Routine surveillance for the detection of acute and recent HIV infections and transmission of antiretroviral resistance

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Objective: To estimate the rate of acute and recent HIV infections and the prevalence of primary antiretroviral resistance.

Design, setting, and subjects: A consecutive sample of individuals presenting for HIV testing at the San Francisco municipal sexually transmitted diseased (STD) clinic in 2004 ($n = 3789$).

Main outcome measures: HIV antibody-positive specimens were screened by BED IgG capture enzyme immunoassay to identify recent infections. HIV antibody-negative specimens were screened by nucleic acid amplification testing (NAAT) to detect acute infections. Newly detected infections were genotyped to detect primary antiretroviral resistance.

Results: There were 11 acute and 44 recent HIV infections among the total 136 newly detected cases. NAAT increased case identification by 8.08% over standard antibody testing. Acute HIV infections were associated with having a known HIV-positive partner, and a history of hepatitis B, syphilis, and chlamydia. The prevalence of primary antiretroviral resistance was 13.2%, with drug-resistant mutations detected in 17 of 129 cases genotyped. Mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) were present in 11 of 17 cases.

Conclusion: The integration of HIV nucleic acid amplification, recent infection, and antiretroviral resistance testing enhanced HIV/STD surveillance. The high proportion of NNRTI mutations detected suggests they may be more common in source partners or more fit for transmission than other forms of drug-resistant HIV-1. Primary antiretroviral resistance monitoring in STD clinic patients may guide the selection of treatment and post-exposure prophylaxis regimens active against viruses being transmitted in the community, and provide health departments with surveillance data in a sentinel population at risk of HIV transmission.

AIDS 2006, **20**:2193–2197

Keywords: HIV-1, acute infection, recent infection, nucleic acid amplification testing (NAAT), BED IgG capture enzyme immunoassay (BED-CEIA)

Introduction

Acute HIV infection refers to the time interval, approximately 7–21 days, between the acquisition of HIV infection and seroconversion. Approximately 50% of individuals with acute HIV infection develop headaches, sore throat, fever, muscle pain, anorexia, rash, or diarrhea [1]. Most individuals with acute infections are not likely

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Received: 7 March 2006; revised: 3 August 2006; accepted: 8 August 2006.

ISSN 0269-9370 © 2006 Lippincott Williams & Wilkins

to be aware of their HIV status. Such undetected acute HIV infections pose a significant transmission risk because high viral loads increase biological transmissibility [2–4]. Individuals unaware of their infection status may engage in risky behaviors, further contributing to transmission risk [2,5].

New advances in laboratory methods capable of detecting acute and recent HIV infections can greatly enhance surveillance and prevention efforts. Nucleic acid amplification testing (NAAT) to detect HIV RNA has been used to identify acute HIV infection [6–9]. BED IgG capture enzyme immunoassay (BED–CEIA) distinguishes between recent (within 155 days) and long-term infections [10,11]. Together, these assays can track the leading edge of the epidemic and inform HIV prevention strategies.

Primary HIV-1 antiretroviral resistance is also a potentially significant clinical and public health concern. The transmission of drug-resistant HIV strains has been documented in studies examining the prevalence of drug resistance among recently infected individuals [12–17]. A challenge to tracking primary antiretroviral resistance is identifying the timing of infection, as transmission may pre-date the advent of certain antiretroviral drugs in some individuals with long-standing HIV infection, and primary drug resistance may persist for a long period of time [18]. Therefore, primary HIV-1 antiretroviral resistance surveillance should ideally be conducted in individuals known to be recently infected.

Recognizing the timely need to address these key aspects of HIV surveillance, prevention, and care, the San Francisco Department of Public Health applied NAAT, BED–CEIA, and viral genotyping to estimate the rate of acute and recent HIV infections and the prevalence of primary HIV-1 antiretroviral resistance at the only municipal sexually transmitted diseases (STD) clinic in the county.

