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MAJOR ARTICLE

Histoplasmosis, An Underdiagnosed Disease Affecting People Living With HIV/AIDS in Brazil: Results of a Multicenter Prospective Cohort Study Using Both Classical Mycology Tests and *Histoplasma* Urine Antigen Detection

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Background. Histoplasmosis is highly endemic in the American continent. This condition is associated with a high mortality, particularly in people living with HIV/AIDS (PLWHA). Diagnosis of histoplasmosis is usually late in South America, as *Histoplasma* antigen detection is rarely available. Here we determined the prevalence, risk factors, and outcome of histoplasmosis in PLWHA in Brazilian hospitals.

Methods. This was a prospective cohort study (2016–2018) involving 14 tertiary medical centers in Brazil. We included hospitalized PLWHA presenting with fever and additional clinical findings. Patients were investigated at each participant center with classical mycology methods. Also, *Histoplasma* antigen detection was performed in urine samples (IMMY). Probable/proven histoplasmosis was defined according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria.

Results. From 616 eligible patients, 570 were included. Histoplasmosis was identified in 21.6% (123/570) of patients. Urine antigen testing increased the diagnostic yield in 53.8%, in comparison with standard mycology methods. Variables independently associated with histoplasmosis were CD4+ count <50 cells/mm^3, use of an antiretroviral (protective effect), and sample collection in the Northeast region of Brazil. Dyspnea at presentation was independently associated with death. Histoplasmosis was more frequent than tuberculosis in patients with low CD4+ counts. Overall 30-day mortality was 22.1%, decreasing to 14.3% in patients with antigen-based diagnosis.

Conclusions. Histoplasmosis is a very frequent condition affecting PLWHA in Brazil, particularly when CD4+ counts are lower than 50 cells/mm^3. Antigen detection may detect earlier disease, with a probable impact on outcomes. Access to this diagnostic tool is needed to improve clinical management of PLWHA in endemic countries.

Keywords. diagnosis; epidemiology; histoplasmosis; HIV.

Histoplasma capsulatum is the etiologic agent of histoplasmosis, a systemic mycosis that is highly endemic in the Americas and is also being identified in other parts of the globe [1, 2]. Histoplasmosis may disseminate in immunosuppressed patients, particularly those with HIV infection. Accordingly, disseminated histoplasmosis has been an AIDS-defining disease since 1987 [3]. Histoplasmosis is often described as the most common endemic mycosis in the United States, with a definite endemic area around the Mississippi valley [4]. More recently, it has become appreciated as a highly prevalent disease in AIDS and a major cause of morbidity and mortality in the Americas [3, 5, 6]. Similar to syphilis and tuberculosis (TB) in the history of medicine, the protean manifestations of histoplasmosis require it to be included in the differential diagnosis of diverse clinical syndromes associated with AIDS in the Americas [2].

Brazil is the largest country in Latin America, with a population of >200 million. It is currently struggling with an HIV

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epidemic, with almost 1 000 000 cases [7]. Nevertheless, the frequency of disseminated histoplasmosis has not been well characterized in Brazil. There are no nationwide studies showing prevalence rates; available data are retrospective and limited to specific regions of the country in single-center studies. Most studies are not population based or representative of the epidemiology as a whole, as data have often been obtained through convenience samples rather than through properly performed epidemiologic surveys [8–10]. Moreover, histoplasmosis is usually diagnosed late in Latin America, mainly due to a lack of non-culture-based diagnostic methods. In this setting, skin lesions are frequently observed; this could reflect a genetic variation of the pathogen, but, more likely, it confirms another marker of late diagnosis [11].

Histoplasma antigen detection by antigen-capture enzyme-linked immunosorbent assay (ELISA) has a high sensitivity using urine samples (95%) [12]. However, its availability is very limited in South and Central America, as its reagents are not commercially available (Miravista Diagnostics, Indianapolis, IN). To overcome these difficulties, other tests have been developed: an ELISA kit (Alpha *Histoplasma* antigen enzyme immunoassay [EIA]; Immuno-Mycologics, Norman, OK) that uses polyclonal antibodies with variable sensitivity (62%–81%); an in-house double-polyclonal-antibody sandwich ELISA from the US Centers for Disease Control and Prevention (CDC), which has high sensitivity (>80%) but is not currently available; and more recently, a commercial *Histoplasma* antigen (*Histoplasma* galactomannan [HGM]) single-monoclonal-antibody sandwich ELISA (Immuno-Mycologics, Norman, OK) [13–16]. The last performed very well in a recent study, conducted in Latin America, with a very high sensitivity and specificity (both >95%) [17].

