

## The first survey of human T-cell lymphotropic viruses (HTLV) in HIV/AIDS patients in Santa Catarina State, Brazil

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### ABSTRACT

Santa Catarina is a Brazilian State that has reported the lowest prevalence of human T-cell lymphotropic viruses (HTLV-1/2) in blood donors (0.04%). Although it presents ports, airports and roads that facilitate the entrance and dissemination of new infectious agents, no information exists concerning the HTLV-1/2 infections in HIV/AIDS patients. This study searched for HTLV-1/2 antibodies in plasma samples of 625 HIV/AIDS patients from the municipality of Tubarao (Southern Santa Catarina), and disclosed 1.1% of positivity (0.48% HTLV-1, 0.48% HTLV-2 and 0.16% untypeable HTLV), and a positive correlation with the male sex (OR 4.16) and intravenous drug use (OR 35.18). Although the percentage of 1.1% appears to be low, it is 27.5 times higher than the percentage detected in blood donors. Since HTLV-1 and HTLV-2 are circulating in HIV-infected individuals in Southern Santa Catarina, and these retroviruses could cause a differently impact on the HIV/AIDS outcomes, the surveillance of HTLV-1/2 is necessary, and it could support public health policies in preventing the transmission and dissemination of these viruses in this State.

**KEYWORDS:** HTLV-1. HTLV-2. HIV. Santa Catarina State. Brazil.

### INTRODUCTION

The first retrovirus identified in human beings, named human T-cell lymphotropic virus type 1 (HTLV-1), has been the etiological agent of at least two important diseases of high lethality and morbidity: the adult T-cell leukemia/lymphoma (ATLL) and the HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)<sup>1,2</sup>. The second human retrovirus, named HTLV-2, although originally isolated from a patient with hairy T-cell leukemia, was later not confirmed to be associated with the specific disease, making its pathogenicity questionable<sup>1</sup>. Nonetheless, when these human retroviruses are associated with HIV, they are claimed to affect the HIV patient's progression towards AIDS. HTLV-1 has been associated with the rapid progression and death due to AIDS<sup>3,4</sup>, whereas the HTLV-2 has been associated with slow progression towards AIDS<sup>5</sup>. Thus, the search for HTLV-1/2 in HIV-infected individuals has a prognostic value.

Brazil, a country of more than 200 million inhabitants, is an important endemic area for HTLV-1 infection and associated diseases in the world, estimated to 800,000 HTLV-1-infected people<sup>2</sup>. HTLV-2 is endemic among the indigenous populations of the Brazilian Amazon region and among intravenous drug users (IDU) in urban areas, co-infected or not with HIV<sup>6</sup>. The Brazilian Ministry of Health recommends HTLV-1/2 serological testing at the beginning

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of HIV-1-infected patients' follow-up, mostly in regions where the HTLV-1 is endemic<sup>7</sup>.

Despite having a heterogeneous geographic distribution in Brazil, HTLV-1/2 discloses the lowest prevalence rate in blood donors (0.4/1.000 blood donors or 0.04%) in the Santa Catarina State<sup>8,9</sup>, which has 47,461 cases of AIDS. The data collected during the period from 2000 to 2017 showed an AIDS notification rate of 26.8 cases per 100,000 inhabitants, which is higher than the national rate (18.3 per 100,000 inhabitants), described during this period<sup>10,11</sup>.

There are no data available regarding HTLV-1 and HTLV-2 in HIV-1-infected individuals from Santa Catarina State. Considering that the airports, ports and roads of this State facilitate the entrance and dissemination of new infectious agents, as occurred with the HIV-1 subtype C in Brazil<sup>12</sup>, a survey on HTLV-1/2 infection is necessary and relevant.

## MATERIALS AND METHODS

A cross-sectional study on HTLV-1/2 serology was conducted by analyzing plasma samples from 625 HIV/AIDS patients with a mean age of 43 years (range 18-84 years, 59.4% men) attending a specialized HIV/AIDS diagnosis and treatment center in the municipality of Tubarao (Centro de Atendimento Especializado em Saude - CAES). The CAES has registered 1,417 patients, including those with tuberculosis and hanseniasis, encompassing 18 municipalities of Southern Santa Catarina: Armazem, Braco do Norte, Capivari de Baixo, Grao Para, Gravatal, Imarui, Imbituba, Jaguaruna, Laguna, Pedras Grandes, Pescaria Brava, Rio Fortuna, Sangao, Santa Rosa de Lima, Sao Ludgero, Sao Martinho, Treze de Maio and Tubarao.

