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Viral and host factors associated with the HIV-1 viral load setpoint in adults from Mbeya Region, Tanzania

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Abstract

Background—The viral load setpoint (VLS) is an important predictor of HIV disease progression, but there is a lack of information regarding the VLS and its possible determinants in African populations.

Methods—Initially HIV negative adults from three distinct groups (female barworkers, females and males from the general population) were followed for up to four years. The VLS was calculated for 108 seroconverters and associations of the VLS with possible risk factors were analyzed using univariate and multivariate regression.

Results—The median VLS for female barworkers, females and males from the general population were 69,850, 28,600 and 158,000 RNA copies/ml respectively. Significant associations with an elevated viral load were observed for male gender (Risk Ratio (RR)=1.83, 95% confidence interval (95%CI)=1.14–2.93), the expression of harmful HLA I alleles (RR=1.73, 95%CI=1.13–2.66) and multiple infection with different HIV-1 subtypes (RR=1.65, 95%CI=1.03–2.66). Barworkers were considerably more often infected with different HIV-1 subtypes than participants from the general population.

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Conclusions—Our study confirms that gender and the expression of different HLA class I alleles are important determinants of the viremia at VLS and it also corroborates an earlier finding that multiple infection with different HIV-1 subtypes is associated with a higher VLS.

Keywords

HIV-1 infection; Acute infection; Viral load setpoint; Multiple infection; HLA class I alleles; Africa

Introduction

Sub-Saharan Africa is most heavily affected by the HIV epidemic. In some countries it has reduced overall life expectancy by more than 20 years¹. If untreated, infected individuals show an extreme heterogeneity in the clinical course and outcome of HIV infection. The identification of factors that influence the natural course of infection is of great importance for prognosis and for the timing of antiretroviral treatment.

The viral load is an important predictor of HIV-1 disease progression. Higher viral loads are associated with faster progression to AIDS and death². During acute HIV-1 infection, the viral load reaches peak levels that subsequently drop to a lower, more stable level of viremia, known as the viral load setpoint (VLS). This is explained by the balance between the virulence of the infecting virus and the host immune system's potential to control the infection³. Because there is no standard method for the calculation of the VLS, researchers use different empirical approaches⁴. Despite these methodological differences, the association between an elevated VLS and faster disease progression to AIDS is widely accepted^{5–7}. The VLS can thus be used as a prognostic marker to identify individuals at risk for rapid disease progression. Such prognostic markers may lead to a better understanding of HIV-1-infection, improved clinical monitoring, and a better timing of the initiation of antiretroviral therapy. Virus- and host-related factors play an important role in determining the VLS. Thus the VLS can differ considerably between individuals and between populations.

The HIV-1 epidemic is characterized by high genetic diversity with multiple subtypes as well as circulating and unique inter-subtype recombinant forms in different parts of the world^{8, 9}. Previous studies suggest that the infecting subtype and multiple HIV infection are important factors that might influence the VLS and HIV disease progression^{9–11}. Possible host-related factors associated with differences in VLS include gender, age, race, other diseases and human genetic variation^{12–14}.

The impact of HLA class I alleles on viral load during the chronic phase of HIV has been examined in two studies in South Africa where different alleles were identified as either “protective” or “harmful” according to their effect on viremia at VLS^{15, 16}. The expression of protective HLA class I alleles is thought to correlate with HIV-specific CD8 T cell responses of potent antiviral efficiency^{17, 18}. However, only very limited data regarding the VLS and its correlates exist for Sub-Saharan Africa¹⁹.

The main objectives of our study were to determine the VLS in our study population and to identify virus and host factors that might have an impact on the VLS. Below we therefore examine the association of the VLS with HLA class I genetic background, infection with different HIV-1 subtypes, and with socio-demographic and behavioral factors.

Methods

Study population

Data for this study were collected from HIV seroconverters who were identified in two different cohorts from Mbeya Region in south-western Tanzania. All laboratory and cohort work done in these two studies was in accordance with the Helsinki Declaration of 1975 as revised in 2000 and was also approved by the appropriate ethics committees of involved partners. All participants provided written informed consent before enrolment.

