HTLV-1 and HTLV-2 infections in patients with endemic mycoses in São Paulo, Brazil: A cross-sectional, observational study

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Summary

Background Brazil is a country endemic for human T-lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2), systemic mycoses such as paracoccidioidomycosis (PCM) and histoplasmosis (HP), and aspergillosis (AP). The prevalence of HTLV-1/-2 infections in individuals with endemic mycoses in Latin America is unknown; however, an association between HTLV-1 and severe PCM and HP has been observed in Peru. Addressing this knowledge gap, we searched for HTLV-1/-2 antibodies in serum samples sent to the Instituto Adolfo Lutz, São Paulo, Brazil, for systemic mycosis diagnosis.

Methods We used 387 sera from a biorepository that had seropositive results for *Paracoccidioides spp.* (G1, *n*=212), *Histoplasma capsulatum* (G2, *n*=95), *Aspergillus spp.* (G3, *n*=61), and at least two of these fungi (G4, *n*=19). We searched for the presence of HTLV-1/-2 antibodies using commercial immunoassays: enzyme immunoassay (HTLV-I+II Murex, Diasorin), western blotting (HTLV Blot 2.4, MP Biomedicals), and line immunoassay (INNO-LIA HTLV I/II, Fujirebio). Demographic characteristics were evaluated in each group.

Findings Different regions in São Paulo were sampled. Most samples were from males (76.2%; p=0.001), except for G3, in which no sex bias was detected. Mean age differences were observed between groups: patients with PCM and HP had a similar mean age (42.8 and 42.0 years, respectively), while those with AP and co-fungal infection were older (55.1 and 52.8 years, respectively, (p<0.001). Noteworthy, males were older than females in G1 (p=0.005). Screening detected HTLV-1/2 antibodies in five samples (1.30%; 95% CI: 0.8–1.8%), with two borderline results. HTLV-1/2 was confirmed in two samples: 2/387 (0.52%; 0.063–1.85%): one HTLV-2, male, 42 years, from G1: 1/212 (0.47%; 0.012–2.60%), and one HTLV-1, male, 51 years, from G3: 1/61 (1.64%; 0.042–8.80%).

Interpretation In the state of São Paulo, HTLV-1 and HTLV-2 seem to circulate in male patients with systemic mycoses, and since HTLV-1 could impact fungal disease severity, the identification of co-infection is important regardless of prevalence.

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Keywords: HTLV-1; HTLV-2; Paracoccidioidomycosis; Histoplasmosis; Aspergillosis; Co-infection

Introduction

Brazil is a country endemic for neglected infections/diseases such as human T-lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2), systemic mycoses such as paracoccidioidomycosis (PCM) and histoplasmosis (HP), and aspergillosis (AP). HTLV-1 and HTLV-2 are retroviruses that cause persistent infections, mainly in T lymphocytes. HTLV-1 is associated with high morbidity and mortality clinical conditions, such as inflammatory and

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Research in context

Evidence before this study

The association between HTLV-1 and severe cases of endemic mycoses (paracoccidioidomycosis and histoplasmosis) has been described in some patients in Peru, a region considered endemic for HTLV-1 and neglected fungal infections/diseases in Latin America. However, the small number of cases did not confirm this association. The prevalence of HTLV-1/-2 in patients with systemic mycoses in Latin America is unknown.

Added value of this study

This study provides the first information regarding the percentage of HTLV-1/-2 infections in patients seropositive for *Paracoccidioides spp.*, *Histoplasma capsulatum*, and *Aspergillus spp.* in the state of São Paulo, Southeast region of Brazil. The overall percentage of co-infection was similar to the prevalence of HTLV-1/2 detected in the general population of another state from the Southeast region. The detection of HTLV-1 and HTLV-2 confirmed the circulation of both viruses in São Paulo. The study also describes the characteristics (origin, sex, and age) of individuals with systemic mycoses in the state of São Paulo and emphasizes the necessity to extend the study in this geographic area and to other regions and populations with a high prevalence of HTLV-1/-2 in Brazil.

Implications of all the available evidence

The presence of HTLV-1 in patients with systemic mycoses is of great concern, since HTLV-1 is associated with immunosuppression and consequently with the development of opportunistic infections and a poor prognosis for other diseases. Compulsory notification of systemic mycoses infection, as well as HTLV-1/-2 in Latin America, could help governments implement public health policies, such as recommending HTLV serology in patients with systemic fungal diseases, to better monitor and treat these co-infected patients.

