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article

Asymptomatic urethritis and detection of HIV-1 RNA in seminal plasma

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Objective: To define risk factors for detection of HIV-1 RNA in semen in men attending the two largest HIV clinics in the West Midlands.

Methods: 94 HIV-1 seropositive men at any stage of infection donated matched semen and blood samples. 36 subjects (38%) were on no antiretroviral treatment, 12 (13%) were on dual therapy, and 46 (49%) were on three or more drugs. Median CD4 count was 291 cells $\times 10^6/l$. 87 subjects underwent a urethritis screen (Gram stained urethral smear and culture for gonococcus, and LCR for *Chlamydia trachomatis* on first pass urine). Quantitative cell free HIV-1 RNA was determined by commercial nucleic acid sequence based assay with a lower detection limit of 800 copies/ml for semen and 400 copies/ml for blood. Independent risk factors for seminal HIV RNA detection were defined by logistic regression.

Results: In univariate analysis, subjects not taking antiretrovirals were 11 times more likely to shed HIV RNA (21/36 (58%) *v* 6/58 (10%); $p < 0.0001$). Seven subjects (8%) had urethritis (including one *C trachomatis* infection). Urethritis was significantly associated with detection of seminal HIV RNA (adjusted OR, 80.2; $p = 0.006$), as was blood plasma viral load (adj OR, 19.3 per factor 10 increase; $p < 0.001$) and age (adj OR, 1.16 per 1 year older; $p = 0.001$). Antiviral treatment status, absolute CD4 and CD8 count, clinical stage, treatment centre, ethnicity, and risk factor were not independent predictors. No subject with undetectable blood viral load had detectable seminal HIV RNA.

Conclusion: Asymptomatic urethritis is independently associated with seminal HIV RNA shedding.

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Keywords: semen; nucleic acid sequence based amplification; HIV transmission

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Introduction

Sexual transmission of HIV by males is determined in part by the amount of infectious HIV in the semen. HIV-1 RNA can be detected in the seminal plasma of the majority of HIV infected men and in general correlates with infectious HIV determined by cell culture^{1,2} and with the amount of HIV RNA in blood plasma.^{2–4} Antiretroviral treatment reduces seminal HIV RNA^{3–5} and seminal HIV proviral DNA⁶ in most patients, but a significant minority continue to shed HIV RNA. Symptomatic genital tract inflammation greatly increases seminal viral load,^{7–9} but the possible role of asymptomatic urethritis in increasing seminal HIV RNA levels has not been explored. We investigated this by measuring the amount of HIV RNA in the semen of HIV seropositive men who were free of genital tract symptoms and agreed to be tested for urethritis.

Methods

STUDY POPULATION, URETHRITIS SCREENING,
SEMEN, AND BLOOD SAMPLES

Seropositive men attending one of two teaching hospital HIV treatment centres in Birmingham, were eligible to enter the study if they were free of genital tract symptoms and willing to provide a semen sample. Subjects were screened for non-specific urethritis, gonorrhoea, and *Chlamydia trachomatis* as follows. A urethral smear for Gram stain was obtained by inserting a plastic loop 2 cm into the distal ure-

thra. "Urethritis" was defined as the presence of more than five pus cells per high power field. Gonococcal isolation was attempted by direct inoculation of urethral material onto selective medium, and the presence of *C trachomatis* plasmid DNA was investigated by ligase chain reaction (LCR) on first pass urine. Semen samples were provided by masturbation into a dry sterile container. A matched blood sample was obtained within 4 hours. Absolute CD4 and CD8 lymphocyte counts were determined by routine flow cytometry on a sample taken within 1 month of semen sampling. Clinical stage was assigned according to the 1993 Centers for Disease Control and Prevention (CDC) criteria.¹⁰ All participants gave fully informed consent and the study was approved by the local research ethics committee.

