Establishment of the Serologic Testing Algorithm for Recent Human Immunodeficiency Virus (HIV) Seroconversion (STARHS) Strategy in the City of São Paulo, Brazil

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Several strategies aim at characterizing the AIDS epidemic in different parts of the world. Among these, the identification of recent HIV-1 infections using the recently described serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) strategy was employed in four testing sites of the City of São Paulo Public Health Department (CSPPHD). Those identified as recently infected were invited to participate in a prospective clinical and laboratory evaluation study. We describe the establishment of the patient identification network and the success in enrolling the participants, as well as their clinical and laboratory characteristics. From May to December 2002, 6,443 persons were tested for HIV in the four participating sites, of whom 384 (5.96%) tested HIV-1 positive; 43 (11.2%) of them were identified as recently infected. Twenty-two were successfully enrolled in the follow-up study, but three of them did not meet clinical and/or laboratory criteria for recent HIV-1 infection. After these exclusions, the laboratory findings revealed a median CD_4^+ T lymphocyte count of 585 cells/μL (inter-quartile range 25-75% [IQR], 372-754), a CD_8^+ T lymphocyte count of 886 cells/μL (IQR, 553-1098), a viral load of 11,000 HIV-RNA copies/mL (IQR, 3,650-78,150), $\log_{10}$ of 4.04 (IQR 3.56-4.88). The identification of recent HIV infections is an extremely valuable way to evaluate the spread of the virus in a given population, especially when cohort studies, considered the gold standard method to evaluate incidence, are not available. This work demonstrated that establishing a network to identify such patients is a feasible task, even considering the difficulties in a large, resource-limited country or city.
Key Words: HIV, recent infection, network, São Paulo.

The identification of individuals recently infected with human immunodeficiency virus (HIV) is an important tool to address the transmission dynamics and to establish a target population to develop virology and immunology studies. Recently, a new approach was developed to help identify such subjects, utilizing the Serologic Testing Algorithm for Recent HIV Seroconversion (recently termed STARHS) strategy, which combines the use of a sensitive enzyme immunoassay (EIA) with a modified, less sensitive (LS) version of EIA, originally using the 3a11 reagents kit (Abbott Laboratories, Abbott Park, IL, USA) [1], but also validated with the HIV-1 Microelisa System (BioMerieux, Raleigh, NC, USA) [2].

STARHS is based on the slow increase in antibodies observed during the early period of infection, then reaching a plateau [3-6]. In the STARHS method, a standard commercial diagnostic EIA is modified to reduce its sensitivity to a point where serum samples with low antibody titers are weakly reactive or negative in the LS assay. A blood specimen that is reactive on a sensitive EIA but non-reactive on the LS EIA identifies a person in the early period of HIV infection. The time between seroconversion on the sensitive EIA and the LS EIA has been estimated to be 170 days (95% confidence interval: 145-200 days) for HIV-1 subtype B [7].

Identifying recent infection subjects and samples enables epidemiological studies [8-13], characterization of the clinical and laboratory presentation, identification of the circulating viral strains [9, 14], and the constitution of a sample repository for immunological and viral evolution studies.

We developed a network involving the Federal University of Sao Paulo (UNIFESP) and the City of São Paulo Public Health Department (CSPPHD) to identify subjects recently infected by HIV-1, using the STARHS strategy, aiming at enrolling these individuals for prospective follow-up. We examined the establishment of the network and the success in enrolling the patients, as well as their clinical and laboratory characteristics.

Material and Methods

Network establishment

The CSPPHD is the largest network providing care for HIV-infected patients in the São Paulo metropolitan area. Twenty-three clinical sites provide health care to infected patients throughout the city, including eight dedicated to offering HIV testing and counseling centers.

Four of these services were selected for this study: Henfil and Campos Elíseos, both located in the central area of the city, Lapa, located in the north, and Pirituba, located in the west. Data collection took place from May to December 2002, but the sites began the collection at different time points, from May to July. At all four sites, persons presenting for HIV testing were informed about the study and then about a new HIV testing technology was used to identify recent infection. A signed informed consent was obtained from all who agreed to the STARHS testing procedure in addition to the HIV testing offered by the testing site.