neurodegenerative HTLV-1-associated myelopathy (HAM), and adult T-cell leukaemia/lymphoma (ATL). In contrast, HTLV-2 is rarely associated with disease.[1,2] Brazil is estimated to have 800,000 to 2.5 million people living with HTLV-1/2, with the prevalence varying according to geographic region, ethnic background, socioeconomic level, and risk factors for acquiring these infections.[1,3,4] For example, in the general population of Salvador, Bahia, a prevalence of 1.48% was found; in blood donors from all over the country, the prevalence ranged from 0.03% to 0.48%, and in pregnant women from 0% to 1.05%.[4] In general, the highest prevalence is found in the North and Northeast regions of Brazil, and the lowest prevalence is in the South region. Studies on the prevalence in the Southeast region of Brazil in the general population are scarce. Recently, the prevalence of 0.53% was detected in the general population of Vitória, Espírito Santo,[5] and of 5.3% in Japanese immigrants and their descendants living in the state of São Paulo.[6] In vulnerable populations (i.e., illicit intravenous drug users, men who have sex with men, male and female sex workers, and people with other sexually transmitted infections, mainly HIV, HBV, and HCV), high prevalence rates are detected, ranging from 0.7% to 5.3%.[4] Interestingly, HIV/HTLV and/or HBV/HTLV and/or HCV/HTLV co-infections are common in people infected with HTLV-1 and HTLV-2.[7,8] However, co-infections with such viruses differ in terms of prognosis: HTLV-1 can accelerate HIV- and/or HCV-associated disease development, whereas HTLV-2 has the opposite effect.[9–12] Recently, a systematic review on clinical and laboratory outcomes of HIV-1 and HTLV-1/2 co-infection showed the association of HIV-1 and HTLV-1 co-infection and HIV-1 and HTLV-1 /2 triple co-infection with shorter survival, higher mortality rate, and faster progression to death, whereas HIV-1/HTLV-2 co-infection appeared to have a neutral association with longer survival, slower AIDS progression, and lower mortality rate.[13] Therefore, the differential diagnosis between the two viral infections is of utmost importance. In addition, in regions and populations endemic for other infections, such as tuberculosis (TB), high percentages of HTLV-1 co-infection and a higher risk of severe TB cases have been reported. A study conducted in Salvador, Bahia, detected more TB cases in HTLV-1 seropositive than in HTLV seronegative individuals (RR 2.6, 95% CI: 1.6–4.2).[14] In Peru, 5.8% of TB patients were infected with HTLV-1, and an association of HTLV-1 infection with a high number of TB deaths in family members (OR 5.4, 95% CI: 1.7–16.8), as well as high numbers of mycobacteria in sputum (3+) were observed in HTLV-1 seropositive patients (OR 4.1, 95% CI: 1.5–11.2).[15]

In Brazil, although PCM, HP, and AP are prevalent, neither mandatory disclosure of disease status nor epidemiological surveillance of these fungal diseases is in place, except in some Brazilian states which have implemented it as part of their public policies.[16] Therefore, there are no epidemiological data on the occurrence, magnitude, and transcendence of such diseases at the Brazilian national level.[17] Despite poor record keeping, an annual incidence of PCM ranging from 1 to 3.7 new cases per 100,000 inhabitants and mortality of 1.65 per million inhabitants were estimated in Brazil in 2017.[16,17] This clustered within the state of Rondônia, where an incidence of 9.4 cases per 100,000 inhabitants was reported, with two municipalities reporting close to 40 cases per 100,000 inhabitants.[18] Regarding HP, one study conducted in 2006 found that the prevalence varied according to geographic regions: South (6.3–29.8%), Southeast (3.0–93.2%), Central-West (4.4–63.1%) and North (12.8–43.4%). Generally, the environmental conditions present in areas of high

endemicity are a moderate climate with constant humidity. Infection with *Histoplasma capsulatum* usually is asymptomatic, but in immunocompromised individuals, such as patients who are receiving corticosteroids, cytotoxic therapy, and immunosuppressive agents or individuals with HIV infection, progressive illness occurs.[19] Regarding AP, in 2016, a total of 1,010,465 cases were described in Brazil (incidence of 211.98 per 100,000 inhabitants): 8664 invasive cases (4.47 per 100,000 inhabitants), 12,032 chronic pulmonary cases post-TB (6.20 per 100,000 inhabitants), 390,486 allergic bronchopulmonary AP cases (201.31 per 100,000/inhabitant), and 599,283 severe asthma cases with sensitization to fungi.[16] Unfortunately, there are no such data on the presence of HTLV-1/2 infection in human severe fungal infections in Brazil.

