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Detection of Acute HIV Infections in High-Risk Patients in California

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Abstract

Background: Given the strong relationship between sexually transmitted diseases (STDs) and the spread of HIV infection, recent outbreaks of syphilis in the United States could lead to increased rates of new HIV infection. STD clinics serving persons at risk for syphilis would be logical sites to monitor rates of acute HIV infection. The detection of acute HIV infection, however, is not routine and requires the use of HIV RNA testing in combination with HIV antibody testing.

Methods: To determine the rate of acute HIV infection, we performed HIV RNA testing on pooled HIV antibody-negative specimens from persons seeking care at San Francisco City Clinic (SFCC) and from men seeking care at 3 STD clinics in Los Angeles. We compared prevalence of acute HIV infection among those groups.

Results: From October 2003 to July 2004, we tested 3075 specimens from persons at the SFCC, of which 105 (3%) were HIV antibody-positive and 11 were HIV RNA-positive/HIV antibody-negative, resulting in a prevalence of acute HIV infection of 36 per 10,000 (95% confidence interval [CI]: 26 to 50 per 10,000) and increasing by 10.5% the diagnostic yield of HIV RNA testing compared with standard testing. From February 2004 to April 2004, 1712 specimens were tested from men at 3 Los Angeles STD clinics, of which 14 (0.82%) were HIV-positive by enzyme immunoassay testing and 1 was HIV RNA-positive/HIV antibody-negative, resulting in a prevalence of 6 per 10,000 (95% CI: 3 to 13 per 10,000) and increasing the diagnostic yield for HIV infection by 7.1%.

Conclusions: In our study, the addition of HIV RNA screening to routine HIV antibody testing in STD clinics identified a substantial increased proportion of HIV-infected persons at high risk for further HIV transmission, who would have been missed by routine HIV counseling and testing protocols. Further evaluation of the addition of HIV RNA screening to routine HIV antibody testing is warranted.

Keywords

sexually transmitted disease; acute HIV infection; ribonucleic acid; syphilis; HIV detection

Detecting persons with acute HIV infection affords an important opportunity for HIV prevention. Acute HIV infection is the stage of disease during which HIV viral replication and shedding occur before detectable HIV antibodies appear.¹ During this time, viral load peaks in blood and genital secretions.²⁻⁵ Because transmission of HIV is influenced by viral load⁶ and persons with acute HIV infection are usually unaware of their HIV status, they may be especially infectious and contribute substantially to the spread of HIV.^{7,8}

The diagnosis of HIV infection is commonly based on seroconversion, the detection of antibodies to HIV which appear 3 to 8 weeks after exposure. Despite the sensitivity of currently used HIV antibody assays, there exists a “window period” during which persons with acute HIV infection have negative test results for HIV antibody. This window period can range from weeks to months depending on the type of test performed and the rapidity with which a person develops HIV-specific antibody.⁹ Currently, 3 types of HIV antibody test systems are routinely used in the United States: first- and second-generation IgG-sensitive assays and a third-generation IgM/IgG-sensitive assay.¹⁰ The window period can be shortened by testing plasma or sera for the presence of HIV-1 RNA. An HIV RNA test with sensitivity to detect 50 copies/mL can identify HIV infection approximately 6 to 11 days before an IgM/IgG-sensitive HIV antibody test and 26 to 31 days before an IgG-sensitive HIV antibody test.¹⁰ A few studies have shown that an HIV RNA screening strategy incorporating multistage specimen pooling is feasible and cost-effective for detecting acute HIV infection.¹¹⁻¹³

There are important public health implications for the detection of acute HIV infection. First, substantial potential exists for secondary HIV transmission by these individuals, which would greatly increase the spread of HIV-1 infection.¹⁴⁻¹⁶ If newly infected persons who are unaware of their HIV infection are engaging in high-risk behaviors with uninfected individuals, a substantial proportion of new infections could result. Persons with acute HIV infection should be counseled about risk-reduction strategies such as abstinence and safer sex. Studies have shown that counseling interventions at the time of the receipt of an HIV-positive test result were associated with a significant reduction in risk behavior,¹⁴ indicating that the identification and counseling of persons with acute HIV infection should be a priority of HIV prevention programs. At the same time, tracing source patients and their exposed partners may help to characterize sexual and social networks in which HIV transmission is active and might facilitate targeted interventions.¹⁷