Here, our goal was to determine the prevalence, risk factors, and outcomes of histoplasmosis in a large cohort of people living with HIV/AIDS (PLWHA). We used for diagnosis both classical mycology tests and a previously validated monoclonal *Histoplasma* galactomannan (HGM) enzyme-linked immunosorbent assay (Immuno-Mycologics [IMMY], Norman, OK) for *Histoplasma* antigen detection.

METHODS

Setting and Study Design

This was a multicenter prospective cohort study with PLWHA from 14 tertiary Brazilian hospitals. Institutions were located in all 5 regions of the country: South—Porto Alegre (Hospital de Clinicas de Porto Alegre, Santa Casa de Misericordia de Porto Alegre, and Hospital Nossa Senhora da Conceicao), Rio Grande (Hospital Universitario de Rio Grande), Santa Maria (Hospital Universitario de Santa Maria), and Curitiba (Hospital de Clinicas da Universidade Federal do Parana); Southeast—Sao Paulo (Hospital Sao Paulo, and Instituto de Infectologia Emilio Ribas); Midwest—Goiania (Hospital de Doenças Tropicais); Northeast—Salvador (Hospital Couto Maia), Natal (Hospital Giselda Trigueiro), and Fortaleza (Hospital São Jose de Doencas Infecciosas); and North—Manaus (Hospital Universitario Francisca Mendes) and Macapa (Laboratorio Central de Saude Pública do Amapa [LACEN]). The study was carried out between October 2016 and February 2018.

We included adult (≥18 years old) patients with documented HIV infection who were admitted to 1 of the participant hospitals. Inclusion criteria required fever plus 1 of the following: weight loss (>10% of usual body weight), diarrhea, miliary pattern on thorax imaging, pancytopenia, lymphadenopathy, splenomegaly, or hepatomegaly. Patients were excluded if they refused to consent to study participation. Outpatients were not studied. Patients were also excluded for inability to perform urine *Histoplasma* antigen tests or for insufficient clinical information. The attending physician was entirely in charge of patients' diagnostic and therapeutic decisions.

Data on patient demographics, clinical manifestations, HIV disease (CD4+ cell counts, HIV viral load, use of antiretroviral drugs), exposure to known risk factors for fungal disease, radiological abnormalities, laboratories, diagnosis by classical mycology methods, use of antifungals, presence of other opportunistic infections, and clinical outcome were collected by the institutions and recorded into a clinical research form.

Laboratory Methods

Urine samples were collected from each patient for *Histoplasma* antigen detection. Urine was collected at each research center, and samples were immediately frozen. Samples were shipped to a reference laboratory (Molecular Biology Laboratory at Santa Casa de Misericordia de Porto Alegre) and run in batches to optimize the use of kits. Antigen detection was performed using the *Histoplasma* galactomannan (HGM) single-monoclonal-antibody sandwich ELISA (Immuno-Mycologics, Norman, OK), according to the manufacturer's instructions. According to test validation performed by Caceres et al., a cutoff of 0.5 ng/mL was used to determine positivity [17]. Physicians were not informed of *Histoplasma* antigen test results.

Clinical Data

Information on demographics and clinical manifestations of disease was compared between patients with histoplasmosis or other diagnoses. Probable/proven histoplasmosis was defined according to modified European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria [18]. The only modification made in the EORTC/MSG criteria was the addition of positive serology (immunodiffusion test for antibody detection) for *Histoplasma capsulatum* to the "probable histoplasmosis" criterion. Any positivity in H or M bands

was considered a positive result. Centers were allowed to use their usual diagnostic tests for histoplasmosis. We evaluated the performance of available classic methods (microscopy, fungal culture, antibody detection, and histopathology) in comparison with *Histoplasma* antigen detection. For the determination of outcomes, covariates were compared in survivors vs nonsurvivors at 30 days. Independent risk factors for death were determined using multivariate analysis.