In contrast, in Peru, which is considered an endemic country for HTLV-1 infection and systemic mycoses, four severe cases of *Paracoccidioides brasiliensis* associated with HTLV-1 infection were reported, one of which included a simultaneous infection of *H. capsulatum*. The authors suggested that immunosuppression caused by HTLV-1 could be the cause of the aberrant conditions of systemic mycoses and recommended that studies on the prevalence of HTLV-1 infection in individuals with systemic mycoses from endemic regions be carried out.[20] Canelo-Aybar et al. (2011) described a case in Peru of gastrointestinal HP in a patient infected with HTLV-1.[21] In 2012, León and collaborators reinforced the need to search for HTLV-1 in patients with endemic mycoses in Latin America, and to assess the immune status of these patients to better understand the involvement of HTLV-1 in the worsening of fungal infections.[22] Dr Eduardo Gotuzzo, from Peru, during the International Health Forum for the Elimination of HTLV 2021 (organized by the Pan American Health Organization/World Health Organization [PAHO/WHO] and the HTLV Channel (https://www.youtube.com/watch?v=pbpruvW5JA0)), emphasized the detrimental association of HTLV-1 and systemic mycoses and the need for more studies concerning this matter.

Given that Instituto Adolfo Lutz (IAL), a public health laboratory in São Paulo, Brazil, specializes in HTLV and immunodiagnosis of systemic mycoses, we conducted the present study to evaluate the association between endemic mycoses and HTLV-1/2 infections in the state of Sao Paulo.

Methods

Study setting, design and study population

This cross-sectional, observational study was conducted retrospectively at the Immunology Center of IAL. This institute has a central laboratory located in the city of São Paulo, which performs highly complex analyses. The IAL also has 12 regional laboratories throughout the state of São Paulo. Patients who attended the IAL came from different specialized health centres located in the state of São Paulo and elsewhere. Three hundred eighty-seven serum samples collected from 2012 to 2021 that were sent to IAL for systemic mycoses diagnosis, were positive for fungal systemic infections and were utilized in HTLV-1/2 infection analysis. The samples were obtained from a biorepository located at the Mycoses Immunodiagnostic Laboratory, Immunology Center, in the central laboratory of IAL, which is the Reference for Systemic Mycoses in the state of São Paulo. They perform serology for *Paracoccidioides brasiliensis, Paracoccidioides lutzii, Histoplasma capsulatum*, and *Aspergillus spp*. The only criterion for sample selection was a volume of more than 200 µL, which was sufficient to conduct all serological HTLV-1/2 assays. The samples belonged to patients suspected of having fungal infections who attended different regional health services of the state of São Paulo, including hospitals located in São Paulo city. Four groups were categorized according to seropositive results for *Paracoccidioides spp.* (G1, *n*=212), *Histoplasma capsulatum* (G2, *n*= 95), *Aspergillus spp.* (G3, *n*=61), and co-infection by at least two of these fungi (G4, *n*=19). Among fungal co-infected samples (G4), 12 had seropositive results for *Paracoccidioides spp.* and *Histoplasma capsulatum*, five for *Paracoccidioides spp.* and *Aspergillus spp.*, and two for *Paracoccidioides spp.*, *Histoplasma capsulatum,* and *Aspergillus spp.* Patients' place of birth according to the group and the 17 Regional Departments of Health (DRS I to DRS XVII) under the Secretary of Health of São Paulo which cover the 645 municipalities in the state of São Paulo are depicted in Figure 1, along with the geographic location of the state of São Paulo, in relation to Brazil and Latin America. Noteworthily, it was not possible to obtain the place of birth of patients hospitalized in the city of São Paulo (data was not provided in the medical request); thus, they were depicted locally in Grande São Paulo (DRS I).

Laboratory methods

The serology for fungal infections was performed using a double immunodiffusion assay (DID), according to the modified Ouchterlony's method.[23] Briefly, glass slides were covered with 3.0 mL of 1% agarose gel type II medium (Sigma Chemical Co., USA) diluted in a buffered saline solution pH 6.9 containing 0.4% sodium citrate and 7.5% glycine. Antigen (12 µL) was placed in the central well, whereas control and patient sera (12 µL) were added to the surrounding wells. Slides were incubated in a humid chamber at room temperature for 48 h. They were then washed with saline solution with several changes over 24 h. Gels were dried and stained in 0.4% Coomassie brilliant blue R-250® (Sigma Chemical Co., USA) in an ethanol-acetic acid-water mixture as solvent. Notably, the antigens and

