 plasma RNA viral loads were above the detection limit (100 copies/ml) in 12 of 42 (29%) patients. This percentage of patients with detectable HIV-2 plasma RNA viral loads is substantially lower than in other studies that have looked at HIV-2 plasma RNA levels in antiretroviral (ARV)-naïve patients (range 45-85%) [2,7-9,11,13-18], suggesting that their study population may not be representative of HIV-2 infection in general, and that their findings of low HIV-2 PBMC DNA levels may be a consequence of having the majority of their patients with low-plasma viral loads.

Using the same CD4 cell count cutoffs as reported by Gueudin *et al.* [1] we reanalyzed additional patients with HIV-1 ($n = 392$) and HIV-2 ($n = 104$) PBMC DNA levels that we previously reported from our Senegal Cohort using a modified Roche Amplicor DNA assay (Roche Amplicor Diagnostic systems Inc., Nutley, New Jersey, USA) with a limit of detection of 1 copy/ μg [11]. In contrast, our data suggest that, at CD4 cellcounts greater than 500 cells/ μl , and between 300 and 500 cells/ μl , the DNA levels are quite similar and, at low CD4 cell counts (<300 cells/ μl), HIV-2 DNA levels are actually significantly higher compared with HIV-1 (Table 1).

Overall, the preponderance of data suggests that levels of HIV-1 and HIV-2 DNA in PBMC are not the major reason for the markedly different levels of plasma virus and rates of disease progression found between these two types of HIV infection.

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Table 1

Median log₁₀ peripheral blood mononuclear cell HIV DNA copies/μg.

Group	HIV-1		HIV-2		P-value	
	Sample size	Median (IQR)	Sample size	Median (IQR)	Unadjusted	Adjusted ^a
All	392	1.45 (0.90–1.96)	104	1.61 (1.04–2.25)	0.052	<0.001 ^b
CD4 cell count (cells/μl)						
>500	63	1.08 (0.48–1.62)	45	1.24 (0.87–1.74)	0.27	0.07
300–500	70	1.33 (0.78–1.78)	21	1.56 (1.15–2.14)	0.31	0.22
<300	229	1.58 (1.00–2.05)	35	2.34 (1.79–2.70)	<0.001 ^b	<0.001 ^b

By HIV status, CD4 cell count, and plasma viral load. IQR, interquartile range; PBMC, peripheral blood mononuclear cells.

^aMultivariate linear regression analyses, adjusting for age, sex, commercial sex work, CD4 cell count and plasma viral load (*n*=438 (all), 102 (CD4 cell count >500 cells/μl), 88 (CD4 cell count 300–500 cells/μl), 248 (CD4 cell count <300 cells/μl)).

^bHIV-2 PBMC DNA levels significantly greater than HIV-1 PBMC DNA levels.