

## Effect of Antiretroviral Therapy on HIV Shedding in Semen

Paulo F. Barroso, MD, PhD; Mauro Schechter, MD, PhD; Phalguni Gupta, PhD; Maria F. Melo, BS; Marcello Vieira, MD; Fernanda C. Murta, RN; Yeda Souza; and Lee H. Harrison, MD

**Background:** The effect of antiretroviral therapy on seminal HIV shedding in the community remains unknown.

**Objective:** To evaluate the effect of antiretroviral therapy on HIV shedding in semen.

**Design:** Prospective cohort study.

**Setting:** University hospital in Rio de Janeiro, Brazil.

**Patients:** 93 HIV-infected men.

**Intervention:** Antiretroviral therapy as prescribed by each patient's physician.

**Measurement:** HIV RNA in semen and blood plasma before and after introduction of therapy.

**Results:** At baseline, HIV RNA was detected in 69 semen samples (74%) and 89 blood samples (96%). Six months after introduction of therapy, HIV RNA was detected in 29 semen samples (33%) and 33 blood samples (38%). The mean reduction in levels of HIV RNA in semen at 6 months was 1.65 log<sub>10</sub> units.

**Conclusions:** Antiretroviral therapy reduces shedding of HIV in semen, which probably in turn reduces HIV transmissibility. However, a substantial proportion of patients may still be infectious and may have drug-resistant strains of the virus.

*Ann Intern Med.* 2000;133:280-284.

For author affiliations, current addresses, and contributions, see end of text.

Sexual activity is the most common method of HIV transmission (1). Strong evidence supports a correlation between blood plasma HIV viral load and transmissibility (2). In addition, small short-term studies have demonstrated that antiretroviral therapy reduces levels of HIV RNA in semen and may thereby reduce HIV transmission (3–5). Recent improvements in antiretroviral regimens have been shown to have dramatic effects on HIV-related morbidity and mortality (6).

However, these advances have led to the existence of a large pool of potentially infectious HIV-seropositive persons. The effect of antiretroviral therapy on HIV shedding in semen is known only in a clinical trial setting; however, it is of major importance to public health, particularly if a substantial proportion of patients continues to shed potentially drug-resistant HIV in semen despite receiving therapy. We evaluated the effectiveness of antiretroviral therapy in a community setting.

### Methods

#### Study Design

The study was conducted from November 1996 through May 1998 at the Hospital Universitário Clementino Fraga Filho (HUCFF), a large teaching hospital of the Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. The institutional review boards of the HUCFF, Johns Hopkins University (Baltimore, Maryland), and the

University of Pittsburgh (Pittsburgh, Pennsylvania) approved the study. Ninety-three HIV-infected men were recruited when they decided to start antiretroviral therapy. The decision to start, change, or stop therapy was made by study patients and their physicians according to the guidelines of the Brazilian Ministry of Health, without input from the study investigators. All patients provided informed consent.

The first (baseline) visit occurred before the introduction of antiretroviral therapy. Follow-up visits were scheduled for 1, 2, 3, and 6 months after initiation of therapy. A detailed interview and a physical examination were performed at each visit. Patients with genital ulcerations or urethral discharge were excluded from the analysis. Semen was collected by masturbation at the study site after at least 48 hours of sexual abstinence, was processed within 30 minutes of collection, and was frozen at  $-70^{\circ}\text{C}$  after liquefaction.

#### Laboratory Methods

We used the NucliSens assay (Organon Teknika, Durham, North Carolina) to measure HIV viral load in whole semen and blood plasma after one freeze–thaw cycle (4). We chose the NucliSens assay because it is less sensitive to inhibition by unknown factors in semen (7). Patients were tested for syphilis with the VDRL test; a positive result was confirmed with a fluorescent treponemal

antibody absorption test (FTA-ABS). A commercial polymerase chain reaction kit was used to detect *Chlamydia trachomatis* infection in urine. We determined CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte counts by using the Becton Dickinson FACScan procedure (Becton Dickinson, San Jose, California).

### Statistical Analysis

In the main analysis, we determined the reduction in HIV viral load from the pretreatment visit to each of the four follow-up visits. Because changes in log-transformed HIV viral load in blood and semen seemed to be normally distributed, 95% CIs were calculated to assess the differences between pre- and post-treatment values. If HIV viral load values were below the detection level (400 copies/mL), they were assigned a value of 282.84 (400 divided by the square root of 2). Spearman rank correlation coefficients were calculated to determine the correlation between HIV viral load in blood plasma and whole semen.