Ethical Approval

Participants signed an informed consent granting permission for using their biological samples in the study, as well as clinical data. The protocol was approved by the ethical committees of the general coordination center (Santa Casa de Misericordia de Porto Alegre) and each participant institution, according to Brazilian research regulation guidelines.

Statistical Analysis

Sample size was calculated in 138 PLWHA, considering an estimated prevalence of 10% of histoplasmosis in PLWHA presenting symptoms or signs of possible disease (margin of error, 5%; confidence level, 95%) [5, 19]. Statistical analyses were conducted using JMP for Mac, version 9 (SAS Institute, Cary, NC). We used descriptive statistics to analyze and present data. The Kolmogorov-Smirnov test was performed to assess for normal distribution. Statistical differences between groups were analyzed using the chi-square or Fisher exact test for categorical data. For continuous data, the Student *t* test or Wilcoxon rank-sum test was employed. All tests were 2-tailed, and a *P* value of ≤.05 was considered significant. A logistic regression model was constructed using a forward stepwise approach. Variables with $P < .20$ were included in the model and remained in the final model if $P < .05$ and/or because of biological significance. Collinearity was assessed when applicable.

RESULTS

From 616 eligible patients, 46 were excluded due to loss of clinical information, accident during sample transportation, absence of urine sample for testing, and/or lack of inclusion criteria after critical revision. Therefore, 570 patients were included in the cohort. From the original 14 medical centers participating in the study, 3 were excluded as no patient was included (Manaus, Macapa, and Curitiba), resulting in 11 centers enrolling patients.

Table 1 presents the general characteristics of patients included in the study. A total of 123 (21.6%) were diagnosed with probable/proven histoplasmosis, including 88 (15.4%) patients with a positive *Histoplasma* urine antigen test. Among 123 patients with histoplasmosis, 109 (88.6%) were properly investigated by classical mycology methods, and 78 of these (71.5%) had confirmed infection at the local institutions. These figures translate into 78 patients in the "proven" category of modified EORTC/MSG criteria and 45 patients in the "probable" category. In the "no histoplasmosis" group, 157/447 (35.1%) were tested with classical methods and found to be negative, and 290/447 (64.9%) were not cultured/biopsied as the attending physician perceived a low pretest probability of disease. A positive test for *H. capsulatum* antigen in the urine was the only criterion for probable/proven disease in 42 (34.1%) patients. In 2 cases (1.6%), positivity was found both on antigen testing and antibody detection; in 1 patient (0.8%), a positive antibody detection test was the only criterion for infection (Figure 1). Among the 78 patients who had been diagnosed at a local hospital, 26/33 (78.8%) had identification of *H. capsulatum* on microscopy, 32/61 (55.7%) patients were culture positive for *H. capsulatum*, 10/16 (62.5%) patients had confirmed infection by histopathology, and 11/48 (22.9%) had positive blood culture. Only 8/41 (19.6%) patients with a local-institution diagnosis had detected antibodies against *H. capsulatum*.

The detection of *Histoplasma* antigen in the urine increased the diagnosis of disseminated histoplasmosis by 53.8% in comparison with classical methods. When all patients with positive antigen were considered (n = 88), 74 (84.1%) were properly investigated with classical methods, and the diagnosis was confirmed locally in 44 of these patients (59.5%). Conversely, urine testing detected only 44 (56.4%) of the 78 patients with a diagnosis made by classical methods. However, it is to be noted that 39.7% of patients with proven histoplasmosis were using antifungals before sample collection (at least 7 days of use), which may have influenced results.

Twenty-two (3.9%) patients had a previous diagnosis of histoplasmosis. In 20 of these (90.9%), data were available on the time between previous diagnosis and current disease, with a median (range) of 12 (1–143) months. Regarding therapy, 9 of these patients (40.9%) were using antifungals before study entry: itraconazole (n = 7, 31.8%), amphotericin B deoxycholate (n = 6, 27.2%), and amphotericin B lipid complex (n = 1, 4.5%).