SAMPLE PROCESSING, QUANTITATION OF HIV RNA
Samples of blood and liquified semen were separated by centrifugation (3000 rpm, 10 minutes) within 6 hours. Aliquots of plasma were stored at -70°C and analysed within 12 months. Quantitative HIV RNA in seminal and blood cell free plasma was determined with a commercial nucleic acid sequence based assay (NASBA; NucliSens HIV-1 QT; Organon Teknika). Samples were processed according to the manufacturer's instructions, except that an additional microcentrifugation step (13 000 rpm, 20 minutes) was required to pellet all seminal cells, and, to reduce inhibition, only 100 μl of seminal plasma was used. The lower

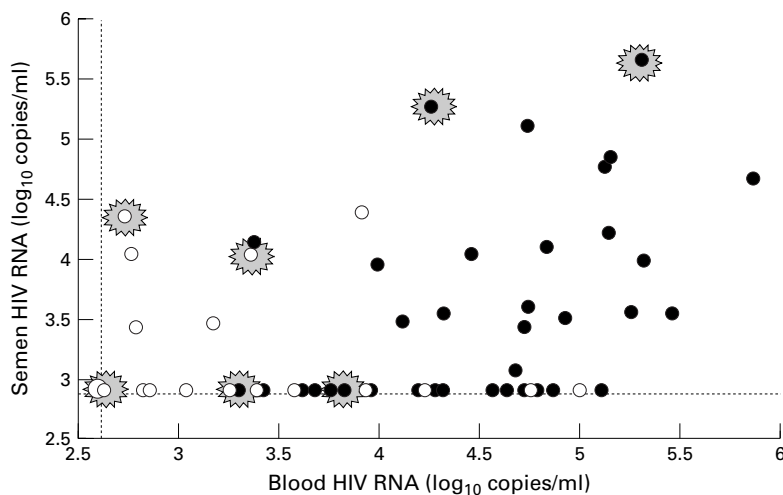


Figure 1 Scatter plot showing relation between seminal and blood plasma viral load in 94 male subjects. Cut off for detection of plasma HIV RNA by NASBA assay (marked by dotted lines) was 800 copies/ml for semen and 400 copies/ml for blood. Those with both values at the lower limit ($n=42$) are shown as a single large circle; one of these had urethritis. Open symbol treated (on dual ($n=12$) or triple or greater ($n=46$) antiretroviral therapy). Solid symbol untreated (on no antiretroviral therapy). Large grey symbol indicates asymptomatic urethritis detected on urethral smear or *C trachomatis* DNA detected in first pass urine by LCR.

limit of detection was 800 copies/ml for semen and 400 copies/ml for blood. Values below the lower limit of detection were set to 799 or 399 copies/ml respectively.

STATISTICAL ANALYSIS

Data were analysed with SPSS 7.5 and (for logistic regression) GLIM4.¹¹ Correlation of quantitative HIV RNA values in blood and semen was examined with Spearman's rank correlation test. Subjects were classified as having either detectable (>799 copies/ml) or undetectable seminal HIV RNA and as taking or not taking antiretroviral therapy. Differences between proportions were compared with the χ^2 test or Fisher's exact test and univariate odds ratios and asymmetric 95% confidence intervals constructed. To determine independent risk factors for seminal HIV RNA detection we constructed a logistic regression model which included the following: presence of urethritis, log blood plasma viral load, antiviral treatment status (naive, previously treated, dual, triple, or quadruple therapy), clinical stage (asymptomatic, symptomatic, or AIDS), absolute CD4 and CD8 cell counts (stratified into quartiles), treatment centre, age, risk factor for acquiring HIV, and ethnicity.

Results

DEMOGRAPHICS

Paired semen and blood samples were obtained from 94 men with a mean age of 36.2 (SD 9.6) years. Fifty eight subjects (60%) were taking

antiretroviral therapy of whom 12 were on dual nucleoside therapy alone and the remainder were on regimens which included HIV protease inhibitors or non-nucleoside reverse transcriptase inhibitors. Median CD4 count was 291 cells $\times 10^6/l$ (range 10–935) and 24 subjects (26%) were asymptomatic. The study groups in each centre were similar in terms of age, CDC stage, risk factor for HIV infection, median blood viral load, and treatment history. Eighty seven men (93%) agreed to the urethritis screen.

ASYMPTOMATIC URETHRITIS

Seven of 87 subjects (8%) had asymptomatic urethritis including one with urethral chlamydial infection. Subjects with urethritis were younger (mean age 29.3 years *v* 36.9 years; 95% CI for difference 2.9–12.4 years; $p=0.005$ by Student's *t* test) but did not differ by CD4 count, clinical stage, or duration of HIV. Among the 53 subjects on antiretroviral treatment tested for urethritis, those with urethritis were over eight times more likely to shed HIV RNA than those without (2/3 with *v* 4/50 without; RR 8.3, $p=0.03$ by Fisher's exact test).

