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Highly active antiretroviral therapy does not completely suppress HIV in semen of sexually active HIV-infected men who have sex with men

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Introduction

Approximately 33.3 million people worldwide are living with HIV/AIDS, and 1.8 million deaths and 2.6 million new infections occur annually [1]. Unprotected intercourse is the most common route through which HIV-1 is transmitted, and semen of HIV-infected men is an important source of infectious HIV [2]. Whereas the HIV/AIDS epidemic in Sub-Saharan Africa is generalized with approximately equal percentages of infections occurring in men and women, the epidemic in the US and many other developed countries is concentrated in men who have sex with men (MSM) [3]. Recent reports suggest that MSM populations in lower and middle income countries are also disproportionately infected with HIV in several locations [4].

MSM are highly susceptible to HIV transmission. In the pre-antiretroviral therapy (ART) era, the rate of HIV transmission among MSM was estimated to be 1 in 100 acts of unprotected anal intercourse [5]), whereas the overall rate of HIV sexual transmission from men to women was much lower (<1 in 1,000 acts of unprotected vaginal intercourse [6]). High peripheral blood and genital HIV viral loads are associated with an increased rate of HIV transmission [7, 8], and ART, which reduces blood and genital viral load, has been

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shown to decrease HIV transmission [7, 9-11]. Yet, evidence suggests that the MSM epidemic has had a resurgence in the era of potent antiretroviral therapy [12, 13].

Genital infections with common STI pathogens including HSV-2, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* have been associated with increased HIV viral load in semen and enhanced HIV transmission in ART-naïve men [14]. Several recent studies have documented the persistence of HIV virions and infected cells in semen from men on stable ART regimens despite undetectable viral loads in peripheral blood [reviewed in [15]], and HIV transmission events have also been reported [16, 17]. Viral persistence in semen may be attributable to HIV compartmentalization in the male genital tract [18], isolated viral replication due to incomplete penetration of antiretroviral drugs [15], and/or activation of resident or infiltrating HIV-infected cells by inflammatory mediators produced locally in the genital tract in response to either symptomatic or asymptomatic infections [19]. MSM on HAART may continue to shed HIV in semen because of continued high risk sex behavior and a high prevalence of STIs and genital inflammation [20-22].

To determine the prevalence of seminal HIV shedding in sexually active HIV-infected MSM on HAART, and risk factors associated with detection of HIV in semen, we recruited participants from a Lesbian/Gay/Bisexual/Transgender (LGBTB) clinic in Boston, MA, that provides ART to a substantial population of HIV-infected MSM at high risk for STIs [22]. We quantified levels of HIV in paired blood and semen samples, and examined the relationship between HIV in semen and an array of clinical, behavioral and biological variables.

Methods

Study Participants

Study participants were recruited from men receiving medical care for HIV infection at Fenway Health in Boston, Massachusetts, USA. Men were eligible for the study if they were HIV-1 infected, on a stable HAART regimen for three months or longer, and were sexually active (i.e., having had sex in the past six months).

This study was approved by the Institutional Review Boards of both Fenway Health and Boston University Medical Campus (BUMC). A research assistant directly asked study participants questions concerning demographics, sexual behavior, HIV therapy, history of STIs and substance use. Participants were asked to report the number of times they engaged in anal, oral or vaginal intercourse without and with condoms in the previous three months. Participants were also asked to report the number of sexual partners with whom they engaged for each behavior, and the HIV serostatus of those partners, if known.

Specimen Collection and Processing

Men were instructed to collect semen by masturbation after a minimum of 24 hours of sexual abstinence. Ten ml of blood were collected at the same visit by venipuncture in test tubes coated with EDTA. All samples were sent to the laboratory at BUMC, and processed within 4 hours of collection.

Semen volume was measured, and sperm and polymorphonuclear granulocytes (PMN) were counted with a microscope [23]. The remaining semen was diluted 1:1 in PBS, and semen cells were pelleted by centrifugation at $600 \times g$ for 10 minutes. Seminal plasma and cell aliquots were stored with and without TRI Reagent/PolyAcryl Carrier at -80°C .

Whole blood was centrifuged ($400 \times g$ for 20 min) and plasma removed. Blood plasma aliquots were stored with and without TRI Reagent/PolyAcryl Carrier at -80°C .