In the multivariate analysis, CD4+ cell count <50 cells/mm^3 (odds ratio [OR], 2.45; 95% confidence interval [CI], 1.60–3.78), use of antiretroviral at study entry (OR, 0.54; 95% CI, 0.34–0.83), and being enrolled in a city in the Northeast region of Brazil (OR, 2.61; 95% CI, 1.53–4.40) were independently associated with probable/proven histoplasmosis.

Clinical presentation of patients was very exuberant, especially in patients with histoplasmosis. Most patients were investigated with imaging techniques and laboratory tests. Table 2 summarizes the main clinical, imaging, and laboratorial findings of patients with histoplasmosis, in comparison with patients with other diseases. With the exception of C-reactive protein and serum hemoglobin, all other tests were significantly more abnormal in probable/proven histoplasmosis patients, compared with patients without histoplasmosis. A multivariate model for clinical variables predicting the occurrence of histoplasmosis is described in Table 3.

Table 1. Patients' Baseline Characteristics in PLWHA With or Without Disseminated Histoplasmosis (Probable/Proven Histoplasmosis)

Variables	No Histoplasmosis (n = 447)	Probable/Proven Histoplasmosis (n = 123)	*P* Value
Age, median [IQR], y	41 [33–48]	39 [33–45]	.162
Female gender, No. (%)	159 (35.6)	33 (26.8)	.069
CD4+ cell, median [IQR], count/mm^3	86 [31–240] (n = 428)	39 [14–91] (n = 115)	<.001
CD4+ cell category, No. (%)	(n = 428)	(n = 115)	<.001
<50 cells/mm^3	146 (34.1)	66 (57.3)	
50–99 cells/mm^3	87 (20.3)	24 (20.8)	
100–149 cells/mm^3	45 (10.5)	13 (11.3)	
150–199 cells/mm^3	28 (6.5)	3 (2.6)	
≥200 cells/mm^3	122 (28.5)	9 (7.8)	
No CD4+ count available	19 (4.2)	8 (6.5)	.297
Receiving antiretroviral treatment, No. (%)	217 (48.5)	42 (34.1)	.005
City of enrollment,[a] No. (%)			<.001
Goiania	76 (17.0)	50 (40.6)	
Fortaleza	39 (8.7)	23 (18.7)	
Porto Alegre	138 (30.9)	13 (10.6)	
Natal	16 (3.6)	13 (10.6)	
Rio Grande	97 (21.7)	10 (8.1)	
Sao Paulo	73 (16.3)	7 (5.7)	
Previous diagnosis of histoplasmosis, No. (%)	10 (2.2)	12 (9.8)	<.001
Previous empirical use of antifungals,[b] No (%)	8 (1.8)	39 (32.0)	<.001
Fluconazole	2/8 (12.5)	6/39 (15.4)	
Itraconazole	2/8 (12.5)	9/39 (23.1)	
d-AmB	6 (75.0)	30/39 (77.0)	
L-AmB	0 (0.0)	2/39 (5.1)	
ABLC	0 (0.0)	1/39 (2.5)	
Reported exposure to environmental risk factors, No. (%)	(n = 269)	(n = 91)	
Rural activity	69 (25.7)	26 (28.5)	.585
Poultry farms	83 (30.9)	30 (33.0)	.708
Caves	6 (2.2)	3 (3.3)	.573
Places with bats	32 (11.9)	17 (18.7)	.103
Tunnels	2 (0.7)	0 (0.0)	>.999
Construction	76 (28.3)	23 (25.3)	.582
Bird exposure	64 (23.8)	14 (15.4)	.092

Abbreviations: ABLC, amphotericin B lipid complex; d-AmB, amphotericin B deoxycholate; IQR, interquartile range; L-AmB, liposomal amphotericin B; PLWHA, people living with HIV/AIDS.

[a]Municipalities with <10 patients in the cohort were omitted from this table.

[b]In the last 7 days before sample collection.