HISIS (longitudinal HIV Superinfection Study)—The seroconverters in this study were part of a larger, well characterized high-risk open cohort of female barworkers enrolled in a prospective study of HIV-1 infection in Mbeya Region²⁰. A total of 753 female volunteers, aged between 18 and 35 years were recruited from September 2000 onwards from 17 high-transmission areas located along the Pan-African Highway. Each participant provided blood samples at enrolment and every three months thereafter, for a period of up to four years. During the study, all participants received health care that included treatment of acute infectious diseases, screening and – if necessary – treatment for sexually transmitted diseases and, since 2003, cotrimoxazole prophylaxis against opportunistic infections for women with CD4+ T cell counts below 200/μl. Since 2005, antiretroviral treatment has been available for participants with AIDS-defining symptoms or CD4 T cell counts below 200 cells/μl. During the collection of data for this study however, all individuals were antiretroviral naïve.

CODE (Cohort-development study)—Between September 2002 and April 2003, 3,096 volunteers (1,778 females and 1,318 males) from the general population were recruited from one rural and two urban sites in and around Mbeya town. CODE participants were followed-up at approximately six-monthly intervals for 3.5 years²¹.

Below we refer to the three different groups of seroconverting participants, HISIS female barworkers, CODE general population females and CODE general population males as “barworkers”, “CODE females” and “CODE males” to simplify the narrative of our observations in this manuscript. The HIV prevalence at enrolment into the two cohorts was 67%, 19% and 14% in these three groups respectively^{21, 22}.

CODE and HISIS participants were only included into the below analysis if they were HIV negative at enrolment (247 barworkers, 1440 CODE females and 1138 CODE males) and got HIV infected during follow up (49 barworkers, 63 CODE females and 38 CODE males). Further requirements were that the last HIV negative visit was less than 9 months before the first HIV positive visit (important in order to estimate the time point of infection with the necessary accuracy), and that participants also had attended at least one study visit after the first positive visit between 5 to 12 months after the estimated time point of infection (necessary for calculation of the VLS). These criteria were satisfied by 46 barworkers, 41 CODE females and 21 CODE males respectively. Of the 42 newly HIV-infected participants who could not be included (three barworkers, 22 CODE females and 17 CODE males), 10 were lost to followup directly after their first HIV-positive visit (one barworker, four CODE females and five CODE males), 19 could not be followed up because their first HIV-positive visit coincided with the last scheduled follow-up (two barworkers, 12 CODE females and five CODE males) and 13 participants missed one or more appointments after their first positive visit, so that the VLS could not be calculated, but returned at a later stage (six CODE females and seven CODE males).

Interviews, clinical examinations and specimen collections

Interviews regarding the social, demographic and behavioral background of participants were conducted in Kiswahili by trained staff members using structured questionnaires. The responses were recorded in English. Clinical examinations and specimen collections took place in a suitable locality.

Laboratory methods

The HIV serostatus was determined using two diagnostic enzyme-linked immunosorbent assay (ELISA) tests (Enzygnost Anti HIV1/2 Plus, Dade Behring, Liederbach, Germany; Determine HIV 1/2, Abbott, Wiesbaden, Germany). Discordant results were verified by HIV-1 Western Blot (HIVblot 2.2 Genelabs/Abbott, Wiesbaden, Germany). HIV-1 plasma RNA load was determined with the Roche Amplicor HIV-1 Monitor Test version 1.5 (Roche Diagnostics, Basel, Switzerland) with a range of quantitation of ≥ 400 to $\leq 750,000$ virus copies/ml. The first HIV positive study visit was defined as the visit where the participant was positive by viral load determination and/or serotesting but the visit before had been negative both by viral load and by serotesting. This, and all subsequent samples over a period of up to 3.5 years were used for viral load determination. The criteria which of these viral load measurements were used for calculation of the VLS are described below.

