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SHORT COMMUNICATION

Improved strategies for HIV diagnosis among men who have sex with men (MSM) in Buenos Aires, Argentina, a population with a high prevalence and incidence of HIV infection

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Background

In Argentina, HIV diagnosis in adults is made using one or two enzyme immunoassay tests and a confirmatory test. These strategies may fail to identify infected individuals during early primary infection, which represents an important public health problem among groups with a high HIV incidence, such as men who have sex with men (MSM) (6.3% persons/year). The general objective of this study was to contribute to reducing HIV transmission among MSM through the identification of antibody-negative, nucleic acid-positive individuals.

Findings

A total of 1549 MSM were recruited for an HIV seroprevalence study. A total of 161 (10.4%) MSM were HIV-positive and 14 (0.9%) were indeterminate. Among the 1374 negative individuals, 16 (1.2%) exhibited reactive results in the screening assay. Indeterminate Western blot (WB) samples and negative WB samples (with discordant results in the screening) were analysed to detect HIV nucleic acid by viral load testing. Up to 23.1% of HIV-indeterminate WB samples and 7.1% of HIV-negative WB samples with discordant results in the screening assays had detectable nucleic acid. Overall, 14.8% of the samples with discordant or indeterminate results were identified as HIV-positive using direct diagnosis. With the identification of four new cases using the nucleic acid detection test, the HIV prevalence in MSM increased by 0.3% (from 10.4 to 10.7%).

Conclusions

The results of this study suggest the importance of including nucleic acid detection in the HIV algorithm for MSM with HIV-indeterminate WB results and those with HIV-negative WB results and discordant results in screening assays, in order to decrease HIV transmission among this population with a high HIV prevalence and incidence.

Keywords: Argentina, HIV diagnosis, men who have sex with men

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Introduction

In Argentina, HIV diagnosis in adults follows the Centers for Disease Control and Prevention (CDC) recommendations which suggest antibody testing using one or two enzyme immunoassay tests (EIAs) and one confirmatory test [Western blot (WB)] [1]. However, strategies limited to

antibody testing may fail to detect infected individuals during early primary infection, with serious implications for public health. The early diagnosis of acute HIV infections may benefit patients by permitting clinical interventions, which can limit viral spread by decreasing viral loads and thus reducing the risk of transmission [2–4].

The most sensitive techniques to identify acute infections are based on the detection of viral nucleic acid by nucleic acid amplification testing (NAAT). Strategies focused on pooled HIV RNA detection can be feasible and cost-effective. However, when the expected number of acute infections is high (as a consequence of high prevalence and incidence rates), use of this algorithm hinders the reporting of results on time and increases the overall cost of testing. In these cases, it is advisable to carry out individual nucleic acid testing.

The first HIV prevalence study on men who have sex with men (MSM) from Buenos Aires revealed a rate of 13.8% [5]. The results of this study also showed that a large number of MSM (≈ 50%) were engaged in unprotected sexual intercourse. A recent study conducted in MSM showed the same trend (HIV prevalence 10.4%; HIV incidence 6.3% persons/year) [6].

In order to decrease HIV transmission among MSM, it is necessary to improve early HIV diagnosis. Therefore, the general objective of this study was to contribute to reducing HIV transmission through the identification of HIV antibody-negative and NAAT-positive MSM during acute infection.

Methods

A total of 1549 MSM were included in an HIV cross-sectional study conducted during 2006–2008 [6]. All the patients had to sign an informed consent form to participate in the study. HIV diagnosis was performed as described previously using two screening tests, an enzyme-linked immunosorbent assay (ELISA) (Genscreen ULTRA HIV Ag-Ab; Bio-Rad, Marnes-la-Coquette, France) and particle agglutination (SFD HIV 1/2 PA; Bio-Rad Fujirebio Inc., Tokyo, Japan). Reactive samples were confirmed by Western blot (WB) (New Lav Blot I; Bio-Rad). WB assays were reported as negative (without bands), positive [with at least two of the following bands: p24, glycoprotein 41 (gp41) and gp120/160] or indeterminate (with bands not meeting the criteria for positivity).