The subjects were asked to return to the testing site 10 to 14 days after giving a blood sample, for access to the results and post counseling. The flow of activities is depicted in the Figure 1 algorithm.

Serological tests

Blood was collected in vacuum tubes, allowed to clot, and transported on the same day to the central laboratory of the CSPPHD. Two enzyme immunoassays (EIA) were run. Samples with two non-reacting EIA results were considered negative. Those with indeterminate or reacting results in one or two EIA assays were submitted to a confirmatory Western-Blot assay.

If the result was indeterminate or positive, the volunteer was asked to donate a second sample for HIV testing, in accordance with Brazilian Ministry of Health Guidelines [15].

Any sample with a positive result obtained at the central laboratory of the CSPPHD was flagged and an aliquot was transported to the Retrovirology Laboratory at the Federal University of Sao Paulo. These samples were again analyzed using the STARHS.

Briefly, the samples were re-tested using the Vironostika HIV-1 Microelisa System (BioMerieux, Raleigh, NC, USA) EIA, which employs testing of a 1:20,000 dilution of the specimen under modified incubation conditions. The Vironostika negative control, CDC calibrator, CDC high positive control and CDC low positive control were all diluted and incubated in the same manner as the specimens described above. Samples were initially screened (one well per sample) and all the controls were run in triplicate. The sample optical density (OD) value was standardized as follows: SOD = sample OD – average negative control OD ÷ average CAL OD – average negative control OD. Samples with screening SOD below 2.0 were re-

tested in triplicate in a confirmatory test. The SOD was calculated using the median OD of the triplicates. A sample with SOD below 1.0 in the confirmatory mode was classified as an infection probably acquired in the last 170 days. The result of STARHS was transmitted to the four sites within 10 days after the blood was drawn.

Since the sample could be submitted to a STARHS assay if it were positive at the first round of testing, all the volunteers were informed about the test and were offered the opportunity to sign an informed consent authorizing it, if the standard immunoenzymatic assay resulted positive.

Volunteer enrollment

At the returning visit of any subject after the first blood was drawn, the test result and counseling were offered at the four testing sites. If the subject tested positive for HIV antibodies, a second blood sample was taken to confirm the first result, again in accordance with Brazilian Ministry of Health Guidelines.

If a seropositive subject was identified as recently infected according to the STARHS assay, the volunteer was offered the option to participate in a protocol involving prospective clinical visits and blood collection at the Outpatient Clinic of the Federal University of Sao Paulo. If the volunteer accepted, a second informed consent was explained and offered, allowing participation in the protocol. A rapid HIV antibody test and a hematocrit were performed using a fingertip puncture. Females were also asked for a urine sample for a dipstick pregnancy test. Volunteers younger than 18 years old, with a negative rapid test, a hematocrit below 28%, or who were pregnant, were not enrolled in the study. The subjects were then submitted to the second blood drawn for further tests, and were asked to come to a scheduled visit at the Federal University of Sao Paulo within 15 days. Samples were submitted to CD_4^+ T lymphocyte counts, and plasma viral load determination.

All the data was stored in a spreadsheet, organized in a data bank and afterwards analyzed using Statistica software, version 6.0 (Statsoft, Inc., Tulsa, OK, USA).

Results

Identification of recently infected cases

From May to December 2002, 6,443 persons were tested for HIV in the four participating sites, of whom 384 (5.96%) tested positive for HIV antibodies. Figure 2 describes the number of tests and distribution in the four sites. Among the 384 patients who tested positive for HIV antibodies, 43 (11.2%) were identified as recently infected using the STARHS testing strategy: 25 were from Henfil, 14 from Campos Elíseos, three from Lapa, and one from Pirituba, corresponding to 58.1%, 32.6%, 7.0%, and 2.3% of the new diagnosed HIV-infections, respectively. The highest percentage of recently infected HIV subjects was 15.8% at Lapa, followed by 13.9% at Henfil, 8.3% at Campos Elíseos, and 5.9% at Pirituba.