Methods

Patient population

A consecutive sample of all individuals presenting for confidential HIV voluntary counseling and testing at the San Francisco municipal STD clinic in 2004 ($n = 3789$) were evaluated. As the study population was individuals seeking HIV testing, newly diagnosed cases were considered to be antiretroviral treatment naive.

Testing algorithm

Figure 1 summarizes the testing algorithm for the evaluation of HIV infection and primary drug antiretroviral resistance. Specimens were screened using standard enzyme-linked immunoassays (Vironostika HIV-1 Microelisa; bioMérieux, Durham, North Carolina, USA) and positive samples were confirmed using immunofluorescence assays (Fluorognost HIV-1 IFA; Sanochemia Pharmazeutika AG, Neufeld, Vienna, Austria). HIV antibody-positive specimens were characterized as recent HIV infections by BED–CEIA (sensitivity 76.8%, dual parameter specificity 72.3–94.4%) [10,11]. HIV antibody-negative specimens were screened by NAAT (sensitivity 75 copies/ml, specificity 97.6%) (Versant HIV 3.0; Bayer Diagnostics, Emeryville, California, USA) [6,7]. Initially, a two-stage pooling strategy was applied, with a 50 specimen master pool and 10 specimen intermediate pools. To expedite the turn around time for results, the current strategy uses 10 specimens in each master pool, followed by the individual testing of specimens in any positive pools. All newly detected HIV infections (antibody positive and RNA-positive/antibody negative) were evaluated for primary antiretroviral resistance by viral genotype population sequencing (Trugene HIV-1 Genotyping Kit; Bayer Diagnostics). The assay detects mutations in the protease and reverse transcriptase sequences of the HIV-1 genome that confer resistance to antiretroviral drugs. The sequencing results were interpreted using guidelines from the manufacturer, International AIDS Society, USA, and the Stanford University HIV-1 Drug Resistance Surveillance Program [19,20]. Phylogenetic analysis of the

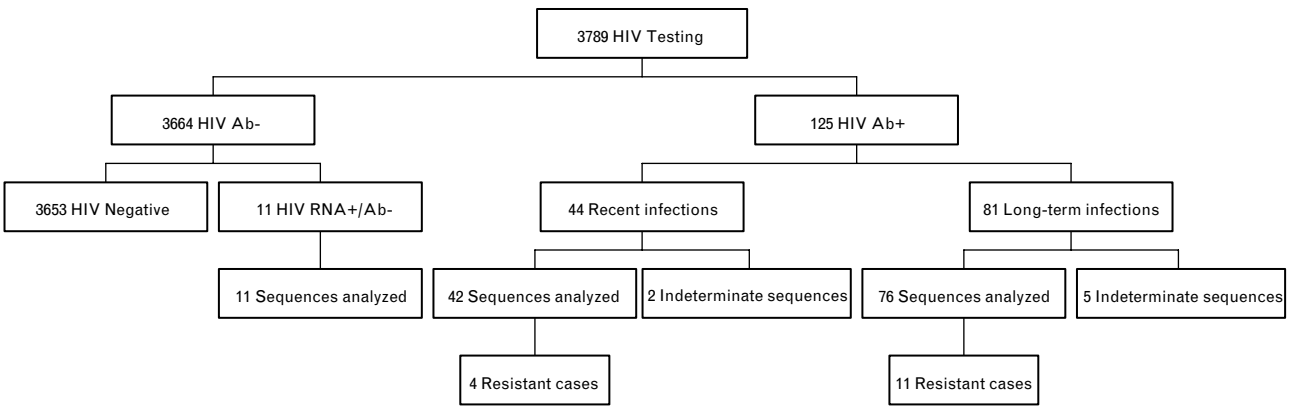

Fig. 1. Testing algorithm for detection of HIV-1 infection and primary antiretroviral resistance.

sequences revealed no specimen contamination but did identify several potential transmission relationships.