Quantitative RT-PCR and PCR

The details of the HIV PCR assays have been described by us previously {RNA and DNA extraction from semen [24]; quantitative HIV RT-PCR and PCR assays [25]}. For HIV-1 RNA, the lowest quantitative limit was 1 copy/μl RNA (equivalent to 80 copies/ml in seminal or blood plasma, and 80 copies/sample in semen cells). For HIV-1 DNA, the lowest quantitative limit was 1 copy/2 μl DNA (equivalent to 100 copies/sample in semen cells). HSV-2 DNA was quantified as described by us previously [23].

Determination of HSV Serostatus

HSV serostatus was assessed by Focus HerpeSelect 1 & 2 Immunoblot IgG Assay (Focus Diagnostics, Cypress, CA, USA).

Assays of Cytokines and Other Immune Factors in seminal plasma

Proinflammatory cytokines IL-6, IL-8, and TNF- α , were measured by Bio-Plex Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA). Levels of innate immunity markers lysozyme (ALPCO, Salem, NH, USA) and SLPI (R & D Systems, Inc., Minneapolis, MN, USA) were determined by ELISA.

Statistical Analysis

For the initial analyses, differences in continuous variables between two independent samples were determined by the non-parametric Mann-Whitney U test, correlations between two continuous variables were determined by Spearman Rank Correlation Coefficient, and categorical variables were analyzed by Fisher's Exact test or Fisher-Freeman-Halton test. Final models used logistic regression [26] to evaluate associations of independent variables with the dichotomous outcome variable, detection of HIV RNA/DNA in semen. Simple and multiple regression models were constructed to include inflammatory biomarkers that were shown previously by descriptive analysis to be associated with our outcome variables, along with risk group indicators (see below) or self-report of unprotected anal sex. Multiple regression models were constructed by stepwise addition of covariates shown via simple regression to be significantly associated with the outcome. In the final model, covariates significantly associated with the outcome were retained along with behavioral risk factors, and presented with adjusted estimates of relative odds (odds ratios) and associated 95% confidence intervals. Additionally, p-values are presented for each adjusted measure of association. SAS (version 9.1.3), StatView (version 5.0.1) (both from SAS Institute, Cary, NC, USA) and StatXact (version 6.2.0, Cytel Software Corporation, Cambridge, MA, USA) statistical software were utilized to perform the statistical computations.

Results

Cohort Characteristics

A total of 101 men were enrolled in the study. The men were predominately Caucasian (74%) and virtually all MSM (97%) by self report; the median age was 43 years, and the median peripheral blood CD4 count was 513 cells/mm³. Eighty percent had been on HAART for >1 year and 72% had been on their current HAART regimen for >6 mos. Twenty-seven percent of the participants were classified as low-risk for STI acquisition (protected intercourse for past 3 months) and 73% were classified as high-risk for STI acquisition (unprotected intercourse in the past 3 months). Nine men, all belonging to the high-risk group, had STI/urethritis [diagnosed with *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum* or nongonococcal urethritis (NGU) within 7 days prior to entry into the study, or experiencing HSV-2 reactivation as detected by HSV-2 DNA in semen]. Sixty-three percent of the men were seropositive for HSV-2 antibodies (Table 1).

Detection of HIV in Blood and Semen: Association with Clinical Characteristics

Of the 101 men, 18% had HIV RNA in blood [median HIV-RNA copy number/ml (range): 560 (80-6.4 × 10⁵)], and 30% had HIV RNA and/or DNA in semen. Table 1 compares clinical, behavioral and seminal HIV variables of men with detectable HIV in blood plasma (BP-HIV⁺), and those with undetectable HIV in blood (BP-HIV⁻). BP-HIV⁺ men had a higher prevalence of HIV in semen than BP-HIV⁻ men [9/18 (50%) vs. 21/83 (25%), $p=0.049$]. None of the clinical or behavioral characteristics differed between the BP-HIV⁺ and BP-HIV⁻ groups except for medication adherence. Five men in the BP-HIV⁺ group were found to have poor adherence to their HAART regimens, and 2 had clinical indications of virologic HAART failure.

Figure 1 compares concentrations of cell-free (CF) and cell-associated (CA) HIV in semen from BP-HIV⁺ vs. BP-HIV⁻ men. Of the 30 men with HIV in semen, 17 had CF HIV-RNA, 17 had CA HIV-RNA and 5 had CA HIV-DNA. Since both cell-free and cell-associated forms of HIV are potentially infectious [27], all three semen HIV variables were combined into a single variable ("any HIV in semen") for the statistical analyses below.

