Short Communication: Current Prevalence and Risk Factors Associated with Human T Lymphotropic Virus Type 1 and Human T Lymphotropic Virus Type 2 Infections Among HIV/AIDS Patients in São Paulo, Brazil

Adele Caterino-de-Araujo,¹ Cláudio Tavares Sacchi,¹ Maria Gisele Gonçalves,¹
Karoline Rodrigues Campos,¹ Mariana Cavalheiro Magri,² Wong Kuen Alencar,³
and the Group of Surveillance and Diagnosis of HTLV of São Paulo (GSuDiHTLV-SP)

Abstract

During the 1990s, high prevalences of HIV/human T lymphotropic virus type 1 (HTLV-1) and HIV/human T lymphotropic virus type 2 (HTLV-2) coinfections were detected in São Paulo, Brazil in association with intravenous drug use (IDU). The current prevalences and risk factors for HIV/HTLV-1/2 were evaluated in 1,608 patients attending the AIDS/STD Reference and Training Center in São Paulo. Blood samples were analyzed for HTLV-1/2-specific antibodies using enzyme immunoassays (EIA Murex HTLV-I+II, Diasorin, and Gold ELISA HTLV-I+II, REM) and immunoblotting (HTLV Blot 2.4, MP Biomedicals and INNO-LIA HTLV-I/II, Innogenetics) and for the *pol* proviral DNA segments of HTLV-1 and HTLV-2 by “in-house” real-time PCR. These analyses revealed that 50 (3.11%) of the samples were HTLV positive, including 25 (1.55%) that were HTLV-1 positive, 21 (1.31%) that were HTLV-2 positive, and 4 (0.25%) that were HTLV positive (untypeable). The median age of the HIV/HTLV-coinfected individuals was 50 years versus 44 years in the overall population ($p=0.000$). The risk factors associated with HIV/HTLV-1/2 coinfections were female gender (OR 3.26, 1.78–5.95), black/*pardo* color (OR 2.21, 1.21–4.03), infection with hepatitis B virus (HBV) (OR 4.27, 2.32–7.87) or hepatitis C virus (HCV) (OR 24.40, 12.51–48.11), and intravenous drug use (IDU) (OR 30.01, 15.21–59.29). The current low prevalence of HTLV-1/2 in HIV-infected patients in São Paulo could be explained in part by programs providing IDUs with sterile needles and syringes and changes in the drug usage patterns of individuals from injecting cocaine to smoking crack cocaine.

Introduction

HUMAN T LYMPHOTROPIC VIRUSES types 1 and 2 (HTLV-1 and HTLV-2) and human immunodeficiency virus (HIV) share the same routes of viral transmission, but they have different clinical outcomes. HTLV-1 is the etiological agent of adult T cell leukemia/lymphoma and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM),¹ and HTLV-2, although it is not associated with a certain disease, has been detected in patients with some neurological disorders, similar to TSP/HAM.^{2–4} HIV is responsible for the AIDS world pandemic, and HIV/HTLV-1/2 coinfections have been associated with a delay or acceleration in the progression of cooc-

curing diseases.^{5–7} In 2012, the UNAIDS reported that there were an estimated 35.3 (32.2–38.8) million infected individuals living with HIV worldwide,⁸ and in Brazil, the Ministry of Health estimated that there were 686,478 AIDS cases from 1980 to June 2013.⁹ In addition, Brazil is the country in Latin America with the highest number of HTLV-1/2-infected individuals, which has been estimated to range from 300,000 to 800,000, with regional variations in rates depending on the genetic background and characteristics of the population.^{1,10}

Since the 1990s, the Instituto Adolfo Lutz (IAL), which is a Public Health Laboratory in São Paulo, has been conducting studies on the prevalence of HIV/HTLV-1/2 and has detected different percentages of positivity of HIV/HTLV-1

¹Centro de Imunologia, Instituto Adolfo Lutz, Secretaria de Estado da Saúde de São Paulo, São Paulo, SP, Brazil.

²Laboratório de Investigação Médica em Hepatologia por Vírus–LIM 47, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil.

³Vigilância Epidemiológica, Centro de Referência e Treinamento em DST/Aids de São Paulo, São Paulo, SP, Brazil.