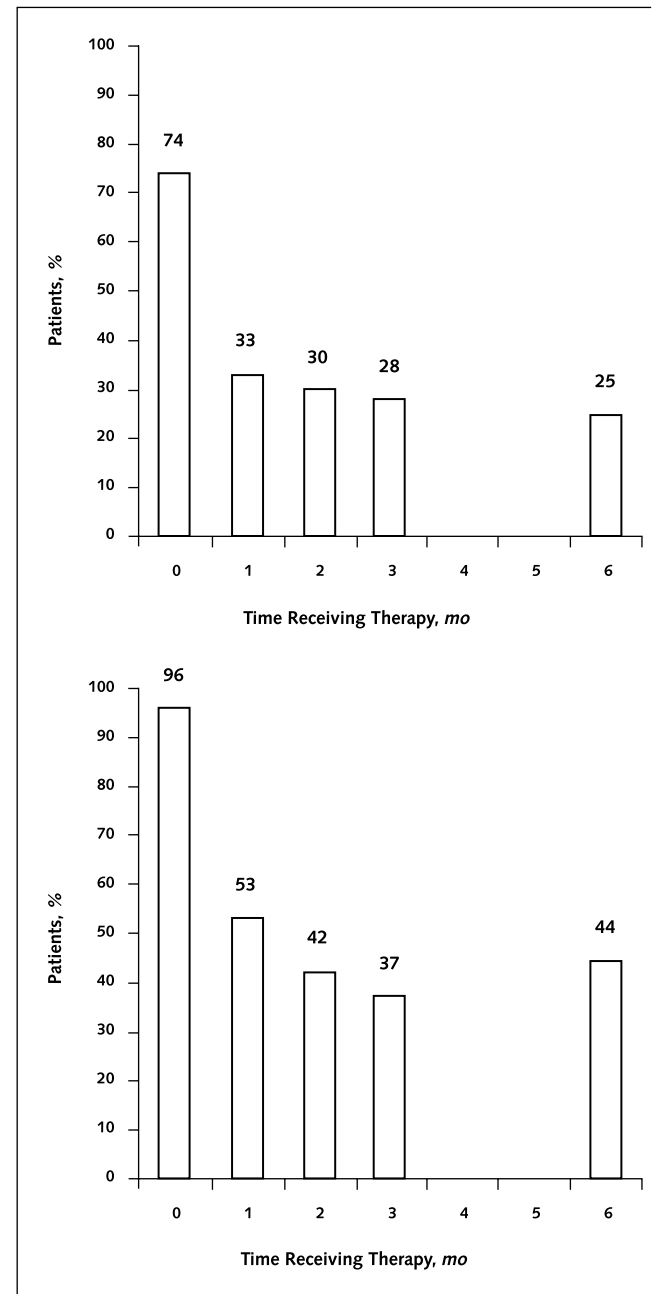
### Results

At the 6-month visit, 88 of 93 patients (95%) had a blood sample available and 85 (91%) provided a semen sample. The median age was 33.8 years. Most patients (95%) had a sexual risk factor for HIV infection. Thirty-five patients (38%) had a CD4<sup>+</sup> count less than 200 cells/mm<sup>3</sup>, 29 (31%) had a CD4<sup>+</sup> count of 200 to 350 cells/mm<sup>3</sup>, and 27 (29%) had a CD4<sup>+</sup> count greater than 350 cells/mm<sup>3</sup>. Ninety-one patients were antiretroviral-naïve, and 2 had a history of nucleoside monotherapy. Eighty patients (86%) began double nucleoside combination therapy, and 13 (14%) began a triple-drug regimen that contained a protease inhibitor. Seventy-two patients (77%) started with zidovudine and didanosine. At the last follow-up visit (6 months), 19 patients (22%) were receiving a triple-drug combination regimen. Baseline values for viral load in semen and blood plasma did not significantly differ by choice of type of therapy. All urine specimens were negative for *C. trachomatis*, and one was positive for *Trichomonas vaginalis*. Serum VDRL and FTA-ABS results were positive in 14 patients. None of these patients reported a history compatible with syphilis in the month before sample collection.