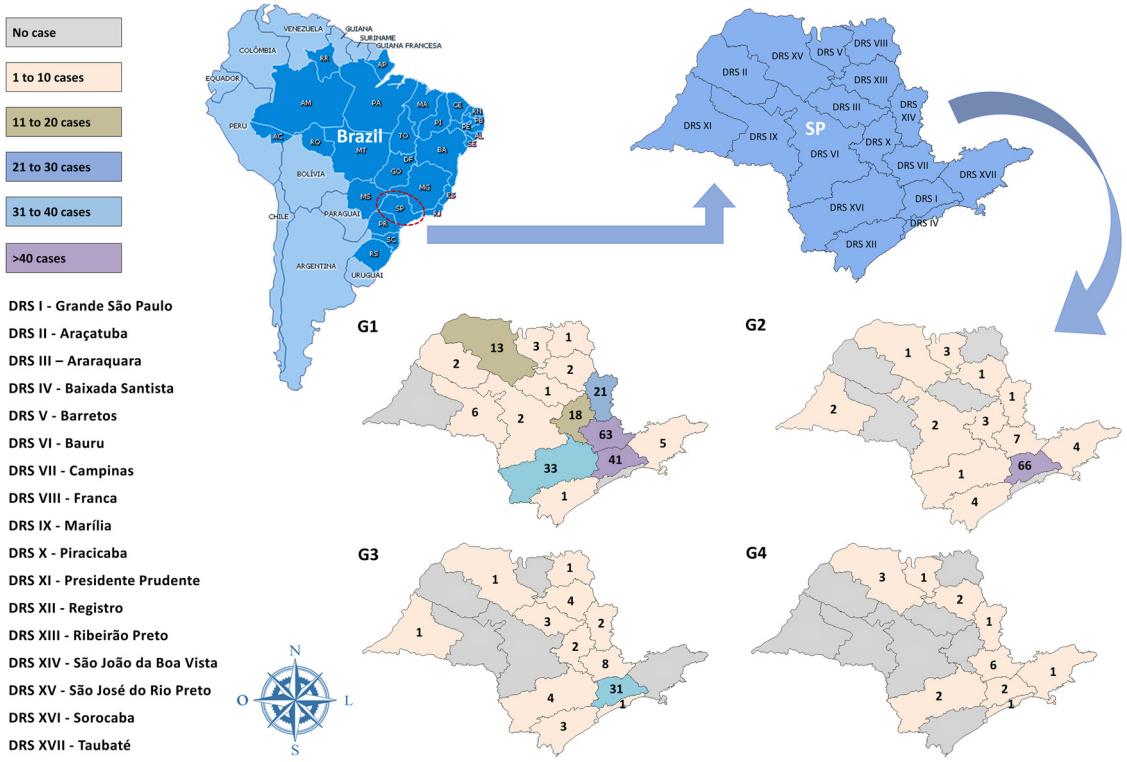

Figure 1. Maps of the Latin America, Brazil, and the state of São Paulo highlighting the origin of patients seropositive for (G1, *n*=212) *Paracoccidioides spp.*, (G2, *n*=95) *Histoplasma capsulatum*, (G3, *n*=61) *Aspergillus spp.*, and (G4, *n*=19) at least two of these fungi. The name and location of the 17 Regional Departments of Health in São Paulo that comprise the 645 municipalities in the state of São Paulo are described, and the number of patients originating from each DRS is depicted.

protocols employed in DID for *Paracoccidioides spp.*, *Histoplasma capsulatum*, and *Aspergillus spp.* were standardized at the IAL, and results were considered positive for serum dilution at ≥1:2 (Figure 2).[24,25]

Anti-HTLV-1/-2 antibody detection was conducted at the HTLV Research Laboratory of the Immunology Center, central IAL, using an enzyme immunoassay for screening (EIA Murex HTLV-I/II, Diasorin, UK) and western blotting (WB) (HTLV BLOT 2.4, MP Biomedicals, Asia Pacific Pte Ltd., Singapore) for confirmation. For inconclusive samples by WB analysis (WB-indeterminate or HTLV-untyped), the line immunoassay (INNO-LIA HTLV-I/II, Fujirebio, Europe N.V., Belgium) was employed.[26] All assays were conducted according to the respective manufacturer's instructions.

Statistical analyses

Statistical analysis was performed using the software Statistical Package for the Social Sciences (SPSS) version 22.0. A descriptive analysis was performed, including frequency distribution for the qualitative variable (sex) and calculation of mean, standard deviation (SD), and 95% confidence interval (CI) for the quantitative variable (age). In addition, differences in the number of males and females in each group were evaluated statistically using the chi-square test. GraphPad Prism software version 5.03 (GraphPad, San Diego, CA, USA) was used for age-group comparisons; the Mann–Whitney U-test was used to compare two groups, and the Kruskal-Wallis analysis of variance (ANOVA) was used for three or more groups. Results with a p-value of ≤0.05 were considered statistically significant. The prevalence of HTLV was estimated by the number of cases diagnosed and confirmed in the tests in relation to the total number of samples tested, and the corresponding 95% CI was calculated.

Ethical review

This study was approved by the IAL Scientific and Ethics Committees for Research [CTC#49N-2021] under the Ministry of Health protocol number CAAE # 56083122.7.0000.0059. Data are presented anonymously.