Patients presented not infrequently with other opportunistic infections, particularly active TB (n = 143, 25.1%). There were 19 cases of histoplasmosis and TB coinfection (15.4% of histoplasmosis cases). As shown in Figure 2, histoplasmosis becomes more frequent than TB in patients with CD4+ cell counts <50 cells/mm^3 (difference not statistically different; P = .085). Pneumocystosis was diagnosed in 57 (10.0%) patients. In addition, 135 (23.7%) patients had bacterial infections, and 78 (13.7%) patients had cytomegalovirus (CMV) disease. CMV disease was reported in 30.5% of patients with histoplasmosis, in comparison with 11.9% of individuals without histoplasmosis (P = .016). Cryptococcosis and invasive candidiasis were uncommon (31 cases each, 5.4%).

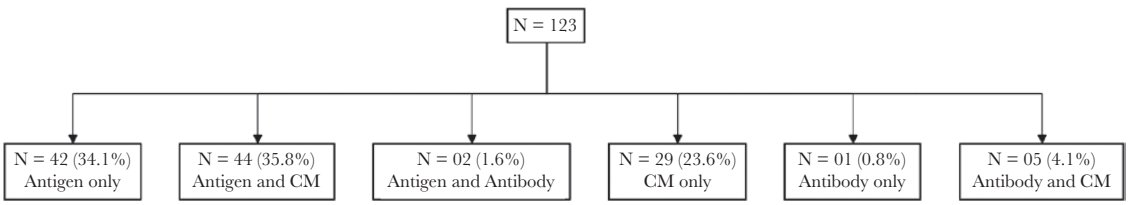

Figure 1. Criteria for diagnosis of histoplasmosis in the 123 patients with probable/proven disease. From the original cohort of 570 patients, 447 were excluded due to negative results. Abbreviation: CM, classical methods (mycological and histopathological examination).

Table 2. Findings on Clinical Examination, Diagnostic Imaging, and Laboratory Tests in PLWHA With or Without Probable/Proven Histoplasmosis at Study Inclusion

Clinical Manifestations	No Histoplasmosis (n = 447)	Probable/Proven Histoplasmosis (n = 123)	*P* Value
Weakness, No. (%)	374 (83.7)	111 (90.2)	.070
Anorexia, No. (%)	254 (56.8)	99 (80.5)	<.001
Night sweats, No. (%)	183 (40.9)	69 (56.1)	.003
Weight loss, No. (%)	336 (75.2)	112 (91.1)	<.001
Amount of weight loss, median [IQR], kg	10 [6–15]	10 [7–15]	.412
Time losing weight, median [IQR], d	60 [30–90]	60 [30–90]	.486
Dyspnea, No (%)	206 (46.1)	53 (43.1)	.555
Generalized lymph node enlargement, No. (%)	69 (15.4)	7 (5.7)	.005
Oral lesions, No. (%)	131 (29.3)	53 (43.1)	.004
Diarrhea, No (%)	149 (33.3)	40 (32.5)	.865
Splenomegaly, No. (%)	55 (12.3)	29 (23.6)	.002
Hepatomegaly, No. (%)	78 (17.5)	43 (35.0)	<.001
Skin lesions, No. (%)	114 (25.5)	48 (39.0)	.003
Papular rash, No. (%)	11 (2.5)	11 (8.9)	.001
Thorax imaging	(n = 348)	(n = 101)	
Normal exam	13 (3.7)	3 (3.0)	.715
Miliary pattern	42 (12.1)	28 (27.7)	<.001
Pleural effusion	51 (14.7)	22 (21.8)	.088
Cavities	27 (7.8)	6 (5.9)	.538
Abdomen imaging	(n = 136)	(n = 56)	
Adrenal enlargement	9 (6.6)	1 (1.8)	.286
Hepatomegaly	85 (62.5)	46 (82.1)	.008
Splenomegaly	79 (58.1)	30 (53.6)	.566
Intra-abdominal lymph node enlargement	47 (34.6)	16 (28.6)	.422
CNS imaging	(n = 48)	(n = 12)	
Cerebral abscess	7 (14.6)	0 (0.0)	.326
Single mass lesion	10 (20.8)	7 (58.3)	.010
Multiple mass lesions	16 (33.3)	3 (25.0)	.735
Laboratory tests			
Pancytopenia	110 (24.6) (n = 447)	65 (52.9) (n = 123)	<.001
C-reactive protein, median [IQR], mg/L	59 [13–106] (n = 378)	59 [28–100] (n = 91)	.312
Lactate dehydrogenase, median [IQR], IU/L	332 [201–602] (n = 355)	901 [428–1880] (n = 107)	<.001
Lactate dehydrogenase >1000 IU/L, No. (%)	41 (11.6) (n = 355)	47 (43.9) (n = 107)	<.001
Alkaline phosphatase, median [IQR], IU/L	104 [75–200] (n = 355)	171 [85–488] (n = 105)	<.001
Ferritin, median [IQR], ng/mL	462 [171–1152] (n = 164)	1389 [488–5446] (n = 39)	<.001
Ferritin >1000 ng/mL, No. (%)	48 (29.3) (n = 164)	21 (53.9) (n = 39)	.004
Aspartate aminotransferase, median [IQR], IU/mL	31 [21–60] (n = 421)	55 [30–116] (n = 120)	<.001
Alanine aminotransferase, median [IQR], IU/L	26 [15–46] (n = 420)	34 [21–59] (n = 119)	.004
Gamma-glutamyltransferase, median [IQR], IU/L	83 [39–179] (n = 348)	112 [70–298] (n = 103)	.001
Hemoglobin, median [IQR], g/dL	10.1 [8.7–12.0] (n = 443)	9.8 [8.4–10.9] (n = 123)	.054
Platelet count, median [IQR], cells/mm^3	191 000 [129 000–281 000] (n = 441)	138 000 [78 000–217 000] (n = 123)	<.001
Leucocyte count, median [IQR], cells/mm^3	5360 [3100–7980] (n = 444)	4400 [2730–7000] (n = 123)	.011
Lymphocyte count, median [IQR], cells/mm^3	895 [510–1450] (n = 438)	564 [345–920] (n = 121)	<.001