Upon identification of a potential recently infected HIV-1 subject, the individual was invited to participate in the follow-up study at the Federal University of São Paulo outpatient clinic. The accumulated number of patients identified and enrolled in this protocol during the study period is depicted in Figure 3.

Enrollment and clinical and laboratory characteristics findings

All the HIV-infected patients with a negative STARHS assay result who came back to know their HIV serostatus were asked to participate in a protocol at UNIFESP, involving prospective clinical exams and laboratory follow-up. Twenty-two patients decided to participate and they were enrolled in the protocol by the end of December 2002, corresponding to 51% of identified cases. Most of the enrollees were identified at Henfil, followed by Campos Eliseos, Lapa, and Pirituba (Figure 3). The average time between the first visit at the service and the subsequent visit for other laboratory tests was 19 days (range, 3-50 days).

The demographical, epidemiological, clinical and laboratory characteristics of the enrolled patients are described in Table 1. Three patients (13%) did not meet the criteria for a recent HIV infection. The first

Figure 1. Algorithm for identification and enrollment of recently-infected HIV subjects. The first visit took place at one of the four sites: Henfil, Campos Eliseos, Lapa, and Pirituba. HIV-positive samples were transported to the Federal University of São Paulo (UNIFESP) for STAHRS. Those patients identified as a potential recent HIV-1 infection were invited to provide a sample and were referred to the UNIFESP outpatient clinic.
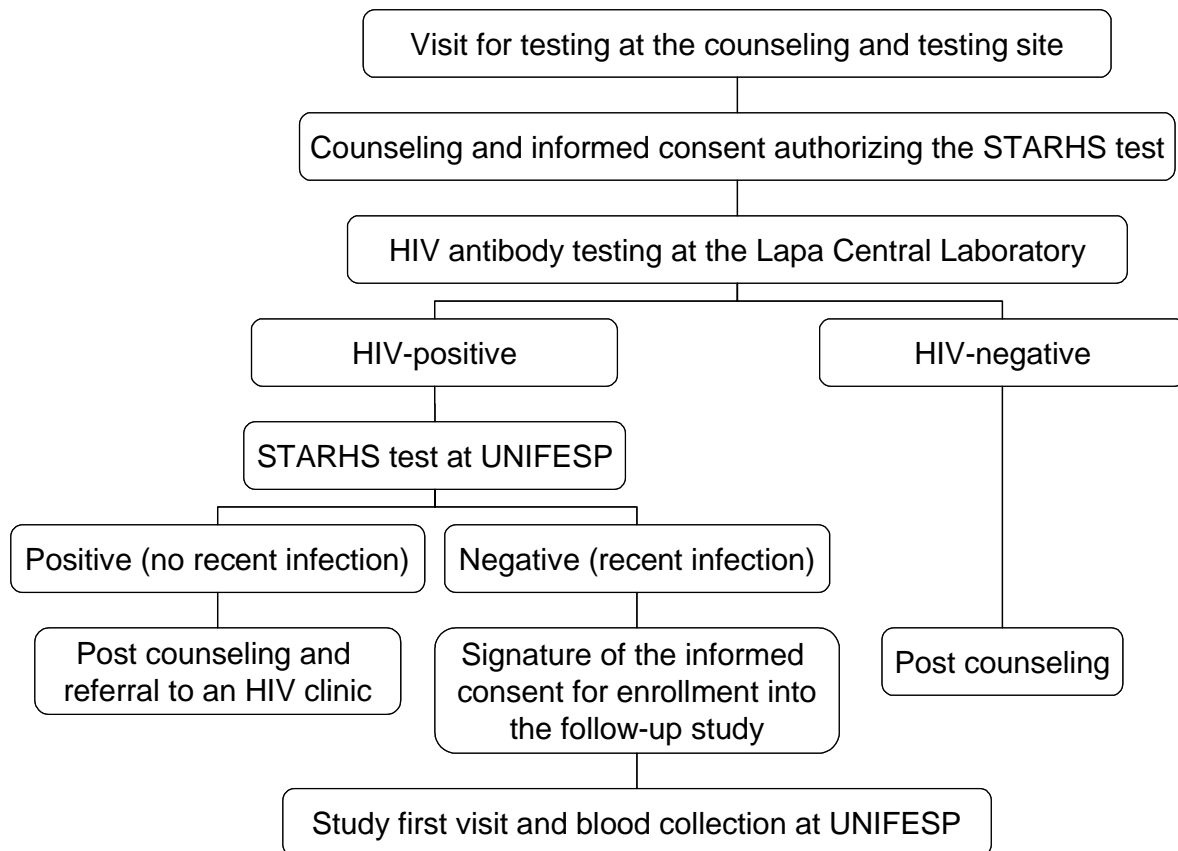
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                    Visit for testing at the counseling and testing site