Blood samples were collected from the patients from October 2016 to July 2017 to determine CD4 and CD8 cell counts and HIV viral load quantification, and only plasma samples were stored in a repository for further analysis. The socio-demographic data and the potential risk factors for HIV infection were obtained from a standardized individual questionnaire, which was applied by a trained interviewer. Data concerning the number of CD4 cell counts, HIV viral load quantification, highly active antiretroviral therapy (HAART) usage, and clinical manifestations were obtained from medical records. The stored plasma samples were sent to the Instituto Adolfo Lutz (IAL), a Public Health Laboratory in Sao Paulo, for the detection of HTLV-1/2 antibodies. These samples were analyzed after obtaining the informed consent from patients, and the protocol approval from the Ethic Committees of both institutions [IAL, CAAE N° 96885118.6.3001.0059, and Universidade do Sul de Santa Catarina (UNISUL) and CAES, CAAE

N° 96885118.6.0000.5369]. All procedures were performed in accordance with the Resolution 466/2012 of the Brazilian National Health Council (CNS) on Ethics in Research with Human Beings.

The plasma samples were screened for HTLV-1/2 antibodies at the HTLV Research Laboratory of the Immunology Department of IAL by using an enzyme linked-immunosorbent assay Gold ELISA HTLV-1/2 (REM Industria e Comercio LTDA, Sao Paulo, Brazil), and confirmed by Western Blot (WB) (HTLV BLOT 2.4, MP Biomedicals, Asia Pacific Pte Ltd, Singapore) and line Immunoassay (INNO-LIA HTLV I/II, Fujirebio, Europe N.V, Belgium). Reactions were performed and interpreted according to the manufacturer's instructions. Noteworthy, HTLV-1/2 molecular confirmatory assay was not performed because of the lack of cells for analysis.

## RESULTS

The screening assay detected eight reactive samples. HTLV-1/2 infection was confirmed in seven samples (1.1%): HTLV-1 (0.48%), HTLV-2 (0.48%), and HTLV positive but untypeable (0.16%). An association between male individuals and IDUs was observed (Table 1). In relation to the age, although the coinfecting patients had a mean age of 49.7 years old, their HIV-mono-infected counterparts had a mean age of 42.9, but no statistic difference was observed. However, six out of seven HIV/HTLV-coinfecting patients were aged 48 to 60 years old; and only one was 29 years old. The distribution of patients according to the age group is in Figure 1, emphasizing that the majority of HIV/HTLV-coinfecting patients were aged 51 to 60 years old.

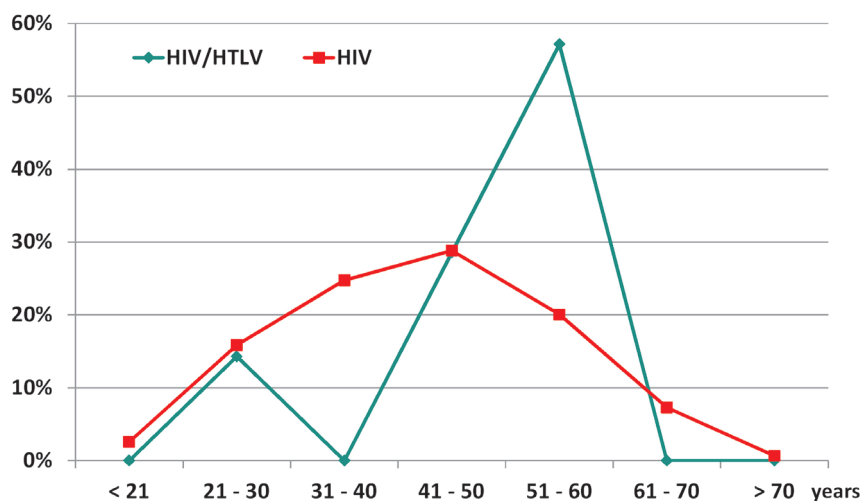
Of note, among HIV/AIDS patients 88.1% were on HAART and 74.1% had undetectable HIV viral load. Among HIV/HTLV-coinfecting patients, all except one were on HAART and had undetectable HIV viral load. The naïve patient was a man of 52 years old, IDU, HIV/HCV/HTLV-1-coinfecting, with a CD4 count of 273 cells/mm<sup>3</sup> and HIV viral load of 77,491 copies/mL, and he died of pneumonia. Another coinfecting patient who died during follow-up was the 29 years old man. Curiously he was HIV/HTLV-2-coinfecting, denied IDU, had a CD4 count of 635 cells/mm<sup>3</sup> and undetectable HIV viral load; had a history of syphilis, tuberculosis and pneumocystosis, and died after a non characterized heart attack. The other coinfecting patients remain alive, on HAART, and with HIV undetectable viral load. None HIV/HTLV-coinfecting patient presented any HTLV-associated neurological diseases.