Because of the relatively short incubation period of bacterial sexually transmitted diseases (STDs) compared with the time required for the development of detectable HIV antibody,

routine HIV antibody testing at the time of STD screening may miss recent HIV infections. Recent increases in STDs, especially syphilis, in men who have sex with men (MSM) in the United States have raised concerns about the effect of those STD increases on HIV incidence.¹⁸ In San Francisco and Los Angeles, syphilis has increased from 26 and 141 cases, respectively, in 1998 to 350 and 387 cases, respectively, in 2004.^{18–20}

To determine the prevalence of acute HIV infection in persons seeking care for STDs, we performed HIV RNA testing in addition to routine HIV antibody testing in specimens from patients at municipal STD clinics in San Francisco and Los Angeles and at the Los Angeles County jail. In addition, we estimated the cost of screening for acute HIV infection relative to routine HIV antibody testing.

METHODS

Study Design and Population

Beginning in October 2003 at the San Francisco City Clinic (SFCC), patients were informed of the new HIV testing protocol whereby HIV antibody-negative specimens would be tested for HIV RNA. All HIV testing at the clinic was voluntary, and patients underwent standard pretest risk assessment and risk-reduction counseling. Counselors and clinicians received training on HIV RNA testing, and patients received information about the meaning of the HIV RNA test in their HIV counseling and testing educational materials. Because of the additional time needed to perform RNA testing and the resultant delay in receiving test results, patients had the option to decline the HIV RNA testing component. The following data were collected as part of routine HIV counseling and testing at the clinic: age, race and ethnicity, occupation, income, zip code, syphilis history, HIV testing history, other STD diagnoses at time of testing, STD history, reasons for testing, behavioral risk factors, HIV status of partners, use of condoms, types of intercourse, and substance use.

In Los Angeles, from February to April 2004, all residual serum specimens from men undergoing routine HIV counseling and testing at 3 STD clinics and the MSM unit of the Los Angeles County Men's Central Jail were tested for HIV infection using HIV antibody and HIV RNA testing. Test results were not linked to personal identifiers; therefore, it was not possible to link results with patients and their descriptive data.

Testing Protocol

HIV enzyme immunoassay (EIA) antibody testing was done on all specimens using the Vironostika HIV-1 Microelisa System (viral lysate) (bioMérieux, Durham, NC), which is an IgG-sensitive HIV-antibody test. From all participants whose HIV antibody results were negative, an aliquot of serum (in Los Angeles) or plasma (in San Francisco) was pooled for HIV RNA testing using the multistage pooling scheme modified from a protocol described by Quinn et al.¹² The main reason for pooling the specimens was lower cost. The San Francisco Public Health Laboratory initially used a 2-stage pooling scheme with a master pool of 50 specimens comprising 5 intermediate pools of 10 specimens each. Master pools with positive results were subsequently tested in intermediate pools. All specimens in any intermediate pool with positive test results were then tested individually. After 6 months, to

reduce the turnaround time for getting test results to the patient, this laboratory switched to a 1-stage pooling scheme, using 10 specimens in each master pool. The Los Angeles County Public Health Laboratory used a 2-stage pooling scheme with a master pool of 90 specimens and subsequent intermediate pools of 10 specimens. Specimens at the San Francisco Public Health Laboratory were screened for HIV RNA using a branched DNA (bDNA) test (Versant HIV-1 RNA 3.0 assay; Bayer Diagnostics, Tarrytown, NY), which has a lower limit of detection of 75 copies/mL, and specimens at the Los Angeles County Public Health Laboratory were screened using reverse transcriptase polymerase chain reaction (RT-PCR; Amplicor; Roche Diagnostics, Branchberg, NJ), which has a lower limit of detection of 50 copies/mL.

Follow-Up and Confirmatory Testing

In San Francisco, participants whose test results were negative with EIA testing but positive with RNA testing were notified and offered partner counseling and referral services and case management, which included enrollment in the clinic's HIV care program. Confirmatory EIA and Western blot testing to document seroconversion was performed on specimens collected on subsequent clinic visits. Participants who were confirmed to be HIV-positive received expedited referral to the University of California San Francisco Options Study (a study on acute HIV infection funded by the National Institutes of Health).