CORRELATION BETWEEN SEMEN AND BLOOD HIV RNA AND ANTIRETROVIRAL TREATMENT

Overall, there was a significant correlation between blood and seminal plasma HIV viral load ($n=94$; Spearman's σ 0.601; $p<0.001$) (fig 1). In no case did we find seminal HIV RNA when blood plasma HIV RNA was undetectable. Antiretroviral therapy significantly reduced the likelihood of detecting seminal HIV RNA (see table 1). Of the six subjects taking antiretrovirals who had detectable seminal HIV RNA all had detectable blood HIV RNA and two had urethritis. Median seminal HIV RNA in these subjects was 11 000 copies/ml (range 2700–25 000), compared with a blood viral load of 1060 copies/ml (range 540–8100). All but one had initiated or changed therapy within the previous 6 weeks.

LOGISTIC REGRESSION ANALYSIS

Eighty seven subjects with complete data were included in the logistic regression analysis which allowed for urethritis, blood plasma viral load, antiviral treatment status, clinical stage, absolute CD4 and CD8 cell counts, treatment centre, age, risk factor for acquiring HIV, and ethnicity. Seminal HIV RNA detection was strongly associated with urethritis (odds ratio 80.2; 95% confidence interval 2.2–2097; $p=0.008$), blood plasma viral load (odds ratio 19.3 per factor of 10 increase; 95% CI,

Table 1 Detection of semen and blood plasma HIV RNA according to antiretroviral treatment status

	Antiretroviral treatment ($n=58$)*	No antiretroviral treatment ($n=36$)	
Median blood plasma viral load (\log_{10} copies/ml; median (range))	2.6 (2.6 to 5.0)	4.7 (3.3 to 5.6)	$p<0.001$ †
Median semen plasma viral load (\log_{10} copies/ml; median (range))	2.9 (2.9 to 4.4)	3.5 (2.9 to 5.7)	$p<0.001$ †
Proportion of subjects with detectable seminal plasma HIV RNA	6/58 (10%)	21/36 (58%)	Odds ratio 11.2 (95% CI 3.7 to 41.5; $p<0.0001$ ‡)

Cut off for detection of plasma HIV RNA by NASBA assay was 800 copies/ml for semen and 400 copies/ml for blood.

*Number of drugs: dual therapy ($n=12$), triple therapy or greater ($n=46$).

†by Mann-Whitney U test.

‡by Fisher's exact test.

2.6–144; $p < 0.001$) and age (odds ratio 1.16 per 1 year older; 95% CI 1.05–1.28; $p = 0.001$). The remaining factors were not independently associated with seminal HIV RNA detection.

Discussion

In this group of 94 HIV seropositive men independent risk factors for detection of seminal HIV RNA were the presence of asymptomatic urethritis, an increase in blood viral load and older age. Eight per cent of subjects tested had unsuspected asymptomatic urethritis, including one with *C trachomatis* infection. Symptomatic genital tract inflammation increases seminal viral load,^{7–9} but sufferers are likely to seek treatment and reduce sexual activity. Asymptomatic infections which remain undetected will blunt any impact of antiretroviral treatment in reducing sexual transmission of HIV. Not only will they increase seminal HIV RNA shedding, but patients who acquire sexual infections are the very ones most likely to be engaging in less safe sex, and are therefore more likely to transmit HIV.

In parallel with other reports, we found that subjects whose blood HIV viral load responded well to antiretroviral therapy also exhibited good suppression of HIV in the seminal compartment.^{3–5} At particular risk of seminal HIV RNA shedding were subjects who had recently changed therapy because of antiretroviral failure. These subjects had comparatively high seminal HIV RNA loads compared with blood. This has major implications for transmission of drug resistant HIV, given that HIV resistance can develop independently in the seminal fluid compartment.¹² Nucleic acid sequencing of the reverse transcriptase and protease genes from seminal plasma HIV RNA in these subjects is in progress. In contrast with other workers we found no independent effect of clinical stage,^{2,13} CD4 count,² or CD8 count¹⁴ on detection of seminal HIV.