### Baseline Measurements

The median baseline viral load was 47 000 copies/mL in plasma and 7800 copies/mL in semen. Viral load was undetectable in 4 blood plasma samples (4%) and 24 se-

**Figure.** Patients with detectable HIV viral load (>400 copies/mL) in semen (top) and blood plasma (bottom) before and after introduction of antiretroviral therapy.



men samples (26%). At baseline, HIV viral load in semen was positively correlated with HIV viral load in plasma ( $\rho = 0.44$  [95% CI, 0.26 to 0.59]) and negatively correlated with CD4<sup>+</sup> cell count ( $\rho = -0.31$  [CI, -0.11 to -0.48]).

**Table. Mean Reduction in HIV Viral Load in 69 Patients with Detectable Baseline Seminal HIV Viral Load according to Type of Combination Therapy\***

Time Point and Therapy	HIV Viral Load in Semen		HIV Viral Load in Blood Plasma	
	Patients	Mean Reduction (95% CI)	Patients	Mean Reduction (95% CI)
	<i>n</i>	<i>log</i> <sub>10</sub> units	<i>n</i>	<i>log</i> <sub>10</sub> units
1 month				
Any	68	1.41 (1.17–1.64)	68	1.54 (1.31–1.77)
Double	59	1.42 (1.17–1.68)	59	1.45 (1.21–1.70)
Triple	9	1.31 (0.60–2.01)	9	2.12 (1.59–2.64)
2 months				
Any	65	1.53 (1.25–1.80)	65	1.63 (1.40–1.87)
Double	57	1.54 (1.24–1.85)	57	1.55 (1.30–1.79)
Triple	8	1.42 (0.83–2.00)	8	2.27 (1.51–3.02)
3 months				
Any	65	1.51 (1.20–1.81)	65	1.65 (1.38–1.91)
Double	51	1.44 (1.10–1.79)	51	1.46 (1.16–1.75)
Triple	14	1.76 (1.03–2.49)	14	2.34 (1.82–2.84)
6 months				
Any	64	1.65 (1.33–1.96)	66	1.52 (1.25–1.80)
Double	48	1.49 (1.12–1.87)	50	1.30 (1.01–1.59)
Triple	16	2.12 (1.54–2.70)	16	2.21 (1.62–2.81)

\* For each type of therapy, none of the differences between semen and blood plasma values were statistically significant.

### Effect of Therapy on HIV Shedding in Semen

Antiretroviral therapy was associated with a 66% reduction in the proportion of patients who had detectable HIV RNA in semen from baseline to the 6-month follow-up visit (Figure). At baseline, HIV RNA was detected in 69 (74%) semen samples and 89 (96%) blood samples. Six months after introduction of therapy, HIV RNA was detected in 29 (33%) semen samples and 33 (38%) blood samples. As early as 1 month after the start of therapy, a reduction of approximately 1.41  $\log_{10}$  units was observed in patients who had detectable HIV RNA in semen at baseline (Table). The viral burden was reduced in patients receiving a triple-drug regimen that contained a protease inhibitor as well as in those receiving a double nucleoside combination regimen. Of patients who had detectable HIV RNA in semen at baseline, 18 of 48 receiving dual therapy (38%) and 2 of 16 receiving a triple-drug, protease inhibitor-containing regimen (13%) had detectable HIV RNA in semen after 6 months of therapy (data not shown).

### Discussion

The main finding of our study is that antiretroviral therapy substantially reduces the concentration of HIV RNA in semen. Seventy-four percent of our patients had

detectable HIV RNA in semen at baseline compared with 25% at the last follow-up visit, a reduction of 66%. All but 2 of our patients were antiretroviral-naive, which may explain the profound effect of therapy on HIV shedding in semen. In addition, the clinically significant proportion of patients who were prescribed antiretroviral therapy but continued to shed HIV may have resulted in part from nonadherence to therapy.

Of interest, a statistically significant reduction in seminal HIV viral load was seen at all time points in patients receiving a double nucleoside regimen. Although protease inhibitor-containing regimens are usually recommended for initial therapy for HIV infection (8), it has been suggested that initial therapy with a double nucleoside regimen could be an option for patients who have an intermediate risk for HIV progression (9). Our finding that double nucleoside combination therapy has a positive effect on HIV shedding in semen suggests that this therapy may also be able to reduce the spread of HIV infection through sexual transmission.