              Counseling and informed consent authorizing the STARHS test

                    HIV antibody testing at the Lapa Central Laboratory

                    HIV-positive                          HIV-negative

                    STARHS test at UNIFESP

        Positive (no recent infection)    Negative (recent infection)

        Post counseling and        Signature of the informed        Post counseling
        referral to an HIV clinic   consent for enrollment into
                                    the follow-up study

                    Study first visit and blood collection at UNIFESP
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Figure 2. Number of HIV-positive tests at the four testing centers from May to December 2002 and respective STARHS results. The percentage of samples suggesting recent HIV-1 infection at each site is indicated.
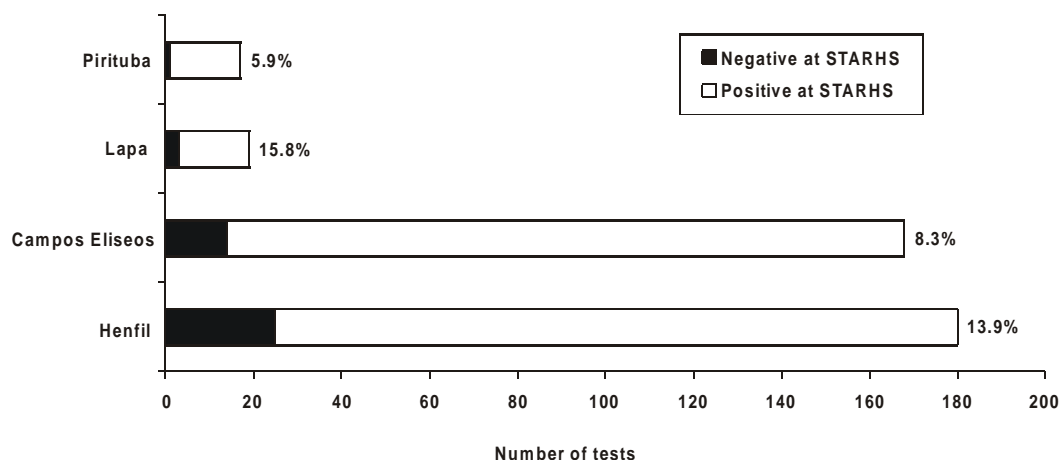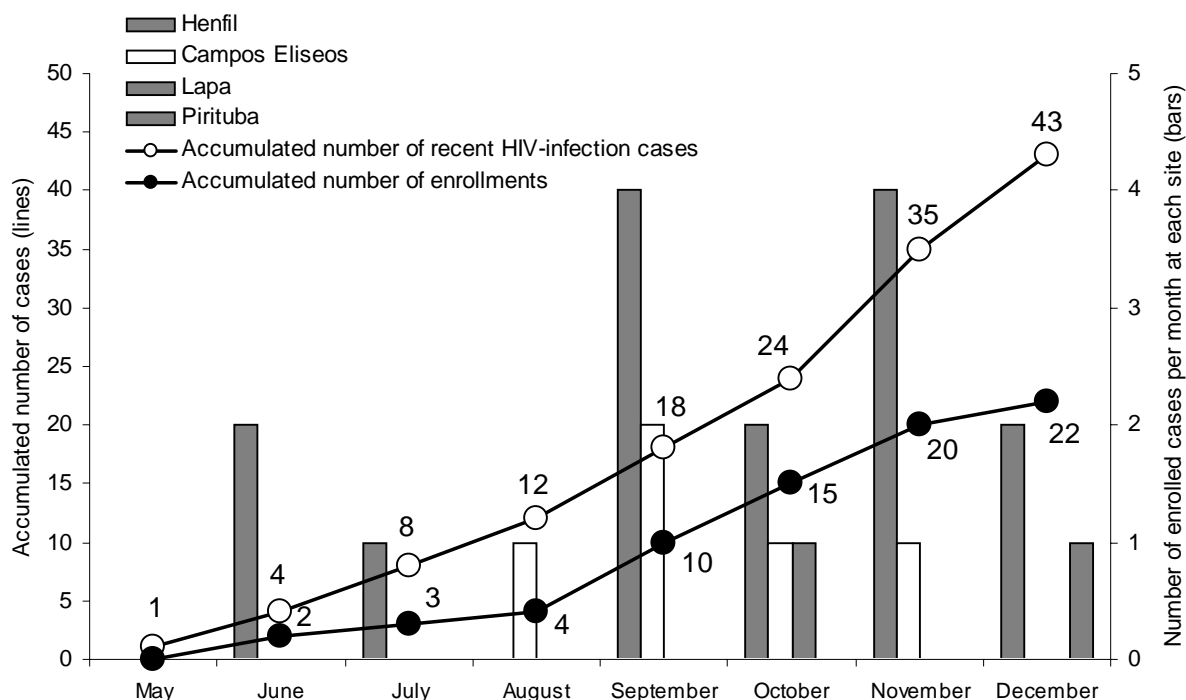
Pirituba   5.9%
Lapa   15.8%
Campos Eliseos   8.3%
Henfil   13.9%