Role of the funding source

The funding source had no role in the design and conduct of the study; collection, management, analysis, or

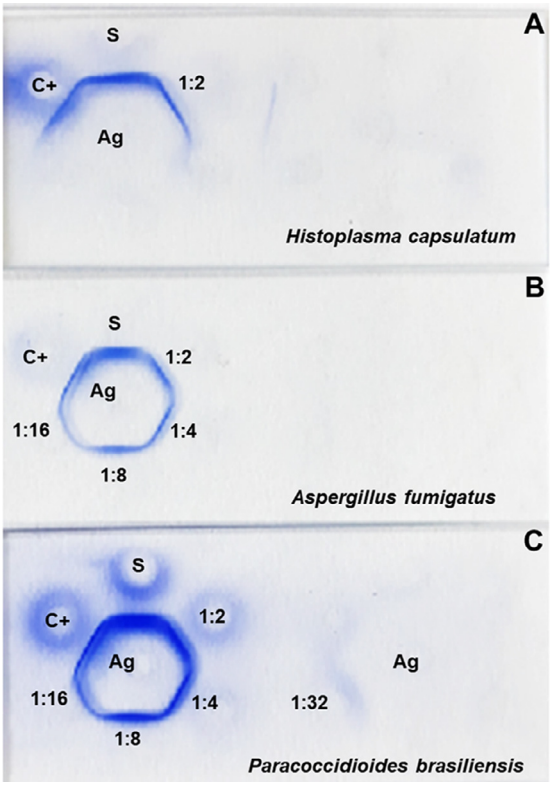

Figure 2. Patterns of semi-quantitative double immunodiffusion assay (DID) for detecting antibodies to *Paracoccidioides spp*., *Histoplasma capsulatum*, and *Aspergillus spp*. Representative slides of samples considered seropositive up to a serum dilution of 1:2 (A), 1:16 (B), and 1:32 (C). Ag, antigen; S, serum sample; C+, positive control.

interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Results

Table 1 presents the characteristics of the study population, in which the samples were analyzed for the presence of HTLV-1 and HTLV-2 specific antibodies. The majority of samples were from male patients (76.2%), except for G3 (AP), in which no difference between the number of male and female patients was detected (57.4% and 42.6%, respectively). Regarding age, differences in the mean ages between groups were observed: patients infected with *Paracoccidioides spp*. and *Histoplasma capsulatum* had almost the same mean age (42.8; 95% CI: 40.3–45.2, and 42.0; 95% CI: 39.1–44.8 years, respectively), while patients infected with *Aspergillus spp*. were older (55.1; 95% CI: 51.4–58.8 years), and patients with at least two fungal infections had a mean age of 52.8; 95% CI: 46.2–59.4 years ($p<0.001$). Difference in ages according to sex was detected in G1 (mean age of 44.0 years among males and 37.3 years in females, $p=0.005$). Differences in relation to age groups are shown in Figure 3. Briefly, most of the male patients from G1 (PCM) were 41–70 years old; G2 (HP), 31–60 years old; G3 (AP), 31–70 years old; and G4 (fungi co-infection), ≥31 years old. Among female patients: in G1 the majority were under the age of 30 years; in G2, 31–50 years; in G3, above 51 years; and in G4, 51–60 years.

Regarding HTLV-1/2 screening, five serum samples reacted positively (1.30%; 95% CI: 0.8–1.8%: three in G1, one in G2, and one in G3), and two of them had borderline and low reactive results (Pt. 178, G1, and Pt. 36, G2, respectively) (Table 2). After WB analysis, one sample confirmed HTLV-1 result (Pt. 30, G3), and two had WB-indeterminate profiles: GD21 and rgp46-II bands (Pt. 189, G1), and a p24 band (Pt. 36, G2), and two were negative. Using LIA analysis of the two samples that had WB-indeterminate results, the sample from Pt. 189 confirmed HTLV-2 infection, and from Pt. 36 remained indeterminate (Table 2).

Overall, two samples had HTLV-1/-2 infections, belonging to individuals born in the cities of Campinas and Américo Brasiliensis, state of São Paulo: one HTLV-2 from G1 (male, 42 years, DID positivity for *Paracoccidioides spp*. until dilution 1:32) and one HTLV-1 from G3 (male, 51 years, DID positivity for *Aspergillus spp*. until dilution 1:16) (Table 2). Thus, the prevalence of HTLV-1/2 in mycoses systemic patients from São Paulo were overall: 0.52% (2/387, 95% CI: 0.063–1.85%); G1: 0.47% (1/212, 95% CI: 0.012–2.60%), and G3: 1.64% (1/61, 95% CI: 0.042–8.80%).

Discussion

Severe human systemic mycoses such as PCM and HP continue to be neglected in Brazil and elsewhere.[27] They have not yet reached the status of neglected tropical diseases (NTDs) by the WHO, whose list includes 20 diseases or groups of diseases, of which only mycetoma, chromoblastomycosis, and other deep mycoses are listed, but without specifying which deep mycoses they refer to (https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1). However, in the recent document elaborated by WHO (2020) that establishes targets, sub-targets, and milestones for NTDs 2021–2030, mycetoma, chromoblastomycosis, sporotrichosis and paracoccidioidomycosis diseases have been included.[28] Regarding other endemic/systemic mycoses, no comment was made in this document.