Comparative analysis of CD4 cell counts among HIV/AIDS mono-infected patients (mean of 507.5 cells/mm<sup>3</sup>) and coinfecting patients (mean of 403.0 cells/mm<sup>3</sup> in HIV/

**Table 1** - Demographic characteristics (number, age, sex and risk factors) of overall HIV-infected and HIV/HTLV-1/2-coinfected patients from Tubarao, Santa Catarina State, Brazil.

Demographic characteristics of patients	HIV (n=618)	HTLV			OR (CI 95%)
		HTLV-1 (n=3)	HTLV-2 (n=3)	HTLV (n=1)	
<b>Age in years<sup>1</sup></b>					
Female	42.7	60.0	-	-	
Male	43.0	50.0	43.0	59.0	
<b>Sex<sup>2</sup></b>					
Female	253 (40.9)	1 (14.3)	-	-	
Male	365 (59.1)	2 (28.6)	3 (42.8)	1 (14.3)	4.16 (0.50 - 92.22)
<b>Exposure to HIV<sup>2</sup></b>					
Sexual	433 (70.0)	1 (14.3)	1 (14.3)	-	
IDU	41 (6.6)	2 (28.6)	2 (28.6)	1 (14.3)	35.18 (5.80 - 271.11)
Mother-to-child	11 (1.8)	-	-	-	
Blood transfusion	5 (0.8)	-	-	-	
Accident with blood	15 (2.4)	-	-	-	
Unknown	113 (18.3)	-	-	-	

<sup>1</sup>Median; <sup>2</sup>Number (%); n: number of individuals; IDU: intravenous drug user; OR: odds ratio; CI: confidence interval of 95%.