The HIV prevalence was 10.4% (161 of 1549 patients) and the HIV incidence was 6.3% persons/year [6]. A total of 14 (0.9%) MSM had an HIV-indeterminate WB and, among the 1374 MSM with HIV-negative results, 16 (1.2%) had discordant results in the screening assay (12 were reactive by Ag-Ab ELISA, three were reactive by the particle agglutination assay, and one was reactive by both techniques, but all were negative by WB) (Table 1).

Three samples were not available for any of the tests and one was only available for viral load measurement, so 14 HIV-negative WB samples with a discordant screening test and 13 HIV-indeterminate WB samples were examined for HIV nucleic acid detection using viral load testing [VERSANT® HIV-1 RNA 3.0 Assay (bDNA); Siemens, Munich, Germany] and for p24 antigen using the ELISA technique (Vironostika HIV-1 Antigen; Biomerieux, Marcy l'Etoile, France). A group of 241 HIV-negative samples (with two negative screening assays) were also tested. Samples with viral load values < 200 HIV-1 RNA copies/mL were considered HIV-negative for the purpose of this study. Table 1 shows the results for each sample.

Results

One of 14 (7.1%) of the HIV-negative WB samples with discordant results in the screening assays had detectable nucleic acid/p24 antigen, and 23.1% (three of 13) of the HIV-indeterminate WB samples were also reactive for p24 antigen and had a viral load > 500 000 copies/mL. Overall, 14.8% (four of 27) of the samples with discordant or indeterminate results were identified as HIV-positive using direct diagnosis. Four new cases were identified by p24 antigen and nucleic acid detection, increasing the HIV prevalence in these MSM by 0.3%, from 10.4% [95% confidence interval (CI) 8.8–11.9%] to 10.7% (95% CI 9.1–12.2%). Among the 241 HIV-negative samples, no cases of viral load > 200 copies/mL were detected. Twenty-five patients had a detectable viral load with values < 200 copies/mL. Of these, 12 returned for further testing and were found to be negative for HIV infection.

Out of a total of 16 patients with HIV-negative WB with discordant results in the screening assays, three (19%) returned for a further HIV test, and were found to be HIV-negative. Patient WB-neg 5, who was retrospectively found to be HIV-positive, did not return for a new diagnosis. Patient WB-neg 14, who had a viral load of 106 copies/mL, did not return. Of the 14 patients with indeterminate results, 12 (86%) returned to have their HIV status determined. Three of them were HIV-positive (WB-ind 1, WB-ind 4 and WB-ind 8) and nine were HIV-negative, including patients WB-ind 10 and WB-ind 13, who had viral load values of 135 and < 50 copies/mL, respectively.

Discussion

Our data suggest that HIV diagnosis needs to be reviewed in order to achieve early detection of HIV infection in

Table 1 Viral load and p24 antigen detection in 14 HIV-negative Western blot (WB) samples with discordant results in the screening assays and 13 HIV-indeterminate WB samples from 1549 men who have sex with men (MSM) included in the HIV seroprevalence study (2006–2008)

Sample ID	HIV diagnostic test			Viral load* (copies/mL)	p24 antigen
	EIA	PA	WB		
WB-neg 1	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 2	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 3	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 4	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 5	**Reactive**	Nonreactive	Negative	**> 500 000**	**Reactive**
WB-neg 6	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 7	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 8	Nonreactive	**Reactive**	Negative	< 50	Nonreactive
WB-neg 9	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 10	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 11	Nonreactive	**Reactive**	Negative	< 50	Nonreactive
WB-neg 12	**Reactive**	**Reactive**	Negative	< 50	Nonreactive
WB-neg 13	**Reactive**	Nonreactive	Negative	< 50	Nonreactive
WB-neg 14	**Reactive**	Nonreactive	Negative	106	Nonreactive
WB-ind 1	**Reactive**	**Reactive**	Indeterminate (gp160/120, p66)	**> 500 000**	**Reactive**
WB-ind 2	Nonreactive	**Reactive**	Indeterminate (p24)	< 50	Nonreactive
WB-ind 3	Nonreactive	**Reactive**	Indeterminate (p24)	< 50	Nonreactive
WB-ind 4	**Reactive**	Nonreactive	Indeterminate (p24)	**> 500 000**	**Reactive**
WB-ind 5	**Reactive**	Nonreactive	Indeterminate (p24)	< 50	Nonreactive
WB-ind 6	**Reactive**	Nonreactive	Indeterminate (p24)	< 50	–†
WB-ind 7	**Reactive**	Nonreactive	Indeterminate (p24)	< 50	Nonreactive
WB-ind 8	**Reactive**	Nonreactive	Indeterminate (p24)	**> 500 000**	**Reactive**
WB-ind 9	**Reactive**	Nonreactive	Indeterminate (p55)	< 50	Nonreactive
WB-ind 10	**Reactive**	Nonreactive	Indeterminate (p24)	135	Nonreactive
WB-ind 11	**Reactive**	Nonreactive	Indeterminate (p24)	< 50	Nonreactive
WB-ind 12	**Reactive**	Nonreactive	Indeterminate (p24)	100	Nonreactive
WB-ind 13	**Reactive**	**Reactive**	Indeterminate (p24)	< 50	Nonreactive