In Brazil, although PCM, HP and AP are not subjected to compulsory notification, recently, the Health Surveillance Guideline elaborated by the Ministry of Health considers PCM and HP as diseases of public health importance.[17] PCM is a fungal disease associated with rural environments and agricultural activities,[29] and HP with soil disturbance.[25] They are acquired by

		Systemic mycoses group				
		Overall ***n*** **= 387**	**G1 (PCM)** ***n*** **= 212(54.8%)**	**G2 (HP)** ***n*** **= 95(24.5%)**	**G3 (AP)** ***n*** **= 61(15.8%)**	**G4 (co-fungi)** ***n*** **= 19(4.9%)**	***p***
Sex Number (%)							
Males		295 (76.2)	174 (82.1)	71 (74.7)	35 (57.4)	15 (78.9)	0.001[a]
Females		92 (23.8)	38 (17.9)	24 (25.3)	26 (42.6)	4 (21.1)	
Age (years)							
Males	**Mean ± SD**	45.3 ± 16.4	44.0 ± 17.3	42.9 ± 14.4	52.6 ± 13.2	53.5 ± 15.0	0.006[b]
	[95% CI]	[43.2–46.8]	[41.4–46.6]	[39.5–46.3]	[48.1–57.2]	[45.2–61.8]	
Females		44.4 ± 18.6	37.3 ± 19.0	38.9 ± 12.0	58.4 ± 15.9	50.0 ± 8.0	<0.001[b]
		[40.3–47.7]	[31.0–43.5]	[33.6–44.2]	[52.0–64.8]	[37.3–62.7]	
Total		*45.0 ± 16. 9*	*42.8 ± 17.7*	*42.0 ± 13.9*	*55.1 ± 14.6*	*52.8 ± 13.7*	*<0.001*[b]
		[43.4–46.6]	*[40.3–45.2]*	*[39.1–44.8]*	*[51.4–58.8]*	*[46.2–59.4]*	
p		0.722[c]	0.050[c]	0.258[c]	0.068[c]	0.885[c]	

***Table 1*: Characteristics of the study population whose serum samples were tested.**
Systemic mycoses groups were categorized by determination of *Paracoccidioides spp.*, *Histoplasma capsulatum*, and *Aspergillus spp.* antibody positivity using double immunodiffusion assays, as described in the Methods section. n, number of individuals; SD, standard deviations; CI, confidence Interval; G, group; G1, PCM, paracoccidioidomycosis; G2, HP, histoplasmosis; G3, AP, aspergillosis; G4, co-fungi: PCM+HP and PCB+AP and PCM+HP+AP; a, chi-square; b, Kruskal-Wallis test of mean age groups; c, Mann–Whitney test of mean ages intragroup.