Our findings have important consequences for the dynamics of the AIDS epidemic. Evidence suggests that reduced HIV viral load in semen is associated with diminished sexual transmissibility of HIV. Previous studies have shown a correlation between blood plasma HIV viral load and heterosexual transmission (2). Furthermore, a high viral load in sexual secretions is positively correlated with risk for sexual transmission of the simian immunodeficiency virus (10).

The longitudinal design is a strength of our study. The inconsistent results of earlier studies on this topic may be explained by cross-sectional design or by laboratory methods (11–13). Another strength of our study design is that it was meant to simulate clinical practice. Because we had no control over the decisions to start or change antiretroviral therapy, our results are probably more representative of the true effectiveness of therapy in the community setting. Our results are generally consistent with those of the few small longitudinal studies that were nested in clinical trials (3–5). The results obtained from clinical trials may not be generalizable because these trials tend to select for adherent patients and may therefore overestimate the effect of therapy (14, 15). However, because we do not know the proportion of persons informed about the study who presented for enrollment, we could also have selected for persons who were more adherent than the general population. There-

fore, our results could overestimate the impact of antiretroviral therapy on HIV shedding in semen.

The main limitation of our study is that the correlation between HIV viral load in semen and sexual infectiousness of HIV has not been established. Replication-competent HIV has been recovered from the semen of HIV-infected men who were receiving antiretroviral therapy and had undetectable HIV viral load in blood plasma and semen (16). In addition, increased high-risk behavior (17) may reduce the effect of antiretroviral therapy on HIV transmission (18). However, strong evidence supports a positive correlation between HIV viral load and risk for transmission (2). From a public health perspective, reduced HIV viral load in semen will probably lead to reduced sexual infectiousness and, consequently, reduced sexual transmission.

Despite these generally positive findings, it was disturbing that HIV RNA was detected in the semen of a substantial proportion of patients who had completed 6 months of therapy, including 13% of those receiving triple-drug therapy. We cannot confirm that this detectable virus was biologically competent. However, the widespread use of antiretroviral therapy may contribute to the selection and transmission of drug-resistant virus and may consequently attenuate any beneficial effects of such therapy on public health (19).

From Hospital Universitário Clementino Fraga Filho, School of Medicine, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland; and University of Pittsburgh, Pittsburgh, Pennsylvania.

**Acknowledgments:** The authors thank Ming Ding for assistance with viral load testing, Ednaura Santos and Elisangela Gonçalves for administrative support, and Clarisse Bressam and Antonieta Bomfim for assistance with data collection. They also thank the patients in the study; Lawrence Moulton for comments and suggestions; and Elizabeth Holt for data management support.

**Grant Support:** In part by a grant/fellowship from Fogarty International Center/USNIH (2D43TW00010) and by the National Program of AIDS/STD-Brazilian Ministry of Health (TC 271/97).

**Requests for Single Reprints:** Paulo F. Barroso, MD, PhD, Laboratório de Pesquisas, Serviço de Doenças Infecciosas e Parasitárias, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Avenida Brigadeiro Trompowski s/n, Cidade Universitária, Ilha do Fundão, CEP 21941-950 Rio de Janeiro, Brazil.

**Requests To Purchase Bulk Reprints (minimum, 100 copies):** Barbara Hudson, Reprints Coordinator; phone, 215-351-2657; e-mail, bhudson@mail.acponline.org.

**Current Author Addresses:** Drs. Barroso, Schechter, and Vieira, Ms. Melo, Ms. Murta, and Ms. Souza: Laboratório de Pesquisas, Serviço de Doenças Infecciosas e Parasitárias, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Avenida Brigadeiro Trompowski s/n, Cidade Universitária, Ilha do Fundão, CEP 21941-950 Rio de Janeiro, Brazil.

Dr. Gupta: Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, 426 Parran Hall, 130 DeSoto Street, Pittsburgh, PA 15261.

Dr. Harrison: Infectious Diseases Epidemiology Research Unit, University of Pittsburgh, 521 Parran Hall, 130 DeSoto Street, Pittsburgh, PA 15261.

**Author Contributions:** Conception and design: P.F. Barroso, M. Schechter, L.H. Harrison.

Analysis and interpretation of the data: P.F. Barroso, M. Schechter, L.H. Harrison.

Drafting of the article: P.F. Barroso, M.F. Melo, L.H. Harrison.

Critical revision of the article for important intellectual content: M. Schechter, P. Gupta, M.F. Melo, M. Vieira, F.C. Murta, Y. Souza, L.H. Harrison.