Measurements

Demographic characteristics and risk behavior information were obtained from intake data collection forms, administered in private by HIV test counselors as a routine part of the HIV voluntary counseling and testing services. Therefore, correlates of acute and recent HIV infection and primary antiretroviral resistance are based on a secondary analysis of existing data. No additional data were collected for this public health surveillance activity. Demographic characteristics included sex, age, race, and ethnicity. Behavioral data included sexual risk behaviors such as unprotected sexual intercourse and sex with a known HIV-positive partner within the past 12 months, substance use such as injection drugs, non-injection drugs, and alcohol within the past 12 months, and a history of hepatitis B and C infection and STD within the past 2 years.

Analysis

Annual HIV seroincidence was calculated on the basis of the BED–CEIA results using the 155-day window period and the seroincidence formula described previously [10,11]. For analysis of recent infection, individuals classified as recently HIV infected were compared with individuals at risk of HIV infection. For an analysis of acute infection, HIV-negative testers were used as the comparison group because acute infection cases would have been considered HIV negative by standard antibody testing before the initiation of the NAAT protocol. Associations were assessed using chi-square and Fisher's exact statistical tests. Primary HIV-1 drug-resistant cases were too few for meaningful statistical comparisons.

Nucleotide sequence accession numbers

Nucleotide sequences obtained in this analysis have been submitted to the GenBank database under accession numbers DQ811957–DQ812085.

Results

Men comprised 82% of the testing population, of which 78% were men who have sex with men (MSM). Twenty-one percent of patients were aged 25 years and younger, 38% were 26–34 years old, and 41% were 35 years and older. The population was ethnically diverse, with 54% of the sample represented by whites, 19% by Latinos, 12% by Asians/Pacific Islanders, and 11% by African-Americans.

Of the 3789 individuals seeking HIV testing, there was a total of 136 newly detected HIV infections (Fig. 1). Newly identified HIV cases represented 3.8% of the testing population and consisted of 11 acute, 44 recent, and 81 long-term infections. The use of NAAT resulted in an 8.08% increase in the rate of HIV case identification over that with standard antibody testing.

On the basis of the BED–CEIA results, the annualized HIV seroincidence estimate was 2.5% [95% confidence interval (CI) 1.8–3.3] in the overall testing population. Among MSM testers, the annualized HIV seroincidence estimate was 3.3% (95% CI 2.3–4.4). Individuals with recent HIV infection compared with individuals at risk of HIV infection were more likely to report unprotected anal intercourse (55 versus 32%; $P = 0.001$), having an HIV-positive partner (30 versus 15%; $P = 0.007$), and the use of amphetamines (30 versus 13%; $P = 0.001$) in the past 12 months.

All 11 acute HIV infections were detected in MSM, of whom four were white, three were Latino, two were African-American, and two were Asian/Pacific Islander. Acute HIV infections were associated with having a known HIV-positive partner (36 versus 14%; $P = 0.046$) in the past 12 months and a history of hepatitis B (9 versus 1%; $P = 0.002$), syphilis (27 versus 3%; $P < 0.001$), and chlamydia (27 versus 10%; $P = 0.047$) within the past 2 years, compared with individuals testing HIV negative.

Viral genotypic sequencing to detect HIV-1 drug-resistant mutations was performed on 136 HIV-positive specimens and interpretable sequences were generated for 129 specimens, corresponding to a 95% assay success rate. The prevalence of primary HIV-1 antiretroviral resistance was 13.2% among the 129 newly detected cases. Among the 17 drug-resistant cases detected, 12 were long-term HIV infections and five were recent HIV infections. No resistance mutations were detected among the 11 acute HIV infection cases.

Mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) were the most common pattern detected, present in 11 of 17 cases. Dual-class resistance was present in three cases and there was one case of multiclass resistance. The specific resistance mutations detected are shown in Table 1. All 17 drug-resistant cases were detected in MSM, of whom nine were white, five were Latino, and two were African-American. Among drug-resistant cases, nine reported having unprotected anal intercourse, three had a known HIV-positive partner, one had a partner who used injection drugs, and one used amphetamines in the past 12 months. Individuals with drug-resistant mutations had a history of chlamydia ($n = 4$), gonorrhea ($n = 4$), and syphilis ($n = 1$) within the past 2 years, and four cited a current STD as the reason for the clinic visit.