CD4+ counts were performed using a FACSCalibur MultiSET System with Trucount tubes (Becton; Dickinson and Company, Franklin Lakes, NJ, USA). The infecting HIV-1 subtype and the presence of infection with multiple HIV-1 subtypes were determined at VLS by the Multi-region Hybridization Assay (MHA), using subtype A, C and D -specific fluorescent probes in 5 genomic regions in a real-time PCR format^{8, 18}. Multiple infections were inferred when more than one subtype-specific probe hybridized to the same genomic region. HLA class I typing was performed as described by Turner et al.²³ and Kijak et al. (High-Throughput High-Resolution Class I HLA Genotyping in East Africa; submitted). HLA class I alleles were classified as either “protective” (A*0205, B*5801, B*8101, B*4201 and B*5703), “harmful” (B*5802, B*4501, B*1801, and B*1503 in subtype C epidemic) or “neutral” (all others) according to previous studies^{15, 16}. Due to logistic reasons CD4+ count, HLA class I typing and viral diversity determination were not done for all participants, resulting in lower participant numbers for these assays.

Serological examinations for syphilis were conducted using the Serodia Treponema pallidum particle agglutination assay (TPPA) (Fujirebio Inc., Japan) and the rapid plasma reagin (RPR) test (VD25; Murex Diagnostics, UK)^{22, 24}. On enrolment all participants were tested for Hepatitis B by ELISA (MONOLISA HBsAg ULTRA, Bio-Rad, Hercules, CA) and positive results were confirmed by a neutralization method of HBsAg (MONOLISA HBsAg Confirmation test, Bio-Rad, Hercules, CA).

Statistical analysis

Data was double-entered into Microsoft Access (Microsoft Corp., Redmond, WA), compared and corrected for entry errors and analysed using Stata 10 statistics software (Stata Corp., College Station, TX). Because both VLS and CD4+ cell counts were not normally distributed, differences of these parameters between groups were tested for significance using the non-parametric Wilcoxon ranksum test. The VLS was defined according to Mei, Wang & Holte⁴ as the median of the participants' viral load between 5 to 12 months after the estimated time point of infection. Because HISIS participants were seen at three-monthly intervals, in most instances their VLS was calculated from more than one VL assessment (two participants with only one assessment, 40 with two assessments and four participants with three assessments) whereas none of the CODE participant had more than one VL assessment during this time span.

Probability values for group differences in binary characteristics (e.g. presence/absence of multiple infections) was calculated using the Pearson's chi-squared test²⁵. Associations between different risk-factors and a binary outcome that indicated whether the VLS was below or above the overall median were analyzed using univariate and multivariate Poisson regression models with robust variance estimates^{26, 27}.

In order to estimate the time point of infection, the average time span from infection to HIV seropositivity was assumed to be 33 days and that to virus-positivity 11 days^{28, 29}. The date of infection was estimated as the midpoint of the resulting time window. To give an example: for participants whose first HIV positive study visit was both virus positive and seropositive, the infection time point was estimated as the midpoint between 11 days before the last negative and 33 days before the first positive visit. For participants, who were virus-positive but had not seroconverted at their first HIV positive follow-up, the time point of infection was estimated as 22 days before this follow-up.

Results

Social and demographic characteristics

Selected characteristics of the three groups of seroconverters at enrolment into their respective cohorts are shown in table 1. The median age was between 22 and 25 years. The prevalence of Hepatitis B and Syphilis TPPA positivity were higher among barworkers (17.4% and 41.3%, respectively) than among CODE participants (7.1% and 12.5% for females, 0% and 4.8% for males).

Comparison of the characteristics shown in table 1 and of initial viral loads and CD4 counts of the included participants with those of the 42 newly HIV-infected subjects who could not be included into this study revealed some differences between these two groups. However, none of these differences was statistically significant at the 95% confidence level.