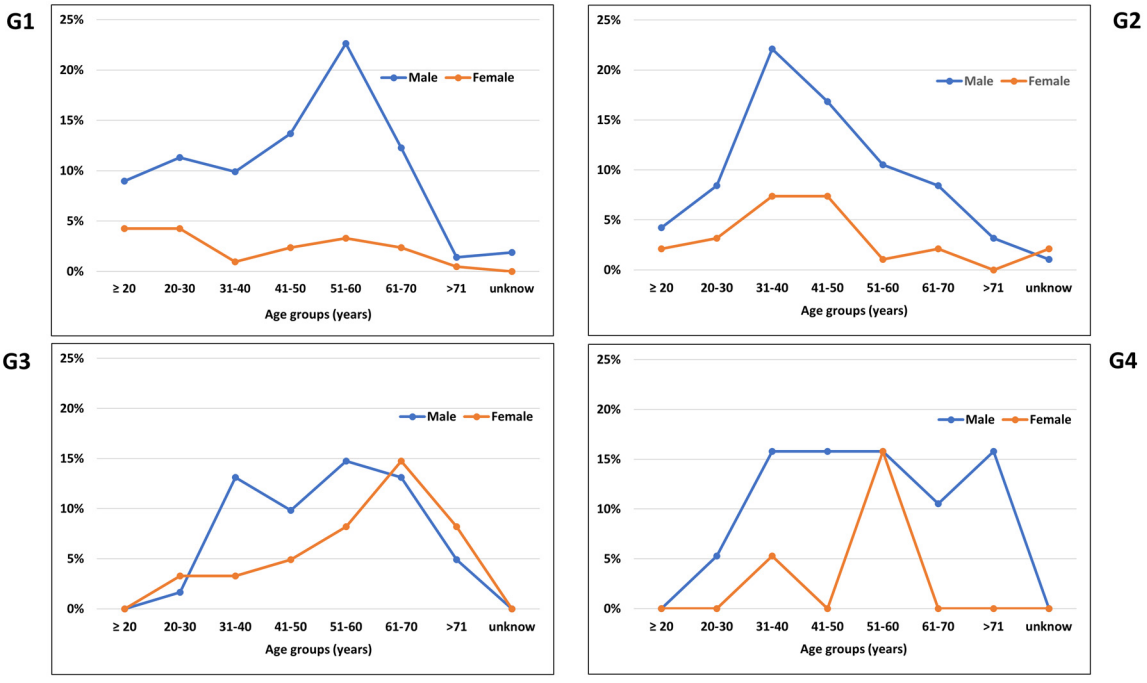

Figure 3. Proportion (%) of male and female patients seropositive for *Paracoccidioides spp.* (G1), *Histoplasma capsulatum* (G2), *Aspergillus spp.* (G3), and at least two of these fungi (G4), per age group, in São Paulo, Brazil.

inhalation of aerosolized microconidia released from disturbance of soil, occurring during several activities such as building, remodelling, demolition, soil excavation, caving, camping, cutting sugar cane or wood, and cleaning sites that harbour the fungi. The risk of infection depends on the following factors: type of activity performed, activity duration, amount of dust containing microconidia or soil exposure, and host immunological conditions such as immunosuppression due to the use of immunomodulatory drugs and HIV infection.[17,25,29] AP is an opportunistic infection acquired by inhaling spores that circulate in the environment and are transmitted through the air. Disease depends on the virulence or pathogenicity of the *Aspergillus* species or strain, as well as on the host's immune status, lung structure, and function. Presently, AP is an emerging

Pt . N/ group	Sex/age in years	City of birth/ DRS code	Fungal DID titer	HTLV-1/2 OD/CO[a]	WB profile[b]	LIA profile[c]	Final result
178/G1	M/45	Piracicaba/DRS X	32	1.1	Negative	NA	Negative
179/G1	M/68	Mogi Mirim/DRS VII	32	5.6	Negative	NA	Negative
189/G1	M/42	Campinas/DRS VII	32	10.4	GD21, rgp46-II,	p24I/II,gp46I/II, gp21I/II, gp46-II	HTLV-2
36/G2	M/51	Unknown/DRS I	2	1.5	p24	gp21I/II	Indeterminate
30/G3	M/51	Américo Brasiliensis/ DRS III	16	10.3	GD21,19,p24,p26,p28, p32,p36,gp46,p53, rgp46-I	NA	HTLV-1

***Table 2*: Characteristics and titers of fungal antibodies of patients whose plasma samples resulted reactive on HTLV-1/2 screening, and final result of HTLV-1/2 confirmatory assays.**

Systemic mycoses groups were categorized by determination of *Paracoccidioides spp.*, *Histoplasma capsulatum*, and *Aspergillus spp.* antibody positivity using double immunodiffusion assays (DID), as described in the Methods section. a, optical density /cutoff ratio (OD/CO) value using EIA Murex HTLV-I/II, Diasorin as screening; b, western blotting (WB) profile using HTLV BLOT 2.4, MP Biomedicals; c, line immunoassay profile using INNO-LIA HTLV-I/II, Fujirebio. HTLV serological results were according to manufacturers' descriptions. Pt. N., patient code number; G, group; G1, PCM, paracoccidioidomycosis; G2, HP, histoplasmosis; G3, AP, aspergillosis; M, male; DRS, Departamento Regional de Saúde; NA, not applicable.

mycosis in intensive care units and postoperative patients, mainly because of the dispersion of spores through hospital ventilation systems, as well as in other non-traditional at-risk groups, including patients with chronic lung diseases, AIDS, and those receiving immunosuppressive drugs.[30]

HTLV-I infection and associated diseases are also neglected in Brazil and other South American countries. Although HTLV-I is not on the WHO list of neglected diseases, a technical report on HTLV-I was elaborated in 2020,[31] and subsequently presented and discussed during one HTLV-I technical update held on March 17, 2021 (https://www.who.int/news-room/events/detail/2021/03/17/default-calendar/htlv-i-technical-update). At present, the most important routes of HTLV transmission in Brazil are (*i*) parenteral (by needle sharing among people who inject recreational drugs, skin scarification, and self-flagellation in indigenous populations and during religious ceremonies), (*ii*) mother-to-child transmission (by breastfeeding for more than six months), and (*iii*) sexual transmission (by sexual intercourse without using condoms).[4,32] A considerable number of public policies have been implemented in Brazil since 1993 to reduce the transmission of HTLV.[33] However, the lack of public awareness, epidemiological data, patient-care reference centres, confirmatory tests, and universal antenatal screening, as well as the absence of cost-effective studies have been identified as weaknesses that still need to be addressed.[33] Of note, recently, the Brazilian Ministry of Health established within the scope of the Unified National Health System (Sistema Único de Saúde, SUS), the HTLV screening, diagnosis, treatment and follow-up in the Maternal and Child Care Network in Brazil.[34]