EIA, enzyme immunoassay; PA, particle agglutination.
*Samples with viral load values < 200 HIV-1 RNA copies/mL were considered HIV-negative for the purpose of this study.
†Three samples were not available for any of the tests and one was only available for viral load.

populations with high prevalence and incidence rates, such as MSM in Buenos Aires, Argentina. The results obtained in this study are consistent with previous reports. A previous study in MSM showed that a total of 36 of 13 677 (0.3%) antibody-negative samples were positive when nucleic acid detection was used [7]. Another study showed that 81% of acute HIV infections identified by nucleic acid detection in sexually transmitted disease (STD) clinics in New York City were found among MSM. That study also demonstrated that, without nucleic acid testing, 9% of HIV infections at STD clinics would have been missed [8]. Another study performed in Thailand in a high-risk population showed that 11 of 6426 subjects (0.2%) were identified as acutely infected with HIV using pooled nucleic acid detection. These acutely HIV-infected subjects were mostly MSM, and the HIV prevalence ranged from 17 to 28% [9].

Although the sensitivity of the fourth-generation ELISA screening test is reported to be 100% by the manufacturer, HIV-positive WB samples with particle agglutination reactivity only have previously been identified in our laboratory (Dr H. Salomon, Head of the Laboratory, Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; personal communication). Although these cases were not very frequent (six of 7820 HIV tests performed over 4 years, representing 0.08%) and no such cases were found in the present study, particle agglutination could be useful to detect some cases that are not reactive by ELISA.

The identification of four new HIV-positive individuals using nucleic acid detection represents 0.3% of this MSM population from Buenos Aires. Although patient follow-up was not specifically planned in the study, a high percentage of individuals with HIV-indeterminate WB results (86%) returned to disclose their HIV diagnosis, including the three HIV-positive individuals. However, this trend was not observed among patients with HIV-negative WB but discordant results in the screening assays, where only 19% of the patients returned. This indicates that patients rely most heavily on the WB result. However, our results suggest that physicians should issue special recommendations not only for those individuals with HIV-indeterminate WB results

but also for those with HIV-negative WB results with reactive screening assays, especially in settings where high prevalence and incidence rates are observed (e.g. MSM). Although we did not obtain any samples with viral loads > 200 copies/mL among the HIV-negative WB tested samples, no conclusions can be drawn about the absence of HIV-positive cases in the group, because only a small percentage of samples (≈ 18%) could be studied. A prospective implementation research study needs to be conducted in order to evaluate the cost-effectiveness of performing nucleic acid detection together with the screening test in this high-risk population.

In this study, although p24 antigen ELISA testing was able to detect the same HIV-positive cases identified by the nucleic acid technique, previous studies suggested that p24 antigen testing could identify from 79 to 90% of acute infections [10]. Thus, the p24 antigen ELISA may be an option for improving early detection of HIV infections only where access to nucleic acid-based detection methods is limited.

In conclusion, the results of this study suggest that the algorithm for early diagnosis of acute HIV infections should include individual nucleic acid detection in MSM with HIV-negative WB with discordant results in the screening assays, as well as in those with HIV-indeterminate WB. An accurate early diagnosis of acute HIV infection may benefit patients by permitting clinical interventions, which can limit viral spread by decreasing viral loads and thus reducing the risk of transmission.

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