FIG. 1. Representative profiles (patterns) obtained by blot analysis using the WB (HTLV Blot 2.4) and immuno line assays (INNO-LIA HTLV-I/II) in plasma samples obtained from HIV/human T lymphotropic virus (HTLV)-coinfecting patients from CRT DST/AIDS-SP in Brazil. The criteria used to determine reactivity for the HTLV Blot 2.4 (MP Biomedicals, USA) and INNO-LIA HTLV-I/II (Innogenetics, Belgium) were provided in the manufacturers' instructions and are described in Materials and Methods.

and HIV/HTLV-2. For instance, from 1992 to 1994, prevalences of 13.2% (7.8% for HTLV-1 and 5.4% for HTLV-2) and 10.1% (4.0% for HTLV-1 and 6.1% for HTLV-2) were reported for HIV/AIDS patients attending the Instituto de Infectologia Emílio Ribas in association with intravenous drug use (IDU).^{11,12} In contrast, in 2007, the AIDS Reference Centers in São Paulo reported a prevalence of 5.8% (3.3% for HTLV-1 and 2.5% for HTLV-2).¹³

This study assessed the current prevalences and risk factors of HTLV-1/2 infections in HIV-infected individuals attending the AIDS/STD Reference and Training Center in São Paulo (CRT DST/AIDS-SP) to conduct surveillance on the current status of these infections and to help physicians in performing accurate patient follow-ups.

Materials and Methods

A cross-sectional study was conducted with 1,608 HIV-infected patients attending the CRT DST/AIDS-SP, at which 5,000 patients were enrolled. The patients were invited to participate in the study during blood collection at the institution for CD4⁺ counting and HIV viral load determinations. After answering a questionnaire to provide demographic, clinical, and laboratory data and signing informed consent forms, a blood sample was collected at the same puncture site as the CD4⁺ count and sent to IAL for analysis. This study was approved by the Ethical Committees of the institutions attended by each participant with the protocol number CAAE 11302512.0.0000.0059.

Briefly, the blood sample was separated into plasma and peripheral blood leukocytes (PBLs). The plasma was screened for the presence of HTLV-1/2-specific antibodies by two enzyme immunoassays, Murex HTLV-I + II (Diasorin S.p.A, Dartford, UK) and Gold ELISA HTLV-I + II (REM, São Paulo, SP, Br) and confirmed by Western blot and immuno line assays (HTLV Blot 2.4, MP Biomedicals, Solon, OH and INNO-LIA HTLV I/II, Innogenetics Biotechnology for Healthcare, Ghent, Belgium). All serological assays were conducted according to the manufacturers' instructions, and the plasma samples were classified as HTLV-1 positive or HTLV-2 positive, HTLV positive (untypeable), or indeterminate according to the criteria of the respective serological confirmatory assays. For the Western blot (WB) 2.4, the HTLV-1-positive plasma samples were defined by the presence of reactivity to gag (p19 with or without p24) and to two env (GD21 and rgp46-I) bands, and HTLV-2-positive samples were defined by the presence of reactivity to gag (p24 with or without p19) and to two env (GD21 and rgp46-II) bands. Samples that demonstrated reactivity to both gag (p19 and p24) and env (GD21) were defined as HTLV positive (untypeable). Any other pattern of bands was considered indeterminate. The INNO-LIA strips contained antigens for validation, confirmation, and discrimination. For validation, the line for each sample was compared to the control lines, and a score was assigned that ranged from - to +3. The confirmatory antigens included gag p19 I/II, gag p24 I/II, env gp46 I/II, and env gp21 I/II. The presence of no bands or a single band (gag p19 I/II, gag p24 I/II, or env gp46 I/II)

TABLE 1. RESULTS OBTAINED BY HUMAN T LYMPHOTROPIC VIRUS SEROLOGICAL AND MOLECULAR CONFIRMATORY ASSAYS IN HIV/HUMAN T LYMPHOTROPIC VIRUS-COINFECTED PATIENTS FROM CRT DST/AIDS-SP, BRAZIL