Univariate Analysis of Variables Associated with Seminal HIV Shedding in BP-HIV⁻ Men

Table 2 presents a univariate analysis of variables associated with HIV in semen from BP-HIV⁻ subjects. The prevalence of HIV in semen was significantly associated with STI risk [5% for the low-risk group vs. 32% for the high-risk group ($p=0.02$), and 75% for the STI/urethritis group ($p=0.003$)]. In addition, detection of HIV in semen was highly associated with LCS ($p=0.0001$) and seminal PMN count ($p=0.003$). The majority (57%) of SE-HIV⁺ men had LCS; the prevalence of seminal HIV in LCS⁺ men was 60% vs. 14% in LCS⁻ men. Detection of HIV in semen was also associated with elevated concentrations of several other seminal inflammatory markers: TNF- α , IL-6, IL-8, and SLPI. Sexual behavior determinants associated with seminal HIV were unprotected insertive anal sex (UIAS), and UIAS with an HIV⁺ partner (UIAS-HIV). Neither UIAS nor UIAS-HIV was significantly associated with seminal PMN count, LCS, or concentrations of any other seminal inflammation markers (data not shown).

Study variables not associated with detection of HIV in semen in BP-HIV⁻ men included duration of antiretroviral treatment and current HAART regimen, use of a protease inhibitor, peripheral blood CD4 counts, and circumcision (Table 2 and data not shown). Detection of HIV in semen was also not associated with HSV-1 or 2 serostatus, but 2 out of three men with HSV-DNA in semen (included in STI group) had HIV detected in semen.

Regression Models

Simple Logistic Regression—The results of simple logistic regression analysis of data from the BP-HIV⁻ cohort indicated that a number of study variables were associated with HIV detection in semen. Specifically, both report of STI/urethritis (OR 11.5, $p<0.006$) and behavioral high risk of acquiring an STI (OR 9.35, $p<0.017$) were associated with HIV detection in semen. In addition, LCS (OR 8.68, $p=0.0003$) and upper quartile concentrations of seminal plasma TNF- α (OR 8.68, $p=0.0003$), IL-6 (OR 5.57, $p<0.004$), and IL-8 (OR 3.42, $p<0.048$) were associated with HIV detection in semen.

UIAS was also associated with HIV detection in semen (OR 3.58; $p<0.03$); the association was considerably stronger when UIAS occurred with an HIV⁺ partner (UIAS-HIV; OR 5.67; $p<0.0025$). Competitive analyses indicated that the association of UIAS-HIV with HIV detection in semen was not due to differential numbers of unprotected anal insertive contacts or partners in the UIAS-HIV subjects (data not shown).

Multiple Regression—Table 3 shows the final multiple regression model. Patients with STI/urethritis were more than 29 times as likely to have HIV in semen (OR: 29.03; 95% CI: 2.60, 523.53) when compared with patients without STI/urethritis. Seminal plasma TNF-levels in the upper quartile remained highly associated with HIV in semen, with a magnitude of association of almost 14-fold when compared with lower concentrations (OR: 13.97; 95% CI: 2.85, 95.02). UIAS-HIV was the strongest behavioral risk factor associated with seminal HIV shedding. UIAS-HIV was associated with a greater than 7-fold risk of having HIV in semen (OR: 7.34; 95% CI: 1.59, 47.73) when compared with patients not reporting UIAS-HIV.

Discussion

Because HIV transmission by semen is a major factor driving the AIDS epidemic, it is important to define variables that determine shedding of infectious HIV into semen. Concomitant STIs, genital inflammation and high peripheral blood viral loads have been associated with seminal HIV shedding in the past, largely in pre-HAART populations [14, 28]. As more men initiate ART and HAART, it is crucial to understand the interplay of therapy, which can suppress blood and genital HIV viral loads, with risk factors that can enhance HIV replication in the genital tract, and the potential for sexual transmission of drug-resistant HIV. Recent evidence indicates that HAART suppresses HIV transmission [7, 10]. The HPTN 052 clinical trial [9] demonstrated that early initiation of ART was associated with a 96% reduction in HIV transmission in HIV discordant heterosexual couples with a low prevalence of STIs. The only study on the effect of HAART on HIV transmission in an MSM population, an observational study conducted in San Francisco over a decade ago, concluded that the transmission rate was decreased by approximately 60% [29].

In our cross-sectional study of sexually active, ART-experienced MSM on a current HAART regimen for >3 mos, 30% of the men had detectable HIV RNA and/or DNA in semen. Eighteen percent of the men had detectable HIV in blood plasma despite being on a HAART regimen, consistent with earlier studies that have shown that substantial numbers (up to 25%) of men will experience virologic failure while on a HAART regimen [30]. These men, as expected, had a significantly higher prevalence as well as increased copy numbers of HIV in semen than did the BP-HIV⁻ men in our study, indicating, as has been shown before [2], that HIV levels in peripheral blood are an important predictor of seminal HIV.