HIV-1 viremia and CD4 counts at viral load setpoint

The mean time between the estimated time point of infection and the mean date of VLS measurements was 8.4 months (range 5.4 to 10.1) for barworkers, 9.1 months (5.7 to 11.9) for CODE females and 8.7 months (6.1 to 9.7) for CODE males. The overall median viremia at VLS for all three participant groups was 69,850 copies/ml which was identical to the median VLS for barworkers (Table 2). Viremia at VLS was lower for female CODE participants (28,600 copies/ml) and higher for male CODE participants (158,000 copies/ml) and this difference between CODE females and males was significant ($p=0.011$). Concordantly, CODE males had a significantly lower median CD4+ T cell count at VLS than the two female groups.

The three groups also differed by their proportion of participants with a VLS below 2000 copies/ml (referred to as "VLS controllers" below). While 26.8% of CODE females were VLS controllers, only 8.7% of the barworkers ($p = 0.025$) and 9.5% of CODE men ($p=0.113$) had viral loads below 2000 copies/ml at VLS (table 2). This is also demonstrated in figure 1, where however only participants with complete data were included.

When excluding the VLS controllers, the median VLS in CODE women is 2.8 times higher (81,100 instead of 28,600 copies/ml) than if VLS controllers are included but only 1.7 times higher in barworkers (116,825 instead of 69,850 copies/ml). The previously observed differences in VLS are thus paralleled by different frequencies of VLS controllers in the three groups.

We also analyzed the development of viremia over time for the three groups individually, excluding VLS controllers and for all VLS controllers separately (figure 2). All seroconverters

for whom we had a valid VLS estimate were included for all time-ranges for which they had VL data. Despite small participant numbers for time intervals beyond two years after infection, the viral load levels remain relatively stable in barworkers and CODE males, they even decline over time in CODE females. However, this pattern could be influenced by a higher drop out rate of participants with elevated viral loads.

HLA class I alleles, HIV-1 subtype and multiple infection

A subsequent analysis of the distribution of HLA class I alleles identified marked but non-significant differences between the three groups (Table 2). CODE males had the lowest proportion of protective (A*0205, B*5801, B*8101, B*4201 and B*5703) and the highest proportion of harmful alleles (B*5802, B*4501, B*1801, and B*1503 in subtype C epidemic).

There was no major difference in subtype distribution between male and female CODE participants with more than 50% of new infections caused by subtype C and only few multiple HIV infections (6.3% and 11.1% respectively). In contrast, participants who were infected with multiple HIV subtypes at VLS represented the largest group (36.4%) in the barworkers, where subtype C caused only 24% of new infections. The large number of multiple HIV infections in female barworkers at VLS is especially striking when considering that this was determined only 5 to 12 months after HIV infection.

Risk factors for elevated VLS

In order to identify potential risk factors for elevated viremia at VLS we initially performed univariate Poisson regression analyses on a binary outcome variable that indicated whether the individual viremia at VLS was above or below the median for all participants (=69,850 copies/ml). If the univariate p-value for at least one stratum of these risk factor variables was ≤ 0.1 , they were included into a multivariate model in order to examine their association with the VLS for mutual independence. The univariate and multivariate results for these variables are shown in Table 3.

Male gender, the presence of harmful HLA class I alleles and infection with multiple HIV-1 subtypes were strongly and significantly associated with the VLS both in univariate and multivariate analysis. CODE males had a 76% higher risk of having an elevated VLS than CODE females. The presence of harmful HLA class I alleles was associated with a 70% increase in elevated VLS and infection with multiple HIV-1 subtypes with a 65% increase when comparing with the respective control groups.

Barworkers were considerably (35%) more likely to have an elevated viral load than general population females and participants with protective HLA class I alleles had a 40% lower prevalence of elevated viral loads than those with only neutral HLA class I alleles. However, both these differences were not significant. The similarity of point estimates and significance of the above associations in univariate and multivariate analysis indicates that their respective influence is largely independent of the other factors.

Potential risk-factors that did not qualify for initial inclusion into the above model were reexamined by introducing them into this model one by one, in order to see whether their association to the viremia at setpoint would change when adjusted for other important variables. However, all other biological factors such as the infecting subtype (A, C, D or recombinant strains), and syphilis and hepatitis B infection at enrolment were unassociated with the viremia at setpoint (data not shown). Similarly neither age nor the other socio-demographic and behavioral factors that we examined (religious denomination, education level, household size, marital status, number of children, use of contraceptives, previous HIV testing) showed an

association with the VLS, nor did they exhibit any strong influence on other variables when added to the multivariate model.