Legend: ■ Negative at STARHS   □ Positive at STARHS

x-axis: Number of tests (0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200)

Figure 3. Dynamics of identification (open circles) and enrollment of recent HIV-infected patients (closed circles). The bars represent the number of enrolled patients at each site by month.

(patient 2001) tested negative for HIV antibodies at the second blood test, with normal values of CD_4^+ and CD_8^+ T lymphocyte counts, and an undetectable viral load; he was then considered uninfected and the first test was considered a false positive. Two other patients (patients 1014 and 2007) had a clinical history and CD4+ T lymphocyte counts that suggested established, chronic infection.

Two patients (10%) had symptoms within the past four months compatible with primary HIV infection within the six months prior to enrollment, according to a physician's judgment. The first (1002) had fever, muscle pain, lymph node enlargement, and oral lesions lasting for five days, followed by spontaneous resolution. The second (1011) reported fever for 20 days, also resolving spontaneously.

After excluding the three patients who did not meet clinical and laboratory criteria for recent infection, the

laboratory findings revealed a median CD_4^+ T lymphocyte count of 585 cells/µL (inter-quartile range 25%-75% [IQR], 372-754), a CD_8^+ T lymphocyte count of 886 cells/µL (IQR, 553-1098), and a viral load of 11,000 HIV-RNA copies/mL (IQR, 3,650-78,150), $\log_{10}$ of 4.04 (IQR 3.56-4.88).

Two patients presented with low CD_4^+ counts. Patient 2006 had a history of a negative test for HIV antibodies one year before, and a CD_4^+ T lymphocyte count of 215 cells/µL, but follow-up evaluations demonstrated increasing numbers (236 and 320 cells/µL, at 31 and 60 days, respectively, after the first follow-up study visit). Patient 1015 reported a highly suspicious contact before the first HIV antibody testing. However, no symptoms compatible with primary infection were reported. The first CD_4^+ count revealed 146 cells/mL, followed by 121 cells/mL 36 days after, which motivated the initiation of antiretroviral therapy.