Among the 83 men with undetectable HIV in blood plasma, 25% had HIV in semen. This is a higher prevalence of seminal HIV shedding than has been reported from other large recent studies of BP-HIV⁻ men on HAART, which have reported 2-3% shedding rates [31, 32]. This is likely due to the high prevalence of STIs and genital inflammation in our sexually active MSM cohort; 9.6% of the men were diagnosed with STI or urethritis, and 24% had genital inflammation (leukocytospermia). Our study provides evidence that genital infections and inflammation are common in HIV-infected MSM that engage in unprotected intercourse, and that these factors can promote compartmentalized shedding of HIV in the genital tract of men on suppressive HAART therapy. We also used a highly sensitive detection assay and measured both cell-free and cell-associated HIV. Cell-associated HIV was included because early reports suggested that HIV-infected cells persisted in semen longer than cell-free HIV after initiation of HAART [24, 33], and also because of a recent resurgence in interest in the sexual transmission of cell-associated HIV [34]. CF-RNA and HIV-infected cells were each detected in 13% of BP-HIV⁻ cases, but only 1/21 subjects with seminal HIV was positive for both forms of virus. This provides further evidence that cell-

free and cell-associated HIV in semen may arise from different sources, as has been suggested in previous reports [35].

In our study, seminal HIV viral copy numbers ranged from 80-2,560 (median 200 copies) in men with undetectable HIV in blood. These seminal HIV levels are considerably lower than those commonly detected in ART-naïve men [24, 36, 37], but could represent an infectious inoculum in MSM since rectal intercourse is an especially effective route of HIV transmission due to the thin rectal epithelium [5, 6]. A series of recent studies substantiate this possibility. A study in macaques comparing the relative transmissibility of SHIV across different mucosal surfaces demonstrated that rectal transmission could be achieved with 5-fold less SHIV than needed for vaginal transmission [38]. A theoretical paper [39] and results from a clinical study [8] both conclude that <1,000 copies of HIV RNA in semen pose a low but real risk of male-to-female HIV transmission; a five-fold reduction in this copy number for rectal transmission (<200 copies) is within the range of values detected in our study. Furthermore, Butler et al. [40] reported a median seminal plasma viral load of 4,300 HIV RNA copies/ml for men transmitting HIV to their MSM partners with a minimum and maximum of 110 and 69,000 HIV RNA copies/ml, respectively. Therefore, the data from our study in combination with these recent reports suggest that sexually active HIV-infected MSM on ART that continue to shed low levels of HIV in semen may be infectious to their sexual partners.

One concern about seminal HIV shedding in men on HAART is that the virus can develop antiretroviral resistance mutations [24, 41, 42], which may contribute to the high prevalence of antiretroviral drug-resistant HIV in ART-naïve HIV-infected individuals in the United States (reported to range from 8 to 24% [43-45]). MSM are at higher risk of acquiring drug-resistant HIV than heterosexual men or women [43, 45]. Our data suggest that HIV replication commonly occurs in the genital tract of sexually active MSM under evolutionary pressure from HAART; this could increase the prevalence of drug-resistant HIV in semen and its transmission to seronegative partners. Although early attempts to culture and sequence HIV in semen from men in this study failed due to low viral copy numbers, further studies are warranted to determine whether HIV in semen from MSM on HAART is infectious in vitro and contains drug resistance mutations..

The multivariate analysis also revealed an independent association between seminal HIV and UIAS-HIV. This raises the possibility that seminal HIV in subjects engaging in UIAS with HIV-infected partners may be attributable to urethral superinfection or contamination with HIV from rectal secretions of sex partners. Reports of high numbers of HIV-target cells in the urethra [46], and HIV-infected cells in urethral secretions from HIV-seropositive men with and without urethritis [47] indicate that the urethra may be a primary HIV infection site. Furthermore, higher concentrations of HIV RNA have been reported in rectal mucosa secretions than in blood and seminal plasma among MSM, and may be independent of ART [48]. Given that many HIV⁺ MSM engage in unprotected anal intercourse with other HIV-infected partners as a way to experience pleasure without having to worry about transmitting HIV to others [20], the potential of acquiring HIV from an HIV⁺ partner during unprotected insertive sex, and subsequently transmitting it to an HIV⁻ partner must be considered. This finding needs to be confirmed, since at present “serosorting” (HIV-infected individuals only having unprotected sex with other infected persons), is considered by some to be a reasonable public health strategy to prevent transmission to uninfected partners [49].