Discussion

The detection of an additional 8.08% of HIV cases by NAAT represents a substantial improvement over current HIV screening protocols using solely standard antibody testing. Enhanced case detection may be especially significant if undiagnosed acute HIV infection

Table 1. HIV-1 drug-resistant mutations detected among newly identified HIV cases.

Case no.	NRTI	NNRTI	PI
1[a]	D67N, K219Q		
2		V108I/V	
3[a]		K103N	
4		K103N, Y181C	
5		V108I	
6		V108I/V	
7[a]	K70R, M184V, K219Q	V106A, G190A	
8			D30N
9[a]		K103N	
10[a]	T215T/S/C		
11		Y181C	L90M
12	T215D	Y181C	L90M
13		K103N	
14	M41L, L210W/L	K103N/S	
15	M184V		
16	D67N, K219Q		
17	T215D		

NNRTI, Non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Recent HIV infection.

results in the risk of secondary transmission or a missed opportunity for referral for early care, particularly because these patients would otherwise receive a negative test result. The 13.2% prevalence of primary HIV-1 antiretroviral resistance was composed of a high proportion of NNRTI mutations, which suggests that NNRTI resistance may be more common in source partners or more fit for transmission than other forms of drug-resistant HIV-1.

The finding that 65% of the resistant cases were associated with NNRTI mutations supports previous reports that NNRTI resistance is on the increase. Among recently infected individuals in San Francisco, NNRTI mutations were present in none of the resistant cases in 1996–1997 but accounted for 35% of resistant cases in 1998–1999 and 48% of resistant cases in 2000–2001 [13].

In our study, the ratio of acute to recent to long-term infections was different from that observed in North Carolina, but similar to Seattle [7,8]. MSM comprised 64% of the San Francisco study population, compared with 100% in Seattle and 3% in North Carolina. The similarity of HIV risk category and demographic characteristics in San Francisco and Seattle may account for the observed parallel in the timing of HIV detection.

Screening STD clinic populations for acute infection creates an opportunity for cost-effective surveillance of new HIV infection and the prevention of secondary transmission. In broad strokes, the total cost per identified case of acute HIV infection in our study was approximately US$5000. By comparison, the lifetime cost for treating a case of HIV infection exceeds US$250 000 [21]. Given that acute HIV infection represents a period of high transmission risk, the potential to prevent a secondary infection justifies the expense of routine NAAT.

Our study illustrates how new technology can be integrated into existing HIV/STD surveillance programmes. The implementation of acute HIV infection and primary antiretroviral drug resistance detection activities was initiated in Autumn 2003, and this integrated surveillance approach has been ongoing. Follow-up with newly identified HIV cases in 2004 provided information on potentially exposed sexual partners and enabled the diagnosis of 10 additional HIV infection cases [9].

The integration of NAAT, recent infection determination, and antiretroviral drug resistance testing at STD clinics enhances HIV/STD surveillance and prevention efforts. Municipal STD clinics represent groups at high risk of acquiring and transmitting infections. The use of NAAT to detect acute HIV infections improves case identification and may help avert secondary HIV transmission through partner notification, venue notification, and risk abatement. The identification of recent infection may help characterize the leading edge of the HIV epidemic within the community and identify sexual networks. Antiretroviral resistance monitoring in STD clinic patients may guide the selection of treatment and postexposure prophylaxis regimens that are active against viruses being transmitted in the community and provide health departments with surveillance data in a sentinel population at risk of HIV transmission.

Acknowledgements

The authors wish to thank the clinicians at the San Francisco City Clinic for providing patient care; the technologists at the San Francisco Department of Public Health Laboratory and the ARI/UCSF Laboratory of Clinical Virology for performing the laboratory assays; and Dr Bharat Parekh of the Centers for Disease Control and Prevention for his technical expertise and advice.

Sponsorship: Funding support for the HIV infection and antiretroviral drug resistance surveillance programme was provided partly from the San Francisco Department of Public Health and the Gladstone Institute of Virology and Immunology.

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