<i>Patient code</i>	<i>WB 2.4^a</i>	<i>INNO-LIA^a</i>	<i>HTLV-1 PCR (C_t)</i>	<i>HTLV-2 PCR (C_t)</i>	<i>Albumin PCR (C_t)</i>	<i>Final Result^b</i>
1	HTLV-2	HTLV-2	0	0	32	HTLV-2
2	HTLV	HTLV	0	0	33	HTLV
3	HTLV-1	HTLV-1	30	0	26	HTLV-1
4	HTLV	HTLV	0	0	26	HTLV
5	HTLV-2	HTLV-2	0	0	30	HTLV-2
6	HTLV-1	HTLV-1	0	0	30	HTLV-1
7	Ind. (rgp46-I, p19, p24)	HTLV-1	0	0	26	HTLV-1
8	HTLV-2	HTLV-2	0	0	31	HTLV-2
9	HTLV-1	HTLV-1	35	0	25	HTLV-1
10	HTLV-1	HTLV-1	29	0	24	HTLV-1
11	HTLV-1	HTLV-1	0	0	30	HTLV-1
12	Ind. (rgp46-II, p24)	HTLV-2	0	39	27	HTLV-2
13	HTLV-1	HTLV-1	35	0	28	HTLV-1
14	HTLV-2	HTLV-2	0	0	29	HTLV-2
15	HTLV-1	HTLV-1	42	0	25	HTLV-1
16	HTLV	HTLV	37	0	28	HTLV-1
17	HTLV-2	HTLV-2	0	35	26	HTLV-2
18	HTLV	HTLV-2	0	0	28	HTLV-2
19	Ind. (GD21, p24)	HTLV-2	0	0	29	HTLV-2
20	HTLV-1	HTLV-1	0	0	29	HTLV-1
21	Ind. (GD21, p19)	HTLV	0	0	32	HTLV
22	HTLV-1	HTLV-1	31	0	25	HTLV-1
23	HTLV	HTLV	0	0	34	HTLV
24	HTLV-1	HTLV-1	37	0	26	HTLV-1
25	HTLV	HTLV-2	0	38	27	HTLV-2
26	HTLV-1	HTLV-1	0	0	31	HTLV-1
27	HTLV-1	HTLV-1	32	0	29	HTLV-1
28	Ind. (p24)	HTLV-2	0	0	27	HTLV-2
29	HTLV-1	HTLV-1	35	0	31	HTLV-1
30	HTLV-1	HTLV-1	30	0	26	HTLV-1
31	HTLV-1	HTLV-1	35	0	25	HTLV-1
32	HTLV-1	HTLV-1	37	0	28	HTLV-1
33	HTLV-1	HTLV-1	34	0	25	HTLV-1
34	HTLV-1	HTLV-1	0	0	25	HTLV-1
35	HTLV-1	HTLV-1	30	0	25	HTLV-1
36	HTLV-1	HTLV-1	38	0	28	HTLV-1
37	Ind. (GD21, p24)	HTLV-2	0	0	31	HTLV-2
38	HTLV-2	HTLV-2	0	33	27	HTLV-2
39	HTLV-1	HTLV-1	35	0	30	HTLV-1
40	Ind. (rgp46-II, p24)	HTLV-2	0	33	26	HTLV-2
41	HTLV-2	HTLV-2	0	33	29	HTLV-2
42	Ind. (rgp46-II, p24)	HTLV-2	0	37	28	HTLV-2
43	HTLV-2	HTLV-2	0	29	27	HTLV-2
44	HTLV-2	HTLV-2	0	40	29	HTLV-2
45	Ind. (GD21, p24)	HTLV	0	40	35	HTLV-2
46	HTLV-1	HTLV-1	0	0	36	HTLV-1
47	HTLV-2	HTLV-2	0	39	32	HTLV-2
48	HTLV-2	HTLV-2	0	0	39	HTLV-2
49	HTLV-1	HTLV-1	36	0	30	HTLV-1
50	HTLV-2	HTLV-2	0	32	31	HTLV-2

^aResults obtained by WB (HTLV Blot 2.4, MP Biomedicals) and immuno line assay (INNO-LIA HTLV-I/II, Innogenetics) according to manufacturers' criteria.

^bFinal result obtained by WB and/or INNOL-LIA and/or real-time PCR assays.

HTLV, human T lymphotropic virus; PCR, polymerase chain reaction; Ind., indeterminate; C_t, cycle threshold value.

denoted negative results, one band (env gp21 I/II) or two bands (except env gp21 I/II) indicated indeterminate results, and two bands (env gp21 I/II and gag p19 I/II, gag p24 I/II, or env gp46 I/II) indicated HTLV positivity. Three discriminatory bands were taken into consideration, including gag

p19-I, env gp46-I, and env gp46-II, and the criteria for HTLV-1 positivity were the presence of gag p19-I and env gp46-I. Samples containing env gp46-II or an env gp46-II band intensity that was greater than those of gag p19-I and env gp46-I were considered HTLV-2 positive. The

representative plasma sample profiles obtained by WB 2.4 and INNO-LIA are presented in Fig. 1.