There are a number of difficulties in interpreting such cross sectional studies of seminal HIV RNA. Firstly, a proportion of men shed seminal HIV RNA intermittently.^{15–16} In spite of this we demonstrated a clear correlation between seminal HIV RNA and blood plasma RNA. It is possible that some of our subjects who had undetectable seminal HIV RNA could have shed cell free HIV at another time. Secondly, although our assay detection limit was 800 copies/ml, it is possible that sexual transmission of HIV could occur at even lower levels of seminal HIV RNA. Thirdly, HIV-1 proviral DNA can be found in semen associated with sperm¹⁷ or mononuclear cells,¹⁸ and this may contribute to transmissibility. We would emphasise that patients taking antiretroviral therapy who have undetectable viral loads should continue to assume that they could transmit HIV infection. We conclude that enhanced awareness of and routine testing for sexually transmitted infections within HIV care settings is essential.

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Contributors: ST and AJW designed the study with assistance from JDCR, DW, and DP, and recruited patients; AJW analysed data and wrote the initial manuscript draft; DW and JDCR oversaw the study in each centre; JW developed techniques for semen analysis with help from DP; AVS undertook the multivariate analysis and modelling. All authors commented on the final manuscript.

- Dyer JR, Gilliam BL, Eron JJ Jr, *et al.* Quantitation of human immunodeficiency virus type 1 RNA in cell free seminal plasma: comparison of NASBA with Amplicor reverse transcription-PCR amplification and correlation with quantitative culture. *J Virol Methods* 1996;**60**:161–70.
- Vernazza PL, Gilliam BL, Dyer J, *et al.* Quantification of HIV in semen: correlation with antiviral treatment and immune status. *AIDS* 1997;**11**:987–93.
- Gupta P, Mellors J, Kingsley L, *et al.* High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. *J Virol* 1997;**71**:6271–5.
- Vernazza PL, Gilliam BL, Flepp M, *et al.* Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS* 1997;**11**:1249–54.
- Gilliam BL, Dyer JR, Fiscus SA, *et al.* Effects of reverse transcriptase inhibitor therapy on the HIV-1 viral burden in semen. *J Acquir Immun Defic Syndr Hum Retroviral* 1997;**15**:54–60.
- Boswell SL, Mayer KH, Goldstein RS, *et al.* The effect of HIV protease inhibitors on seminal proviral DNA. In: *Proceedings of the Fourth Conference on Retroviruses and Opportunistic Infections, Washington, DC, 1997*:102 (Abstract 426).
- Cohen MS, Hoffman IF, Royce RA, *et al.* Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet* 1997;**349**:1868–73.
- Atkins MC, Carlin EM, Emery VC, *et al.* Fluctuations of HIV load in semen of HIV positive patients with newly acquired sexually transmitted diseases. *BMJ* 1996;**313**:341–2.
- Dyer J, Eron JJ, Hoffman IF, *et al.* Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1 infected men in Malawi. *J Infect Dis* 1998;**177**:224–7.
- Centers for Disease Control. 1993 Revised classification system for HIV infection and expanded case definition for AIDS among adolescents and adults. *MMWR* 1992;**41** (RR-17):1–19.
- Francis B, Green M, Payne C. *GLIM4. The statistical system for generalized linear interactive modelling*. Oxford: Clarendon Press, 1993.
- Eron JJ, Vernazza PL, Johnston DM, *et al.* Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. *AIDS* 1998;**12**:F181–9.
- Anderson DJ, O'Brien TR, Politch JA, *et al.* Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen. *JAMA* 1992;**267**:2769–74.
- Krieger JN, Coombs RW, Collier AC, *et al.* Seminal shedding of human immunodeficiency virus type 1 and human cytomegalovirus: evidence for different immunologic controls. *J Infect Dis* 1995;**171**:1018–22.
- Krieger JN, Coombs RW, Collier AC, *et al.* Intermittent shedding of human immunodeficiency virus in semen: implications for sexual transmission. *J Urol* 1995;**154**:1035–40.
- Gupta P, Patterson BK, Rinaldo CR, *et al.* Seminal cell-associated viral transcriptional activity and the level of seminal chemokines in the shedding of HIV-1 in semen. In: *Proceedings of the 12th World AIDS conference, 1998*, abstract 23399.
- Bagasra O, Farzadegan H, Seshamma T, *et al.* Detection of HIV-1 proviral DNA in sperm from HIV-1-infected men. *AIDS* 1994;**8**:1669–74.
- Quayle AJ, Xu C, Mayer KH, *et al.* T lymphocytes and macrophages but not motile spermatozoa are a significant source of human immunodeficiency virus in semen. *J Infect Dis* 1997;**176**:960–8.