Discussion

In HIV infection the VLS is an important and generally accepted predictor for the progression to AIDS. It is also an important marker for the evaluation of vaccines and microbicides that do not protect from infection but may ameliorate viral load. A comparison of our VLS results with those from other parts of Sub-Saharan-Africa shows that the median VLS of CODE females is at the lower end of the spectrum of 4.45 to 4.61 log₁₀ RNA copies/ml found in three other studies from East Africa concerning females from the general population^{3, 9, 30}. In contrast, the barworkers in our study, had a median VLS that was well above the range found in four other studies from Sub-Saharan-Africa concerning high risk females (3.71 to 4.33 log₁₀ copies/ml)^{10, 19, 31, 32} and the VLS of CODE males also was considerably higher than those from two other studies concerning general population males in other parts of East Africa (4.74 and 4.76 log₁₀ copies/ml)^{3, 9}. Our finding that general population males had a higher VLS than females is in accordance with two studies from East Africa that included both sexes and also with findings from other parts of the world, although this difference does not seem to imply a difference in HIV progression between sexes^{3, 9, 33}. A final biological or behavioral explanation for this phenomenon is still lacking.

Participants with multiple HIV-1 infections had a higher risk of elevated viremia compared to those with a single infection. This important finding is in agreement with the results of a previous smaller study¹⁰ and is also consistent with the finding that multiple HIV infections can accelerate disease progression³⁴.

Although one would expect more multiple infections with different HIV subtypes in high risk populations, the difference in prevalence of multiple HIV infections between female barworkers (36.4%) and the other two groups (6.3% and 11.1%) so early after seroconversion is striking. It would be interesting to determine whether these multiple infections are mostly acquired during one infection event (i.e. transmission from an individual that is already multiply infected), or whether the multiple infection is caused by an additional (super-) infection. If the latter was true this would hint to a higher susceptibility to HIV infection shortly after initial seroconversion. In any case our data indicate that specifically within such sub-populations there is a high risk for coinfection with multiple HIV strains, and that this is also a risk factor for elevated viral loads. Because high viral loads are associated with increased transmission risk, an existing multiple infection is thus likely to increase the risk of HIV transmission.

Unfortunately, the proportion of multiple infections is not fully comparable between the two cohorts. Cohort-specific differences in visit intervals (three-monthly in HISIS and six-monthly in CODE) and thus the number of MHA tests performed may result in a lower sensitivity for detection of multiple HIV infections in CODE participants. Nevertheless, even if only one of the MHA assessments for barworkers had been used, as in CODE participants, where only one assessment was available, the proportion of multiple infections in the barworkers would still have been three times as high as in male and female CODE participants combined (24% vs. 8%, p=0.105).

The influence of host genetic polymorphisms within the HLA class I allele gene locus on HIV viral load and on disease progression during chronic infection is well documented^{15–17, 35–38}. Particularly the expression of HLA class I allele B57 correlates with the absence of a symptomatic HIV-1 seroconversion illness³⁷. This is important because patients with more severe symptoms during acute HIV-1 infection and longer duration of the acute infection

syndrome tend to progress more rapidly to AIDS³⁷. In addition there is an association between HLA-B57 and favorable clinical, virological and immunological events during acute HIV-1 infection¹⁷. Our analysis demonstrates that the expression of harmful HLA class I alleles that are associated with poor viral control during the chronic phase of HIV is significantly associated with poor control even early after HIV infection. This indicates that viral control is not lost gradually over time but rather that it has never been efficient in these individuals in the first place.

Female barworkers had a 35% higher risk of having an elevated VLS than CODE females after adjustment for HLA type and multiple infections ($p=0.341$). Although non-significant, this might hint to a real difference between general population females and high-risk females, that our model was unable to account for. One possible reason for this difference could be a higher prevalence of genital tract co-infections in this group, that, apart from syphilis, we did not assess during this study.