The PBLs from all of the patients showing reactive results on the screening assays were analyzed for the presence of HTLV-1/2 proviral DNA by “in-house” real-time polymerase chain reaction (PCR), as previously described. This PCR employed probes specific for the *pol* segments of HTLV-1 and HTLV-2, albumin as an internal control, and a *TaqMan* system (Applied Biosystems, USA). The results were expressed as C_t values (cycle threshold). The sensitivities of this assay for detecting HTLV-1, HTLV-2, and albumin were 10 ng, 1 ng, and 10 pg of DNA, respectively.¹⁴

HIV-infected patients were considered coinfecting with HTLV-1, HTLV-2, or HTLV untypeable when their blood sample showed positive results by at least one confirmatory assay.

Statistical analysis

The laboratory results and the questionnaire data were recorded and analyzed with Epi Info version 3.5.4 software. Comparative analysis of assays was conducted to determine the sensitivity of each assay, taking into consideration the final HTLV result (serological and/or molecular). Differences related to the demographic variables were evaluated statistically by Chi-squared or Fisher's exact tests as appropriate. A p value of ≤ 0.05 was considered significant. Logistic regression analysis was used to identify the factors associated with HTLV-1/2 infection by calculating the odds ratios (ORs) and 95% confidence intervals (CIs).

Results

Considering the findings of all HTLV-1/2 laboratory analyses, 50 out of 1,608 HIV-infected individuals (3.11%) were confirmed to have HTLV coinfection, of whom 25 (1.55%) were positive for HTLV-1, 21 (1.31%) for HTLV-2, and 4 (0.25%) for HTLV untypeable. Table 1 presents the results obtained in each confirmatory assay. Considering the final HTLV results, the sensitivities of the INNO-LIA, WB 2.4, and real-time PCR confirmatory assays were 96%, 76%, and 60%, respectively.

Table 2 presents the characteristics of the HIV/HTLV-coinfected individuals, the percentage of individuals receiving highly active antiretroviral therapy (HAART), and the sensitivity of each confirmatory assay according to the type of HTLV. Older age, female gender, black/*pardo* color, IDU or partner of IDU, and infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) were the more frequent characteristics observed in this population.

Figure 2 shows the percentages of individuals tested in the present study according to age group. Differences among individuals in the overall population and also among the HIV/HTLV-coinfected individuals were detected. Although the median age was 44 years (range of 19 to 83 years) in the overall population, and 63.5% of the individuals were over 40 years of age, among those that were HIV/HTLV coinfecting, the median age was 50 years (range of 28 to 68), and 96% of these individuals were >40 years of age ($p=0.000$).

The risk factors associated with HTLV-1/2 infection in HIV-infected individuals are presented in Table 3, confirming that female gender, black/*pardo* color, IDU, and hepatitis

TABLE 2. DEMOGRAPHIC, CLINICAL, AND LABORATORY CHARACTERISTICS OF HIV/HUMAN T LYMPHOTROPIC VIRUS-COINFECTED PATIENTS FROM CRT DST/AIDS-SP, BRAZIL

Demographic, clinical, and laboratory characteristics of patients	HTLV-1 ^a (n = 25)	HTLV-2 ^a (n = 21)	HTLV ^a (n = 4)
Age in years ^b	49.3 (28–66)	50.6 (45–68)	50.5 (48–53)
Gender ^c			
Male	15 (60%)	9 (52.9%)	2 (50%)
Female	10 (40%)	12 (47.1%)	2 (50%)
Color ^c			
Black/ <i>pardo</i>	14 (56%)	11 (53.4%)	2 (50%)
White	8 (32%)	9 (42.8%)	2 (50%)
Yellow	3 (12%)	1 (4.8%)	0
Exposure to HIV ^c			
Sexual	5 (20%)	1 (4.8%)	1 (25%)
IDU	11 (44%)	11 (53.4%)	1 (25%)
Partner IDU	3 (12%)	7 (33.3%)	2 (50%)
Blood transfusion	3 (12%)	0	0
Others	3 (12%)	2 (9.5%)	0
HCV coinfection ^c	17 (68%)	15 (71.4%)	3 (75%)
HBV coinfection ^c	12 (48%)	9 (42.8%)	1 (25%)
Use of HAART ^c	23 (92%)	21 (100%)	3 (75%)
WB + ^c	23 (92%)	12 (57.1%)	3 (75%)
INNO-LIA + ^c	24 (96%)	20 (95.2%)	4 (100%)
PCR + ^c	18 (72%)	12 (57.1%)	2 (1 HTLV-1 and 1 HTLV-2)