Statistical Analysis

Persons for whom the EIA test was positive were defined as having chronic HIV infection. Persons for whom the EIA test was negative but RNA test was positive were defined as having acute HIV infection. Collectively, these individuals were considered to be HIV infected. Not HIV-infected persons were defined as persons for whom the EIA test was negative and the RNA test was negative. Descriptive statistics were generated using SAS version 8.2 (SAS Institute, Cary, NC). Univariate analyses were done using Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA). Participants were excluded from analyses for which data were missing.

Cost Analysis

Cost data, including supplies, labor, and equipment, were assessed to calculate the additional cost of adding HIV RNA testing to routine HIV antibody testing.

Human Subjects Protection

This study was done as part of a public health response to syphilis outbreaks in California. It was classified as non-research, in accordance with the human experimentation guidelines of the US Department of Health and Human Services.

RESULTS

San Francisco

During a 10-month period (October 2003-July 2004), 3075 specimens were received for HIV testing. Of these, EIA testing showed 2969 (97%) to be HIV-negative and 105 (3%) to be HIV-positive. One specimen had inconclusive results and could not be categorized.

Of the 2969 specimens with negative EIA results, 2722 were pooled for HIV RNA testing; 236 patients had declined to have this HIV RNA testing done, and 11 specimens were of insufficient quantity to complete the testing protocol. Of the 2722 specimens tested, 11 (0.40%) had positive HIV RNA results, identifying an additional 10.5% of HIV-infected persons.

Of the 11 patients with positive RNA test results, 9 were informed, offered partner counseling and referral services, and enrolled in medical care. Follow-up confirmatory testing using EIA and Western blot tests for those 9 (100%) participants showed that they had seroconverted to HIV-positive results; all entered medical care. All 9 were also referred to the University of California San Francisco Options Study, and 4 were enrolled.

Characteristics of the HIV-infected persons and HIV-uninfected persons stratified by test results are shown in Table 1. HIV-infected persons (EIA⁺ and RNA⁺) were significantly more likely than HIV-uninfected persons to be male (relative risk [RR] = 6.45; $P < 0.001$), Hispanic (RR = 1.62; $P = 0.015$), or MSM (RR = 21.98; $P < 0.001$); to have an STD at the time of HIV testing (RR = 2.53; $P < 0.001$); and to have had early syphilis at the time of HIV testing (RR = 5.24; $P < 0.001$). HIV-uninfected persons were significantly more likely than HIV-infected persons to be heterosexual (RR = 7.69; $P < 0.001$), to be bisexual (RR = 2.32; $P = 0.015$), and to have a previous HIV-negative test (RR = 3.03; $P < 0.001$). Among HIV-infected persons (EIA⁺ and RNA⁺), there were no significant differences between those with chronic (EIA⁺) and acute (RNA⁺) HIV infection. Among non-Hispanic whites, Hispanics, African Americans, and Asians, the proportions of acute infections among all HIV infections detected varied and were 6%, 9%, 13%, and 30%, respectively. Among MSM and heterosexuals, the proportions of acute infection among all HIV infections were 10% and 17%, respectively. None of the persons with acute HIV infection identified themselves as bisexual. Of persons with acute HIV infection, 3 (27%) reported having had 20 or more sex partners in the past 12 months and 4 (36%) reported having had sex with anonymous partners. Of the 4 persons who had acute HIV infection and an HIV-positive steady partner, only 1 reported consistent condom use with his partner. This same man reported an episode of condom breakage 1 month before his positive HIV RNA test, which is suggestive of the transmission event. One (9%) person with acute HIV infection did not identify himself as gay or bisexual; however, he reported having had oral intercourse with a male partner. In addition, he reported that within the past 12 months, he had had multiple STDs and 20 female partners with whom he engaged in vaginal, oral, and anal intercourse.

Los Angeles

From February 2004 to April 2004, 2523 specimens from persons seeking HIV counseling and testing were tested for HIV infection. Of these, EIA testing showed 2501 (99%) to be

HIV-negative and 22 (0.87%) to be HIV-positive. Of the 2501 HIV-negative specimens, 2148 were pooled for RNA testing; 353 specimens were not pooled because the remaining quantity of sera was insufficient. One (0.05%) specimen obtained from an STD clinic that predominantly serves gay men had positive RNA testing results despite negative EIA testing results.