Regarding HTLV-I/-2 and co-infections, a recent comprehensive review addressed the impact of HTLV-I on several co-infections and vice-versa, like sexually transmitted (Syphilis, Chlamydia, HPV, HSV) infections, *Mycobacterium tuberculosis* and *Mycobacterium leprae, Schistosoma mansoni* and *Strongyloides stercoralis*, and HBV and HCV.[12] However, nothing was described concerning the association between HTLV and endemic/systemic mycoses, probably because PCM and HP are clinically confused with TB, and also because of the low number of studies available in the literature, all of which are from Peru.[20–22] Thus, the present study bridges the knowledge gap, by providing new information concerning the prevalence of HTLV-I/-2 and systemic mycoses co-infections in São Paulo, Southeast region of Brazil. The HTLV-I/2 prevalence detected was 0·52%, corroborating the prevalence detected in the general population from a neighbouring state, Espírito Santo, also in the Southeast region. There, a prevalence of 0·53% and a predominance of HTLV infection in male patients were detected,[5] corroborating the present data. Although HTLV-I infection in endemic regions/populations has been associated with female sex, and the age of >45 years,[4,6] no case of HTLV-I/2 was detected in female patients from the present study. This could be partially explained by the analyzed population. Most were men, and probably individuals who had more contact with agricultural and/or soil disturbance activities. Furthermore, the women in the present study were younger than the men, therefore, had fewer years of chance of acquiring HTLV infection.

In accordance with the sex and ages described in HTLV-I/2 seropositive cases of the present study, previous studies of HTLV surveillance carried out in patients with HBV, HCV and/or HIV, from the same geographic area (state of São Paulo), showed HTLV-I/-2 co-infection mainly in men with the ages varying from 40 to 60 years.[10,26,35] Moreover, the detection of HTLV-I and HTLV-2 in systemic mycoses co-infected individuals corresponds with the virus types that circulate in

patients presenting other co-infections like HIV, HBV and HCV in São Paulo.[5–8,26,31–32]

There were several limitations to our study. The low percentage of HTLV/systemic mycoses co-infection detected in the present study compared to that of other co-infections previously described (HTLV/HIV [3.1% to 4.2%], HTLV/ HBV [1.9%], and HTLV/HCV co-infection [4.0%]), could be partially explained by the differences in transmission routes of such pathogens and by the limited number of tested individuals.[26,35] HIV, HBV, and HCV share routes of transmission with HTLV-1/2, while fungi have a different route. In addition, there was a limited number of systemic mycoses samples available for analysis (n=387), and this small sample size may have influenced the results obtained. Thus, the results on the prevalence were indicated by adding exact binomial 95% CIs demonstrating their statistically low precision. Worth noting is that the positive DID results up to a serum dilution of 1:32 for *Paracoccidioides spp.*, and of 1:16 for *Aspergillus spp.*, the last one detected in the individual infected with HTLV-1, which may be related to the aggressiveness of the aspergillosis in this patient. Interestingly, the lowest positive DID result (1:2 for *H. capsulatum*) was detected in a patient sample sent to IAL by Kidney Hospital from São Paulo; so we can suppose small production/detection of antibodies due to hemodialysis and/or immunosuppressive drugs used after kidney transplantation. Unfortunately, these are just hypotheses, as these data were not included in the medical request.

Then again, the low number of HTLV-1/-2 seropositive samples and the study design does not allow for any firm conclusions. Nonetheless, despite these limitations, this study contributed to the knowledge of the characteristics of individuals that developed systemic mycoses in the state of São Paulo and confirmed the presence of HTLV-1 and HTLV-2 in co-infected patients from this geographic area, and since HTLV-1 could impact on fungal diseases severity, the identification of co-infection is important regardless of prevalence. We are now interested in expanding this study by collecting more samples from the state of São Paulo and other regions of Brazil, mostly from the North and Northeast regions, considered high HTLV-1/-2 prevalence areas. In the future, we will evaluate the impact of these co-infections on the development and severity of systemic mycoses, as previously described in Peru.[20–22]

Contributors

ACA: conceptualization, funding acquisition, project administration, investigation, methodology, formal analysis, writing of the original draft, writing review, and editing; KRC: figure artwork, formal analysis, writing review, and editing; ICA: methodology, writing review, and editing; APV: data acquisition, methodology, data curation, formal analysis, writing review, and editing.

Data sharing statement

The Instituto Adolfo Lutz does not release datasets related to patients. Researchers who are interested in knowing about the serodiagnosis of systemic mycoses at IAL should contact Adriana Pardini Vicentini (adriana.vicentini@ial.sp.gov.br), and for potential collaboration should contact the corresponding author (adele.caterino@ial.sp.gov.br).

Editor note

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Declaration of interests

The authors declare that they have no commercial or other associations that might pose conflicts of interest.

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