Figure 1 illustrates that VLS differences between the three groups were small when not considering the VLS controllers. However, this should not be interpreted as an explanation of the VLS differences between barworkers, CODE females and CODE males, because being able to control viremia at VLS is not an independent explanatory factor but just another way to categorize our outcome, the viremia at VLS.

In conclusion our data show that gender, expression of different HLA class I alleles and multiple infection with different HIV-1 subtypes are strongly associated with the viremia at VLS in our study population. The first two of these findings confirm results of previous studies from other parts of the world. The latter finding substantiates the results of a smaller study from South Africa¹⁰ but has – to our knowledge – not been reported from other studies yet. The infecting HIV-1 subtype, hepatitis B and syphilis infection before seroconversion and the various socio-demographic and behavioral factors that we examined did not appear to influence the viremia at VLS in our study population. Another important result that merits further investigation in other settings is the high prevalence of infection with different HIV-1 subtypes early after seroconversion in the barworkers cohort.

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Figure 1. Viral load setpoint, multiple infection and presence of harmful HLA type 1 alleles
Only participants with complete assessments are included.

Figure 2. Median viral load over time in the three study groups

All participants with a VLS < 2000 copies/ml (VLS controllers) are graphed separately and their data was not included into their respective groups. Whiskers indicate non-parametric 95% confidence limits; numbers denote N for each group and time-range. Please note that data for later time-ranges are unreliable due to low participant numbers.

Table 1

Characteristics of the study population at enrollment

n	Barworkers		CODE females		CODE males		All combined	
	n	%	n	%	n	%	n	%
Median age in years (IQR)	25 (22 to 28)		22 (20 to 28)		24 (21 to 27)		24 (20 to 28)	
Education								
None	1	2.2	5	12.2	4	19.0	10	9.3
Primary	42	91.3	32	78.0	16	76.2	90	83.3
Secondary	3	6.5	4	9.8	1	4.8	8	7.4
Denomination								
Catholic	14	30.4	7	17.1	2	9.5	23	21.3
Protestant	30	65.2	26	63.4	13	61.9	69	63.9
Muslim	2	4.3	3	7.3	0	0.0	5	4.6
No religion	0	0.0	5	12.2	6	28.6	11	10.2
Marital status								
Single	5	10.9	13	31.7	13	61.9	31	28.7
One spouse	41	89.1	27	65.9	7	33.3	75	69.4
≥ 2 spouses	0	0.0	1	2.4	1	4.8	2	1.9
Hepatitis B status								
positive	8	17.4	2	7.1	0	0.0	10	11.2
negative	38	82.6	26	92.9	15	100.0	79	88.8
Syphilis TPPA status								
positive	19	41.3	5	12.5	1	4.8	25	23.4
negative	27	58.7	35	87.5	20	95.2	82	76.6

n = number of study subjects; IQR = inter quartile range

Table 2

Virus- and host-related characteristics at viral load setpoint

	Barworkers	p^a	CODE females	p^b	CODE males	p^c	All combined
HIV-1 virus copies per ml ^d							
n examined	46		41		21		108
Median	69,850	0.133	28,600	0.011	158,000	0.109	69,850
Median log ₁₀	4.84		4.46		5.2		4.84
IQR for log ₁₀	(4.04 to 5.33)		(3.05 to 5.29)		(4.37 to 5.58)		(4.06 to 5.45)
Mean log ₁₀ (SD)	4.67 (0.85)		4.27 (1.15)		4.99 (0.88)		4.58 (1.01)
VLS controllers ^e	8.7%	0.025	26.8%	0.113	9.5%	0.912	15.7%
CD4+ cells/μl							
n examined	20		32		21		73
Median	516	0.792	491	0.028	381	0.050	478
IQR for median	(391 to 623)		(395 to 761)		(250 to 499)		(362 to 644)
Mean (SD)	553 (237)		594 (289)		421 (224)		533 (265)
HLA class I alleles							
n examined	32		40		21		93
neutral HLA only	28.1%	0.094	47.5%	0.730	42.9%	0.268	39.8%
protective HLA	28.1%	0.281	17.5%	0.747	14.3%	0.239	20.4%
protective & harmful HLA	12.5%	0.737	10.0%	0.479	4.8%	0.346	9.7%
harmful HLA	31.3%	0.556	25.0%	0.287	38.1%	0.607	30.1%
Infecting subtypes							
n examined	33		16		9		58
A only	9.1%	0.712	12.5%	0.269	0.0%	0.348	8.6%
C only	24.2%	0.027	56.3%	0.973	55.6%	0.072	37.9%
D only	0.0%	0.147	6.3%	0.667	11.1%	0.053	3.5%
recombinant strains	30.3%	0.390	18.8%	0.835	22.2%	0.634	25.9%
multiple infections	36.4%	0.025	6.3%	0.667	11.1%	0.146	24.1%