^aFinal result obtained by WB, INNO-LIA, and/or real time PCR assays as described in the Materials and Methods section.

^bMedian (minimum and maximum).

^cNumber (%).

n, number of individuals; IDU, intravenous drug user; HCV, hepatitis C virus; HBV, hepatitis B virus; HAART, highly active antiretroviral therapy; WB, Western blot; PCR, polymerase chain reaction.

FIG. 2. Percentages of overall HIV-infected and HIV/human T lymphotropic virus type 1/type 2 (HTLV-1/2)-coinfected patients from CRT DST/AIDS-SP, Brazil according to age group.

B virus (HBV) and hepatitis C virus (HCV) infections are risk factors for HTLV infection.

Discussion

The results of this study confirm that the rate of HIV/HTLV coinfection in São Paulo has steadily declined in the past decades, from 13.2% in 1992 to 10.1% in 1994 to 5.8% in 2007, and it is presently 3.1%.¹¹⁻¹³ In addition, our results demonstrated that IDU remains the major risk factor for HTLV infection in these patients.

Interestingly, the majority of the HIV/HTLV-coinfected patients in the present study reported that they had become HIV infected between 1990 and 1991 (data not shown) and that they had shared syringes and needles while injecting illicit drugs (cocaine) or had participated in sexual intercourse with an IDU. Thus, it could be hypothesized that they acquired HIV and HTLV at least 25 years ago by the above-

mentioned exposure routes. In agreement with this hypothesis, the majority of the HIV/HTLV-coinfected patients (96%) were over 40 years old.

The decrease in the percentage of HIV/HTLV coinfection during the past decades could be explained in part by the Brazilian response to the HIV/AIDS epidemic among IDUs, including the establishment of harm reduction programs that provide IDUs with sterile needles and syringes. The city of Santos in São Paulo state was the first to attempt to control the spread of HIV/AIDS between and from IDUs in 1989, and subsequently several harm reduction projects have been politically and financially supported by the federal government of Brazil.¹⁵ Another important factor that has contributed to the decrease in HIV/AIDS and HTLV infections among IDUs is an altered pattern of drug use (from injecting cocaine to smoking crack cocaine). One study conducted in Santos during the 1990s pointed out that when cocaine injection decreased and crack cocaine use increased, the prevalence of HIV infection decreased in this geographic area.¹⁶ It is likely that the same situation occurred with the HTLV infections in São Paulo.

Notably, serology for HTLV has been mandatory in the blood banks of Brazil since 1993, and a reduction in the percentage of positive cases among blood donors has been observed since then. Two studies that have been conducted during different periods assessing blood donors from São Paulo have reported prevalences of 0.32% and 0.1% from 1995 to 2000 and from 2007 to 2009, respectively.^{17,18} Until now, the only study of HTLV prevalence in the general population of Brazil was conducted in Salvador, Bahia,¹⁹ preventing comparative analysis between the general population and the HIV-infected population from São Paulo. Future studies investigating whether the decrease in HTLV prevalence in the general population accounts for the reduction in HTLV prevalence among HIV-infected individuals are warranted.

Another interesting result in the present study was the equal distribution of HTLV-1 and HTLV-2 in the HIV-coinfected patients, which differs from data reported by other AIDS services in Brazil. For example, the majority of HIV/HTLV-coinfected individuals from Salvador, Bahia (northeast Brazil) are HIV/HTLV-1 coinfecting,^{5,6} while in Londrina, Paraná (southeast Brazil), the majority are HIV/HTLV-2 coinfecting.²⁰ These differences are probably due to the genetic backgrounds of the populations who colonized these regions and to the different categories of HTLV exposure. Because São Paulo (the largest city in Latin America) has a mixed population and continuously receives migrants and immigrants from all foreign countries, both HTLV-1 and HTLV-2 circulate in this region.