Final approval of the article: P.F. Barroso, M. Schechter, P. Gupta, M.F. Melo, M. Vieira, F.C. Murta, Y. Souza, L.H. Harrison.

Provision of study materials or patients: P.F. Barroso, M. Schechter, M. Vieira, F.C. Murta.

Statistical expertise: P.F. Barroso, L.H. Harrison.

Obtaining of funding: P.F. Barroso, M. Schechter, L.H. Harrison.

Administrative, technical, or logistic support: P.F. Barroso, M. Schechter, P. Gupta, M.F. Melo, M. Vieira, F.C. Murta, Y. Souza, L.H. Harrison.

Collection and assembly of data: P.F. Barroso, M.F. Melo, M. Vieira, F.C. Murta, Y. Souza.

## References

1. Royce RA, Sena A, Cates WJ Jr, Cohen MS. Sexual transmission of HIV. *N Engl J Med*. 1997;336:1072-8.
2. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med*. 2000;342:921-9.
3. Gupta P, Mellors J, Kingsley L, Riddler S, Singh M, Schreiber S, 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.
4. Gilliam BL, Dyer JR, Fiscus SA, Marcus S, Zhou S, Wathen L, et al. Effects of reverse transcriptase inhibitor therapy on the HIV-1 viral burden in semen. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;15:54-60.
5. Vernazza PL, Gilliam BL, Flepp M, Dyer JR, Frank AC, Fiscus SA, et

- al. Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS*. 1997;11:1249-54.
6. **Detels R, Munoz A, McFarlane G, Kingsley LA, Margolick JB, Giorgi J, et al.** Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. *JAMA*. 1998;280:1497-503.
7. **Gupta P, Ding M, Cottrill M, Rinaldo C, Kingsley L, Wolinsky S, et al.** Quantitation of human immunodeficiency virus type 1 DNA and RNA by a novel internally controlled PCR assay. *J Clin Microbiol*. 1995;33:1670-3.
8. **Carpenter CC, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hammer SM, et al.** Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel. *JAMA*. 2000;283:381-90.
9. **Schechter M, Struchiner CJ, Harrison LH.** Protease inhibitors as initial therapy for individuals with an intermediate risk of HIV disease progression: is more necessarily better? *AIDS*. 1999;13:97-102.
10. **Sodora DL, Gettie A, Miller CJ, Marx PA.** Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. *AIDS Res Hum Retroviruses*. 1998;14(Suppl 1):S119-23.
11. **Anderson DJ, O'Brien TR, Politch JA, Martinez A, Seage GR 3d, Padian N, 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.
12. **Krieger JN, Coombs RW, Collier AC, Ross SO, Chaloupka K, Cummings DK, et al.** Recovery of human immunodeficiency virus type 1 from semen: minimal impact of stage of infection and current antiviral chemotherapy. *J Infect Dis*. 1991;163:386-8.
13. **Hamed KA, Winters MA, Holodniy M, Katzenstein DA, Merigan TC.** Detection of human immunodeficiency virus type 1 in semen: effects of disease stage and nucleoside therapy. *J Infect Dis*. 1993;167:798-802.
14. **Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al.** Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med*. 1997;337:734-9.
15. **Fatkenheuer G, Rockstroh J, Salzberger B.** Reply: Virological treatment failure of protease inhibitor therapy in an unselected cohort of HIV-infected patients. *AIDS*. 1998;12:1402-3.
16. **Zhang H, Dornadula G, Beumont M, Livornese L Jr, Van Uitert B, Henning K, et al.** Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med*. 1998;339:1803-9.
17. **Page-Shafer KA, McFarland W, Kohn R, Klausner J, Katz MH, Wohlfeiler D, et al.** Increases in unsafe sex and rectal gonorrhea among men who have sex with men—San Francisco, California, 1994–1997. *MMWR Morb Mortal Wkly Rep*. 1999;48:45-8.
18. **Blower SM, Gershengorn HB, Grant RM.** A tale of two futures: HIV and antiretroviral therapy in San Francisco. *Science*. 2000;287:650-4.
19. **Salomon H, Wainberg MA, Brenner B, Quan Y, Rouleau D, Cote P, et al.** Prevalence of HIV-1 resistant to antiretrovirals drugs in 81 individuals newly infected by sexual contact or injecting drug use. Investigators of the Quebec Primary Infection Study. *AIDS*. 2000;14:F17-23.

© 2000 American College of Physicians—American Society of Internal Medicine