n = number of study subjects; IQR = inter quartile range; SD=standard deviation; VLS = viral load setpoint

^{a,b,c}p values for the difference between

^abarworkers and CODE females,

^bCODE females and CODE males and

^cbarworkers and CODE males respectively. The Wilcoxon ranksum test was used for testing differences in age, viral load and CD4+ counts, and the Pearson's chi-squared test for all other tests. Please note that due to multiple testing the overall probability of at least one significant result ($P \leq 0.05$) in the above comparisons is 84.2% under the "universal null hypothesis" i.e. when assuming that in fact there are no differences whatsoever between the three groups regarding the tested variables.

^dReporting the median, median log10 and the mean may seem redundant, but facilitates comparison with the literature where all of these are used to characterize the VLS. However, it should be noted that the mean is not really suited as a measure of central tendency for data that do not conform to a gaussian distribution.

^eVLS < 2000 copies/ml

Table 3

Associations of multiple HIV infection and presence of protective and harmful HLA class I alleles with elevated viral load setpoint

Covariate	univariate models				multivariate model ^a				median VLS ^b	% elevated VLS ^b	
	n	RR	95% CI	P	n	RR	95% CI	P			
Study group											
CODE females	41	1			40	1			26000	37.5	
CODE males	21	1.83	(1.14 to 2.93)	0.012	21	1.76	(1.09 to 2.85)	0.021	158000	71.4	
Barworkers	46	1.28	(0.79 to 2.07)	0.313	32	1.35	(0.73 to 2.51)	0.341	104900	53.1	
HLA alleles present											
Neutral only	37	1			37	1			35400	43.2	
Protective	19	0.61	(0.26 to 1.41)	0.248	19	0.60	(0.28 to 1.29)	0.192	23400	26.3	
Prot. & harmful	9	1.29	(0.64 to 2.57)	0.480	9	1.38	(0.70 to 2.69)	0.352	72500	55.6	
Harmful	28	1.73	(1.13 to 2.66)	0.012	28	1.70	(1.11 to 2.59)	0.014	195000	75.0	
Multiple HIV Infection ^b											
No	44	1			43	1			58650	44.2	
Yes	14	1.65	(1.03 to 2.66)	0.038	14	1.65	(1.03 to 2.63)	0.036	183475	71.4	
Not determined	36	1.16	(0.72 to 1.86)	0.544	36	1.29	(0.78 to 2.13)	0.316	77400	50.0	

Note: Results of univariate and multivariate poisson regression models using robust variance estimates. Due to multiple testing the overall probability of at least one significant result ($P \leq 0.05$) is 30.2% for the univariate and multivariate model respectively under the "universal null hypothesis" i.e. when assuming that in fact there are no differences in VLS between strata of the above variables.

n = Number of participants in stratum; RR = risk ratio for having a VLS $\geq$ the median; 95% CI = 95% confidence interval; P = P-value; % elevated VLS = % of participants with VLS above the overall median of 69,850 copies/ml; median VLS = (unadjusted) median VLS in stratum

^a showing all variables that were included in the model

^b multiple HIV infection according to MHA assay. MHA data was only available for 58 participants

^b median VLS and % with elevated VLS for participants who were included into multivariate model