The identification of female gender, black/*pardo* color, and infection with HBV and HCV as risk factors for HTLV infection supports the findings of previous studies conducted in other parts of Brazil and elsewhere.^{1,5,6,10,21-24} Females are more vulnerable to HTLV infection/disease^{1,10} as well individuals of low income and black/*pardo* individuals.^{1,10,19} In addition, females could acquire HTLV infection during unprotected sex in exchange for drugs. The strong association between HTLV and HCV is due to the fact that these viruses share the same route of transmission.²²⁻²⁵

Concerning the sensitivities of the assays for detecting HTLV-1 and HTLV-2 in HIV-infected individuals, we confirmed the results of previous studies conducted in our

TABLE 3. RISK FACTORS ASSOCIATED WITH HUMAN T LYMPHOTROPIC VIRUS-1/2 INFECTION IN HIV-INFECTED PATIENTS FROM CRT/DST AIDS-SP, BRAZIL

Characteristics of patients	HIV (n = 1558)	HIV/HTLV (n = 50)	OR (95% CI)
Age in years ^a	44 (19–83)	50 (28–68)	
Gender ^b			
Male	1214 (77.9%)	26 (52%)	0.31 (0.17–0.56)
Female	344 (22.1%)	24 (48%)	3.26 (1.78–5.95)
Color ^b			
Black/ <i>pardo</i>	541 (34.7%)	27 (54%)	2.21 (1.21–4.03)
White	958 (61.5%)	19 (38%)	0.38 (0.21–0.71)
Yellow	59 (3.8%)	4 (8%)	
IDU ^b	43 (2.8%)	23 (46%)	30.01 (15.21–59.29)
HCV coinfection ^b	136 (8.7%)	35 (70%)	24.40 (12.51–48.11)
HBV coinfection ^b	242 (15.5%)	22 (44%)	4.27 (2.32–7.87)

^aMedian (minimum and maximum).

^bNumber (%).

n, number of individuals; IDU, intravenous drug user; HCV, hepatitis C virus; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval.

laboratory that have demonstrated difficulties in diagnosing HTLV-2 in HIV/AIDS patients.^{11–14,20} The low sensitivity of the PCR assay in the present study was not due to low-quality DNA samples because the internal albumin control showed positive results. This finding may be explained by the use of HAART, which caused the viral load of HIV and probably that of HTLV to be under the detection limit of the respective assays used, as previously described.^{14,26} In fact, all except three patients had detectable HIV viral loads in the present study (data not shown).

In contrast, the INNO-LIA was shown to be more efficient in confirming and discriminating between HTLV-1 and HTLV-2 in HIV-infected individuals. These data corroborate those from one pilot study conducted in our laboratory, showing the higher capacity of INNO-LIA to discriminate between HTLV-1 and HTLV-2 in the blood samples showing indeterminate results via WB 2.4 analysis (data not published). One explanation for this finding could be that the antigens spotted onto the nylon strips of the INNO-LIA kit were more sensitive in detecting antibodies against anti-Brazilian HTLV-2 isolates or that this kit has less stringent criteria for determining HTLV positivity.

Interestingly, a reduction in HTLV-indeterminate results detected by WB 2.4 was found in the present study compared with previous studies conducted in our laboratory of HIV-infected patients. In the current study, 0.6% of WB-indeterminate samples were detected in contrast to the detection of 3.2%, 4.3%, and 5.4% of these samples in previous studies.^{11–13} One explanation for these findings could be the improved screening kits employed in the present study, which use a sequential antigen sandwich ELISA based on recombinant proteins and synthetic peptides of HTLV-1 and HTLV-2 selected to maximize specificity and sensitivity to both of these viral types.

Although real-time PCR showed a lower sensitivity than the WB and INNO-LIA assays, PCR was able to confirm HTLV infection in two serological HTLV-positive (untypeable) samples (one HTLV-1 and one HTLV-2, Table 1). Thus, in patients infected with HIV, INNO-LIA and PCR are complementary assays.