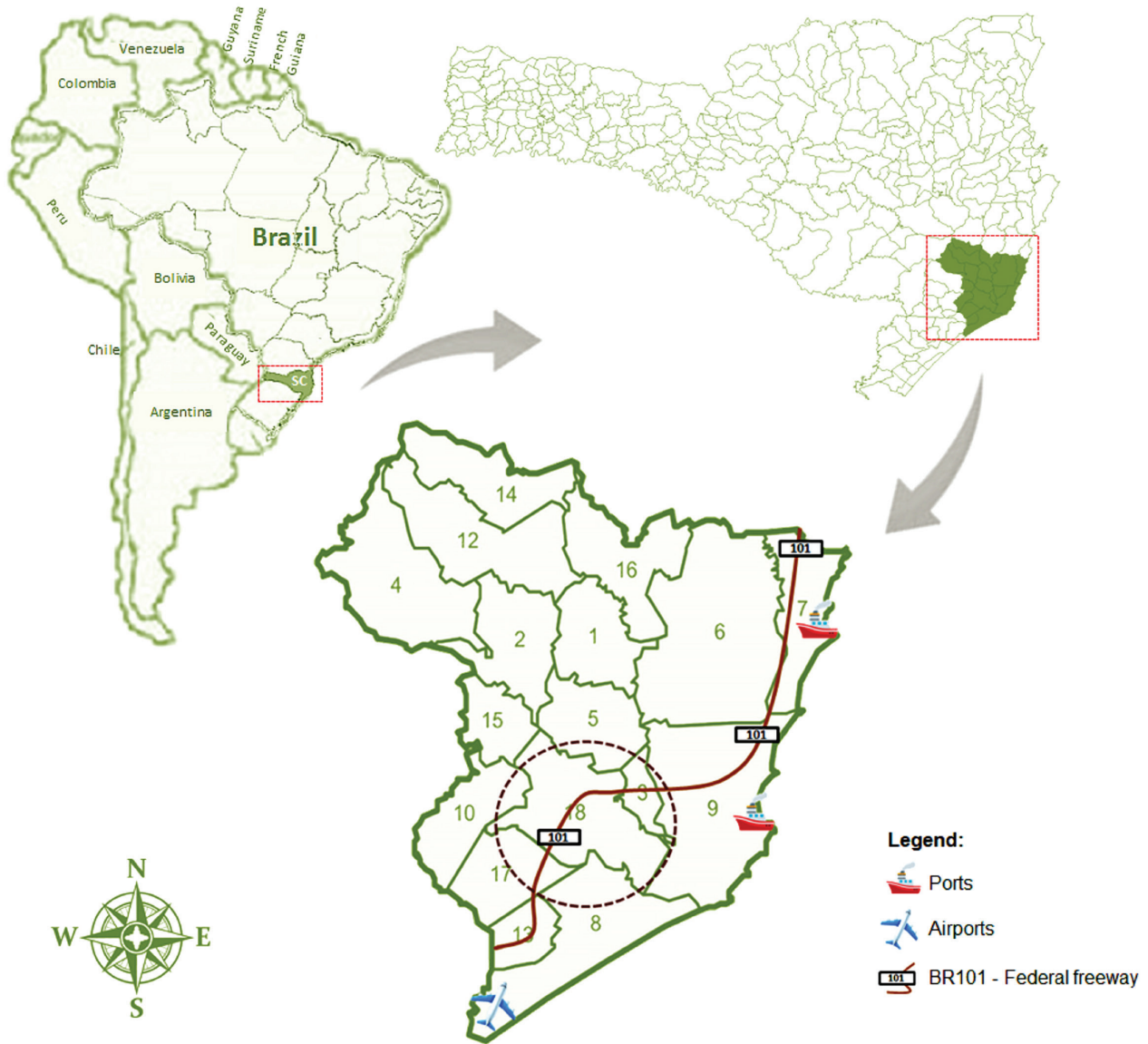
**Figure 1** - Percentages of overall HIV-infected and HIV/HTLV-1/2-coinfected patients from Southern Santa Catarina, Brazil, according to the age group.

HTLV-1, 475.0 cells/mm<sup>3</sup> in HIV/HTLV-2, and 145.0 cells/mm<sup>3</sup> in HIV/HTLV co-infected patient) were not feasible because of the low number of HIV/HTLV-1/2-coinfected individuals. Thus, neither clinical nor statistical correlations were performed.

## DISCUSSION

Although the percentage of 1.1% of HTLV-1/2 seropositivity appears to be low, it is 27.5 times higher than the positivity detected in blood donors in Santa Catarina State<sup>8,9</sup>, and this is of concern. In addition, both HTLV-1 and

HTLV-2 circulate in this region, and this is somewhat similar to that observed in Sao Paulo State where both, HTLV-1 and HTLV-2 were detected in HIV/AIDS patients<sup>13-15</sup>. In fact, this could be due in part to the mixed population living in Sao Paulo, which is composed of immigrants and migrants from elsewhere in the country and in the same way, this is the same situation found in Southern Santa Catarina. This region has an airport, two ports (Imbituba and Laguna), and the federal freeway BR101 beside others, allowing the contact with foreign people within Brazil, coming from Latin American countries and abroad (Figure 2). In addition, the transmission route and the age of HIV/HTLV-coinfected patients from



**Figure 2** - Maps of the Latin America, Brazil, and Santa Catarina State, with emphasis on the Laguna region, and the 18 municipalities from where blood samples were collected. Location of the airport, the ports [Imbituba (North) and Laguna (South)], the freeway BR101 and the municipality of Tubarao are depicted.