Table 1. Characteristics of the patients enrolled in the follow-up study

Patient ID	Age (years)	Gender	Time between visits (days)	Risk behavior*	History of symptoms	CD$_4^+$T cells/μL	CD$_8^+$T cells/μL	HIV-RNA (copies/mL)
1001	34	M	6	MSM	No	372	491	59,300
1002	38	M	4	MSM	Yes	619	952	14,300
2001	27	M	36	Hetero	-	661	471	<80
2002	21	M	41	Hetero	No	558	886	16,000
1004	34	F	14	Hetero	No	489	987	97,000
2003	70	M	50	Bisex	No	969	559	11,000
1005	24	M	29	MSM	No	424	547	340
1006	24	F	7	Hetero	No	362	443	1,900
1007	37	M	13	MSM	No	788	1,147	1,000
1008	34	M	7	Hetero	No	349	570	4,400
1009	25	M	42	MSM	No	837	1,636	49,000
2004	36	M	21	Bisex	No	709	116	6,800
2005	27	M	22	Bisex	No	726	894	3,500
1010	38	M	3	MSM	No	1,442	877	3,800
1011	27	M	15	MSM	Yes	698	4,680	178,000
1012	28	M	27	MSM	No	910	633	6,330
2006	69	M	8	Hetero	No	215	329	248,000
1013	19	M	14	MSM	No	585	1,772	2,870
1014	40	M	22	Hetero	No	21	444	>750,000
1015	23	M	11	MSM	No	146	1,532	428,000
1016	23	M	11	MSM	No	372	1,049	426,000
2007	34	F	18	Hetero	No	10	144	34,600

* - MSM: men who have sex with men; Hetero: heterosexual; Bisex: bisexual.
** - NA: Not available.

Discussion

The identification of recent HIV infections is an extremely valuable way to evaluate the spread of the virus in a given population, especially when cohort studies, considered the gold standard method to evaluate incidence, are not available. Among the several observations, one could address important issues, such as the estimation of incidence rate, the characterization of the high-risk population, the profile of circulating viruses, and the identification of primary resistance to antiretroviral agents in patients recently infected by HIV-1.

We describe a successful collaborative work to identify recently-infected HIV-1 patients in the São Paulo metropolitan area, a region recognized as the Brazilian AIDS epidemic epicenter as a consequence of the high proportion (22%) of the AIDS cases reported to the Brazilian Ministry of Health [16]. Therefore, the identification of such patients will contribute to a better understanding of HIV-1 epidemic characteristics and dynamics in this area. It is necessary, however, to exert caution due to the potential inclusion of patients with very advanced disease, who can resemble recently infected subjects according to

STARHS. Hence, it is always advisable to obtain clinical and laboratory information to consider a recent infection case, using the proposed algorithm.

Patient laboratory findings were compatible with recently infected patients, who presented with high median CD_4^+ T lymphocyte counts (585 cells/µL) and low viral loads (11,000 HIV-RNA copies/mL). These results are in accordance with the findings of Fiebig et al. [17], who classified HIV-1 acute and recent infection in plasma donors in six distinct stages. Using the proposed criteria, patients identified with STARHS are classified as stage V or VI, in whom the median viral loads were 18,700 and 12,150 HIV-RNA copies/mL, respectively, similar to what was found in our study.

One of the major characteristics of the targeted patient population is that the majority is constituted of men who have sex with men (MSM). This could be explained by the special attention that the four participating sites have concentrated on the MSM population. In the city of São Paulo, MSM account for approximately one third of the overall reported AIDS cases, and this type of sexual activity is the main transmission route in the population served by the participating sites in this study [18]. New strategies have been designed and implemented to target other populations, including heterosexuals and intravenous drug users, considering their role in the city of São Paulo epidemic.

Recent HIV Infection Study Group Participants (by institution)

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