In conclusion, we detected a high prevalence of seminal HIV shedding in a cohort of sexually active HIV-infected MSM on HAART. HIV in semen was associated with detection of HIV in blood (due to poor drug adherence and/or virologic HAART failure), and with STIs and genital inflammation in men who were fully suppressed (undetectable

HIV in blood). In light of recent evidence that even low amounts of HIV in semen could pose a transmission risk in MSM, who are more vulnerable to HIV infection than heterosexual men, this information has potential clinical significance for the HIV epidemic in MSM. HIV-infected men who engage in unprotected intercourse may use HAART and viral load status in their sexual decision-making, and being on HAART or having an undetectable blood viral load may relax concerns about transmitting HIV [50]. Therefore, MSM at risk for transmitting HIV may believe that they have a low risk based on incorrect assumptions that HAART eliminates HIV from semen. Until more information on transmission risk in MSM is available, it would be prudent to advise sexually active HIV-infected MSM to use condoms and other risk-reduction strategies throughout all stages of HIV disease regardless of HIV treatment status, and to promote the aggressive diagnosis and treatment of STIs.

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Figure 1.

Cell-free and cell-associated HIV RNA and DNA in semen of men with detectable (BP-HIV⁺) and undetectable (BP-HIV⁻) HIV in blood plasma. Cell-Free HIV is expressed as HIV-1 copies per ml seminal plasma. Cell-associated HIV RNA and DNA are numbers of copies per sample. The dotted line indicates the lower limit of HIV detection, and values under this line represent samples with “undetectable” HIV. BP-HIV⁺ men had significantly higher concentrations of cell-free HIV RNA (mean $\pm$ SE: $4,438 \pm 3,388$ vs. 51 ± 17 , $p < 0.03$), cell-associated HIV RNA ($1,604 \pm 1,006$ vs. 50 ± 31 , $p < 0.005$) and cell-associated HIV DNA (41 ± 26 vs. 5 ± 4 , $p < 0.009$).

Table 1
Comparison of Men with and without Detectable HIV RNA in Blood Plasma (n=101)

Variable	All Subjects (n=101)	BP HIV Positive (n=18)	BP HIV Negative (n=83)	P-value ^a
Age	43 (24-59) ^b	45 (27-55)	43 (24-59)	0.43
Race				
White	75/101 (74%)	14/18 (78%)	61/83 (74%)	0.80
Black	19/101 (19%)	4/18 (22%)	15/83 (18%)	
Native American	1/101 (1%)	0/18 (0%)	1/83 (1%)	
Asian/Pacific Islander	1/101 (1%)	0/18 (0%)	1/83 (1%)	
Other	5/101 (5%)	0/18 (0%)	5/83 (6%)	
Duration of ART				
>1 Year	81/101 (80%)	15/18 (83%)	66/83 (80%)	>0.99
Duration of Current HAART Regimen				
>3 Months	101/101 (100%)	18/18 (100%)	83/83 (100%)	>0.99
>6 Months	73/101 (72%)	11/18 (61%)	62/83 (75%)	0.26
>12 Months	50/101 (50%)	11/18 (61%)	39/83 (47%)	0.31
Peripheral CD4 Count (cells/mm³)	513 (108-1,604) ^b	394 (125-921)	524 (108-1,604)	0.08
HIV Detected in Semen Total^c	30/101 (30%)	9/18 (50%)	21/83 (25%)	0.049
Cell-Free RNA	17/101 (17%)	6/18 (33%)	11/83 (13%)	0.07
Cell-Associated RNA	17/101 (17%)	7/18 (39%)	10/83 (12%)	0.01
Cell-Associated DNA	5/100 (5%)	3/17 (18%)	2/83 (2%)	0.03
Sexual Orientation				
Homosexual	90/101 (89%)	16/18 (89%)	74/83 (89%)	0.64
Bisexual	8/101 (8%)	1/18 (6%)	7/83 (8%)	
Heterosexual	3/101 (3%)	1/18 (6%)	2/83 (2%)	
Circumcision	84/101 (83%)	14/18 (78%)	70/83 (84%)	0.50
STI Risk Group				
Low Risk Group	27/101 (27%)	6/18 (33%)	21/83 (25%)	0.56
High Risk Group ^d	74/101 (73%)	12/18 (67%)	62/83 (75%)	
STI Status				
Current STI/Urethritis	9/101 (9%)	1/18 (6%)	8/83 (10%)	>0.99
HSV-1 Seropositive	72/101 (71%)	15/18 (83)	57/83 (69%)	0.26
HSV-2 Seropositive	64/101 (63%)	11/18 (61%)	53/83 (64%)	>0.99