In conclusion, the current prevalence of HTLV-1/2 in HIV patients attending CRT DST/AIDS-SP is 3.11%. HCV and IDU were strongly associated with HIV/HTLV-1/2 coinfections. Changes in the behavior of HIV-infected individuals in Brazil during the AIDS epidemic could partially account for the obtained results.^{9,15,16} Concerns pertaining to the shift from injecting cocaine to smoking crack cocaine deserve the attention of public health authorities. Unprotected sex in exchange for drugs could account for the increased incidence of HTLV and other sexually transmitted diseases, which mainly occurred in women. The best laboratory algorithm for detecting HTLV infection in HIV/AIDS patients seems to be the use of both the INNO-LIA and PCR assays. We are now expanding our studies of HIV-infected individuals to other AIDS Reference Centers in São Paulo to confirm and propose the best laboratory algorithm for the diagnosis of HTLV infection in HIV/AIDS patients in Brazil.

Acknowledgments

This study was supported by grants from the Ministério da Saúde/SVS/DDAHV BRAK57 #CA 125/13; DECIT/SCITIE/

MS, CNPq, FAPESP, SES-SP/PPSUS #2012/51220-8; FAPESP IC #2013/19775-2, FAPESP TT3 #2013/21014-0, and TT3 #2014/15845-9; and CNPq PD #303545/2012-7.

The Group for the Surveillance and Diagnosis of HTLV of São Paulo (*GSuDiHTLV-SP*) is composed of other members who contributed to some of the phases of this study, including Alexandre de Almeida, Carlos H. Barreto-Damião, Fábio T. Higa, Lucila O. Fukasawa, Luana P.O. Coelho, Luis F.M. Brígido, Marcela B. Santana, Maristela M. Salgado, Nadia A. Costa (Instituto Adolfo Lutz), Leda F. Jamal, Maria de Fátima Jorge, Maria Lúcia R. Mello, Risia C.S. Oliveira (CRTA), and Telma M. Oshiro (FMUSP).

The authors are indebted to Terezinha Pereira de Araujo and Lúcia Cupertino Barreto for their technical assistance and to Elisabete Amorim Leandro Lima and Márcia Maria Gonçalves Ribeiro for their management support.

Author Disclosure Statement

No competing financial interests exist.

References

- Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, and Murphy EL: Global epidemiology of HTLV-1 infection and associated diseases. *Oncogene* 2005;24:6058–6068.
- Orland JR, Engstrom J, Friley J, *et al.*: Prevalence and clinical features of HTLV neurologic disease in the HTLV outcomes study. *Neurology* 2003;61:1588–1594.
- Roucoux DF and Murphy EL: The epidemiology and disease outcomes of human T-lymphotropic virus type II. *AIDS Rev* 2004;6:144–154.
- Posada-Vergara MP, Montanheiro P, Fukumori LMI, *et al.*: Clinical and epidemiological aspects of HTLV-II infection in São Paulo, Brazil: Presence of tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) simile diagnosis in HIV-1-co-infected subjects. *Rev Inst Med Trop S Paulo* 2006;48:207–210.
- Brites C, Alencar R, Gusmão R, *et al.*: Co-infection with HTLV-1 is associated with a shorter survival time for HIV-1-infected patients in Bahia, Brazil. *AIDS* 2001;15:2053–2055.
- Brites C, Sampaio J, and Oliveira A: HIV/human T-cell lymphotropic virus coinfection revisited: Impact on AIDS progression. *AIDS* 2009;11:8–16.
- Beilke MA: Retroviral coinfections: HIV and HTLV: Taking stock of more than a quarter century of research. *AIDS Res Hum Retroviruses* 2012;28:139–147.
- Global report–UNAIDS report on the global Aids Epidemic 2013: Available at www.unaids.org/globalreport/HIV_prevalence_map.htm.
- HIV–AIDS, Boletim Epidemiológico, Brasília, 2013: Ano II–nº 01 até semana epidemiológica 26ª–dezembro de 2013.
- Gessain A and Cassar O: Epidemiological aspects and world distribution of HTLV-1 infection. *Front Microbiol* 2012;3:article 388.
- De-Araujo AC, Casseb JSR, Neitzert E, *et al.*: HTLV-I and HTLV-II infections among HIV-1 seropositive patients in São Paulo, Brazil. *Eur J Epidemiol* 1994;10:165–171.
- Caterino-de-Araujo A, Santos-Fortuna E, Zandoná-Meleiro MC, *et al.*: Sensitivity of two ELISA tests in relation to Western blot in detecting HTLV-I and HTLV-II infections among HIV-1-infected patients from São Paulo, Brazil. *Diagn Microbiol Infect Dis* 1998;30(3):173–182.