Of the 2523 specimens received for testing, 1712 (68%) were from 3 STD clinics in the Los Angeles area and 811 (32%) were from the Los Angeles County jail. Of the 1712 specimens received from the STD clinics, 14 (0.82%) were HIV-positive according to EIA testing and 1 (0.06%) was positive according to RNA testing despite a negative EIA test result. Therefore, this strategy increased the number of cases of HIV infection detected in STD clinics by 7.1%. Of the 811 specimens received from the Los Angeles County jail, 8 (0.98%) were HIV-positive according to EIA testing and none were positive according to RNA testing; therefore, RNA testing did not increase the number of cases of acute HIV infection detected.

Overall, acute HIV infection prevalence at the SFCC was estimated at 11 per 3075 or 36 per 10,000 persons at risk (95% CI: 26 to 50 per 10,000); prevalence at Los Angeles STD clinics was estimated at 1 per 1712 or 6 per 10,000 persons at risk (95% CI: 3 to 13 per 10,000), which represents a significant difference ($P < 0.01$).

Cost of Additional Screening in San Francisco

Seven of the 30 master pools at the San Francisco Public Health Laboratory had positive HIV RNA test results, creating the need to test 35 intermediate pools. Of the intermediate pools, 7 had positive results, requiring testing of 70 individual specimens, which revealed 7 positive specimens. Detecting these 7 positive specimens among the original 1500 specimens required a total of 135 HIV RNA tests. After the laboratory switched to the 1-stage pooling protocol, the remaining 1223 specimens resulted in 125 master pools, of which 4 had positive results, requiring 40 individual tests or 165 total HIV RNA tests. At approximately \$120 per HIV RNA test, including supplies and labor, the 2-stage protocol cost \$2314 per case identified and the 1-stage protocol cost \$4950 per case identified. The total cost of pooled HIV RNA testing was \$34,800 or \$12.78 per specimen compared with the cost of antibody screening alone, which was \$2.14 per specimen.²¹

DISCUSSION

We report the use of pooled HIV RNA testing to screen for acute HIV infection in high-risk populations in California. In San Francisco, the addition of HIV RNA screening to routine HIV antibody testing among persons seeking care at a municipal STD clinic increased by 10.5% the number of HIV-infected persons identified and facilitated their participation in public health activities, research, and medical care. In Los Angeles STD clinics, the number of HIV-infected persons identified increased by 7.1%. In the Los Angeles County jail, however, this number was not changed. Therefore, this study demonstrated the utility of adding HIV RNA testing to routine HIV antibody testing in high-risk populations.

This study suggests that persons with acute HIV infection are being missed by routine HIV counseling and testing procedures currently in practice in San Francisco and Los Angeles,

and likely in many parts of the United States. These data also support the fact that pooled HIV RNA testing is a cost-effective means to increase the sensitivity of HIV testing and detect acute HIV infection among high-risk populations, particularly in areas where an IgG-sensitive HIV antibody test is used. Screening with a more sensitive HIV antibody test (IgM/IgG) may have detected nearly three quarters of these HIV-infected persons who had false-negative HIV antibody results.²² The average cost of a more sensitive HIV antibody test is approximately \$8 per specimen; therefore, switching to a more sensitive HIV antibody test may also be cost-effective. Because a more sensitive antibody test does not detect HIV infection before seroconversion and cannot characterize the duration of infection, however, HIV RNA testing may still increase the sensitivity for HIV detection and be a worthwhile addition to HIV prevention efforts. Although more studies need to evaluate the diagnostic yield of adding RNA testing to IgM/IgG-sensitive HIV antibody testing, identifying persons with acute HIV infection should remain a priority.

The presence of an STD is a significant risk factor for HIV acquisition. Genital ulcerative diseases, particularly herpes and syphilis, and inflammatory diseases such as gonorrhea increase HIV acquisition.^{23,24} In this study, HIV-infected persons were significantly more likely to have an STD at the time of HIV testing than persons who were not infected with HIV. This indicates that the use of HIV RNA testing can readily identify persons with acute HIV infection and other acute sexually transmitted infections. These dually infected persons are highly infectious and even more likely to transmit HIV to others.^{15,24} Studies in the United States and abroad have shown that STD clinic attendees have a high prevalence of acute HIV infection.²⁵⁻²⁷ This study also supports the notion that STD clinics may be ideal locations to screen for acute HIV infection so as to avert further HIV transmission.