^aBP HIV Positive vs. BP HIV Negative; significant p-values in bold type

^bMedian (Range)

^cCell-Free and/or Cell-Associated HIV RNA and/or HIV DNA

^dUnprotected receptive and/or insertive intercourse within six months prior to study entry

Table 2
Univariate Analysis of Variables Associated with HIV in Semen of Men with Undetectable HIV in Blood Plasma (n=83)

Risk Factor	HIV in Semen ^a		P-value ^b
	Positive (n=21)	Negative (n=62)	
Duration of ART			
>1 Year (n=66)	17/21 (81%)	49/62 (79%)	0.74
Duration of Current HAART Regimen			
>3 Months (n=83)	21/21 (100%)	62/62 (100%)	>0.99
>6 Months (n=62)	17/21 (81%)	45/62 (73%)	0.57
>12 Months (n=39)	9/21 (43%)	30/62 (48%)	0.80
Peripheral CD4 Count (cells/mm³)			
200 (n=7)	2/21 (10%)	5/62 (8%)	0.99
>200 (n=76)	19/21 (91%)	57/62 (92%)	
STI Risk Group			
Low Risk Group (n=21)	1/21 (5%)	20/62 (32%)	0.02
High Risk Group ^c (n=62)	20/21 (95%)	42/62 (68%)	
High Risk Sexual Behavior			
UIAS ^d (n=36)	14/21 (67%)	22/62 (36%)	0.02
UIAS-HIV ^e (n=28)	13/20 ³ (65%)	15/62 (24%)	0.002
STI Status			
Current STI/Urethritis (n=8)	6/21 (29%)	2/62 (3%)	0.003
HSV-1 Seropositive (n=57)	14/21 (67%)	43/62 (69%)	0.79
HSV-2 Seropositive (n=53)	11/21 (52%)	42/62 (68%)	0.29
Genital Inflammation			
Leukocytospermia			
Yes (n=20)	12/21 (57%)	8/62 (13%)	0.0001
No (n=63)	9/21 (43%)	54/62 (87%)	
PMN (#/ml × 10 ⁶)	1.12 (ND-12) ^f	0.04 (ND-6.8)	0.003
TNF- (pg/ml)	8 (2-77)	6 (ND-78)	<0.0001
IL-6 (pg/ml)	105 (11-2,773)	23 (3-1,400)	<0.0001
IL-8 (pg/ml)	1,152 (134-41,482)	546 (42-4,270)	0.005
Lysozyme (ng/ml)	157 (59-242)	180 (50-413)	0.21
SLPI (ng/ml)	10.5 (5-279)	5.8 (4-107)	0.009

ND: Not detectable

^aCell-Free and/or Cell-Associated HIV RNA and/or HIV DNA

^bSignificant p-values in bold type

^cUnprotected receptive and/or insertive intercourse within three months prior to study entry or ongoing STI

^dUnprotected insertive anal sex with HIV⁻ or HIV⁺ person within three months of sample

^eUnprotected insertive anal sex with HIV⁺ person within three months of sample

^fMedian (Range)

Table 3
Final Multivariate Analysis Model of Risk Factors Associated with Detection of HIV in Semen from HIV-infected MSM on HAART with Undetectable HIV VL in Blood

Parameter	Unadjusted OR (95% Confidence Interval)	Unadjusted P-value	Adjusted OR (95% Confidence Interval)	Adjusted P-value
High TNF- levels in seminal plasma				
Yes (levels in upper quartile)	8.68 (2.50, 32.81)	0.0003	13.97 (2.85, 95.02)	0.0003
No (levels in lower three quartiles)	1.0 (referent)		1.0 (referent)	
STI Status				
Patient with STI/urethritis	11.52 (1.83, 127.80)	0.006	29.03 (2.60, 523.53)	0.003
Patient without STI/urethritis	1.0 (referent)		1.0 (referent)	
Unprotected Insertive Anal Sex with HIV⁺Person				
Yes	5.67 (1.74, 20.24)	0.003	7.34 (1.59, 47.73)	0.007
No	1.0 (referent)		1.0 (referent)	