13. Jacob F, Santos-Fortuna E, Azevedo RS, and Caterino-de-Araujo A: Serological patterns for HTLV-I/II and its temporal trend in high-risk populations attended at Public Health Units of São Paulo, Brazil. *J Clin Virol* 2008; 42(2):149–155.
14. Costa EAS, Magri MC, and Caterino-de-Araujo A: The best algorithm to confirm the diagnosis of HTLV-1 and HTLV-2 in at-risk individuals from São Paulo, Brazil. *J Virol Methods* 2011;173:280–286.
15. Mesquita F, Doneda D, Gandolfi D, *et al.*: Brazilian response to the human immunodeficiency virus/acquired immunodeficiency syndrome epidemic among injection drug users. *Clin Infect Dis* 2003;37(Suppl 5):S382–S385.
16. Mesquita F, Kral A, Reingold A, *et al.*: Trends of HIV infection among injection drug users in Brazil in the 1990s: The impact of changes in patterns of drug use. *J Acquir Immune Defic Syndr* 2001;28:298–302.
17. Catalan-Soares B, Carneiro-Proietti ABF, Proietti FA, and the Interdisciplinary HTLV Research Group: Heterogeneous geographic distribution of human T-cell lymphotropic viruses I and II (HTLV-I/II): Serological screening prevalence rates in blood donors from large urban areas in Brazil. *Cad Saúde Pública*, Rio de Janeiro 2005;21:926–931.
18. Carneiro-Proietti ABF, Sabino EC, Leão S, *et al.*: Human T-lymphotropic virus type 1 and type 2 seroprevalence, incidence, and residual transfusion risk among blood donors in Brazil during 2007–2009. *AIDS Res Hum Retroviruses* 2012;28:1265–1272.
19. Dourado I, Alcantara LC, Barreto ML, *et al.*: HTLV-I in the general population of Salvador, Brazil: A city with African ethnic and sociodemographic characteristics. *J Acquir Immune Defic Syndr* 2003;34:527–531.
20. Morimoto HK, Morimoto AA, Reiche EMV, *et al.*: Difficulties in the diagnosis of HTLV-2 infection in HIV/AIDS patients from Brazil. Comparative performances of serologic and molecular assays, and detection of HTLV-2b subtype. *Rev Inst Med Trop S Paulo* 2007;49(4): 225–230.
21. Dourado I, Andrade T, Carpenter CL, and Galvão-Castro B: Risk factors for human T cell lymphotropic virus type I among injecting drug users in Northeast Brazil: Possibly greater efficiency of male to female transmission. *Mem Inst Oswaldo Cruz* 1999;94:13–18.
22. Etzel A, Shibata GY, Rozman M, *et al.*: HTLV-1 and HTLV-2 infections in HIV-infected individuals from Santos, Brazil: Seroprevalence and risk factors. *J Acquir Immune Defic Syndr* 2001;26:185–190.
23. Segurado AC, Braga P, Etzel A, and Cardoso MRA: Hepatitis C virus coinfection in a cohort of HIV-infected individuals from Santos, Brazil: Seroprevalence and associated factors. *AIDS Patient Care STDs* 2004;18(3): 135–143.
24. Morimoto HK, Caterino-de-Araujo A, Morimoto AA, *et al.*: Seroprevalence and risk factors for human T-cell lymphotropic virus type 1 and 2 infection in human immunodeficiency virus (HIV)-infected patients attending AIDS Referral Center Health Units in Londrina and other communities in Paraná, Brazil. *AIDS Res Hum Retroviruses* 2005;21(4):256–262.
25. Moreira M, Ramos A, Netto EM, and Brites C: Characteristics of co-infections by HCV and HBV among Brazilian patients infected by HIV-1 and/or HTLV-1. *Brazil J Infect Dis* 2013;17(6):661–666.
26. Machuca A and Soriano V: In vivo fluctuation of HTLV-I and HTLV-II proviral load in patients receiving anti-retroviral drugs. *J Acquir Immune Defic Syndr* 2000;24: 189–193.

Address correspondence to:

Adele Caterino-de-Araujo

Centro de Imunologia

Instituto Adolfo Lutz

Av. Dr. Arnaldo, 355, 11º andar

01246-902, São Paulo, SP

Brazil

E-mail: caterino@ial.sp.gov.br; caterino@usp.br