Differences in the study samples and methods between San Francisco and Los Angeles may have contributed to a lower prevalence of acute HIV infection among persons tested in Los Angeles. Los Angeles tested residual stored sera, whereas San Francisco processed and tested fresh plasma. Because plasma is the recommended specimen, the results using plasma may be more accurate than those using sera. Second, Los Angeles used larger specimen pools than San Francisco; the resultant dilution may have reduced the sensitivity. The lower limit of detection of the pooling strategy used in Los Angeles was 6750 copies/mL. Although this is low enough to detect persons with acute HIV infection,⁵ it may be high enough to miss persons partially treated with antiretroviral therapy, persons who are slow progressors, and persons with recent HIV infection who were not detected by the IgG-sensitive HIV antibody test. Third, each site used a different commercial assay. Fourth, patients seeking care at the Los Angeles STD clinics are most likely different from those at the SFCC. The SFCC predominantly serves MSM, whereas the Los Angeles STD clinics likely serve a more diverse population with a higher proportion of heterosexuals, who may be at lower risk of contracting HIV (Lisa Smith, PhD, 2005, verbal communication).

The costs of HIV screening with routine antibody testing and its expansion have been justified.^{28,29} The cost for the identification of acute HIV infection varied according to the size of the master pool. Costs with both pools, however, were less than \$5000 per case identified. Given that the lifetime costs of HIV infection exceed \$200,000, only 1 case prevented for 40 cases identified would justify the costs for HIV RNA screening. Acute HIV

infection is highly infectious, and it seems reasonable to expect that the identification and counseling of cases would curtail subsequent HIV transmission.

Of the 40,000 new HIV infections estimated to be occurring each year in the United States, ³⁰ the proportion transmitted by those who are acutely infected is unknown; however, a substantial potential for the secondary transmission of HIV during that period exists. Empiric estimates of rates of transmission during early HIV infection suggest that approximately 17,000 new infections may result from persons who are acutely infected.³¹ Therefore, considerable efforts and resources should be directed at evaluating the identification, counseling, partner prevention services, and, possibly, early treatment of persons with acute HIV infection.

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TABLE 1.

Characteristics of Patients Tested for HIV, SFCC, October 2003 to July 2004

Characteristic	HIV Infected		Not HIV Infected	
	EIA ⁺ (%) (n = 105)	RNA ⁺ (%) (n = 11)	EIA ⁺ (%) (n = 2711)	RNA ⁺ (%) (n = 2711)
Age, years (mean)	34	34	34	33
Gender				
Male [‡]	102 (97)	11 (100)	2299 (85)	
Female	3 (3)	0 (0)	387 (14)	
Transsexual	0 (0)	0 (0)	23 (1)	
Race				
Non-Hispanic white	52 (50)	3 (27)	1537 (57)	
Hispanic [‡]	32 (30)	3 (27)	522 (19)	
African American	14 (13)	2 (18)	276 (10)	
Asian	7 (7)	3 (27)	330 (12)	
Native American	0 (0)	0 (0)	25 (1)	
Pacific Islander	0 (0)	0 (0)	14 (1)	
Self-identified sexual orientation				
MSM [‡]	91 (87)	10 (91)	1448 (54)	
Heterosexual [‡]	5 (5)	1 (9)*	815 (30)	
Bisexual [‡]	8 (8)	0 (0)	408 (15)	
Lesbian	0 (0)	0 (0)	3 (1)	
Previous HIV-negative test result	64 (66)	9 (82)	1898 (87)	
History of STDs in the past 12 months	7 (7)	0 (0)	105 (4)	
>5 sex partners in the past 12 months	49 (63)	4 (67)	1277 (62)	
Early syphilis diagnosed at visit for HIV testing [‡]	10 (10)	0 (0)	40 (1)	

* Patient self-identified as heterosexual but reported having had oral sex with men and oral, vaginal, and anal sex with women.

[‡]Differences between HIV infected and HIV uninfected were significant.