Sao Paulo and Southern Santa Catarina are quite similar. In both States, there was a strong association with IDU (OR 30.01 and OR 35.18, respectively), and the mean age of 50 years old was observed<sup>13</sup>. Interestingly, the only patient of 29 years old acquired HIV and probably HTLV-2 by sexual route, and although on HAART, presenting undetectable viral load and a good CD4 cells count, he died as a consequence of a fulminant heart attack, probably due to lipodystrophy.

Worthy of note, only one study concerning HTLV-1/2 in at-risk individuals from Santa Catarina is available. It was conducted by our group, analyzing 90 female sex workers from the same geographical region of this study. One HTLV-1-positive pregnant woman was detected, and she was advised not to breastfeed her offspring<sup>16</sup>.

Other studies from Southern Brazil were carried out in the municipality of Londrina, Parana State, Brazil. High percentages of HIV/HTLV-coinfections in 758 HIV/AIDS patients (5.7%) were found, and there was also association with IDU (OR 10.72) and HCV (OR 30.36). A high prevalence of HTLV-2 as compared to HTLV-1 (4.9% versus 0.8%, respectively) was detected<sup>17,18</sup>.

One study conducted in Porto Alegre, Rio Grande do Sul State, Brazil showed the occurrence of 2.4% of HIV/HTLV coinfection (1.4% HTLV-1, 0.5% HTLV-2, and 0.5% untypeable HTLV) in 2,985 patients, seen at three HIV counseling centers in Porto Alegre, associated with intravenous cocaine use ( $p < 0.001$ ) and HIV positivity ( $p < 0.001$ )<sup>19</sup>.



More recently, another study employing molecular assays for confirming HTLV-1/2 infections, conducted on 580 HIV-infected individuals from the metropolitan area of Porto Alegre, showed 2.9% of HIV/HTLV-1/2 coinfection (1.9% HTLV-1 and 1.0% HTLV-2)<sup>20</sup>. All of these studies confirm the occurrence of HTLV-1 and HTLV-2 in Southern Brazil.

Noteworthy, at present the major routes of HTLV transmission in Brazil are the sexual and the vertical<sup>21</sup>, but from 1980 to 2000, the parenteral route predominated, mostly among IDU who became infected with HTLV-2 in the South and Southeast regions in Brazil, as previously described<sup>13,15,18,22,23</sup>. On the other hand, in the Northeast Brazil, HTLV-1 predominated among IDU<sup>24</sup>, and these findings could be related to the differences in HTLV-1 and HTLV-2 endemic areas, as well to the different patterns of drug usage. In fact, studies from 2000, described the Brazilian Harm Reduction Programs that provided sterile syringes and needles to IDU, and the change in drug use from intravenous cocaine to crack cocaine. They emphasize the drastic reduction in the number of HIV, and probably HTLV infected individuals in Brazil<sup>13,25,26</sup>. Herein, the older age of HIV/HTLV-coinfected patients, and the strong association with the male sex and IDU corroborate these data.

Taken together, the results detected in this first survey on HTLV-1/2 in HIV/AIDS patients from Southern Santa Catarina revealed the occurrence of HTLV-1 and HTLV-2 circulating among HIV-infected individuals. Given that these retroviruses could cause different impacts on HIV/AIDS outcomes, the surveillance of HTLV-1/2 is necessary, and it could support public health policies in preventing the transmission of these retroviruses. The authors of the present study are currently interested in expanding these data, searching for sexually transmitted diseases, including HTLV-1/2 in other at-risk populations, such as sex workers, lesbians, gays, bisexual men, and transgender individuals from this geographical area.

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## AUTHORS' CONTRIBUTIONS

ACA, CEMM and FST contributed to the study

conception and design; CEMM, FST, ADS, DJT attended patients, applied the questionnaire and obtained the data and the informed consent from patients; GBS and KRC performed the lab experiments; ACA and KRC analyzed the data and wrote the manuscript. All authors have read the paper and approved the final version.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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