Abbreviations: CNS, central nervous system; IQR, interquartile range; PLWHA, people living with HIV/AIDS.

Overall mortality in patients with histoplasmosis at 30 days was 22.0%. Mortality in patients diagnosed only by urinary antigen was 14.3% (6/42 patients), in comparison with patients diagnosed by classical methods (26.9%, 21/78; P = .114). Clinical features and their association with death in 30 days are presented in Table 4. In a multivariate analysis, only the presence of dyspnea at clinical presentation (OR, 2.83; 95% CI, 1.19–7.06) was independently associated with death.

Regional disparities inside Brazil were demonstrated (Figure 3). Centers located in the Northern states (Northeast and Midwest regions) had a higher prevalence of histoplasmosis than centers in the Southern states (South and Southeast regions). Even though there was a difference in mortality across centers, this difference was not present when only patients with histoplasmosis were evaluated (data not shown).

Table 3. Multivariate Model for the Clinical Prediction of Probable/Proven Histoplasmosis in the PLWHA Cohort

Clinical Variable	Odds Ratio (95% Confidence Interval)
CD4+ <50 cells/mm^3	2.11 (1.17–3.82)
Pancytopenia	1.79 (1.00–3.21)
Miliary pattern on thorax imaging	2.72 (1.35–5.46)
Hepatomegaly on clinical examination	2.47 (1.28–4.76)
Generalized lymphadenopathy	0.37 (0.11–0.96)
Lactate dehydrogenase >1000 IU/L	3.60 (1.94–6.69)

Abbreviation: PLWHA, people living with HIV/AIDS.

DISCUSSION

This is the largest prospective cohort study of AIDS patients with disseminated histoplasmosis ever conducted [20–23]. We found a high rate (21.6%) of probable/proven histoplasmosis in febrile PLWHA admitted to Brazilian hospitals. These findings have tremendous impact in terms of public health and disease awareness in South America, considering that Brazil is the largest country and has the greatest population in this region.

Disseminated histoplasmosis is an opportunistic infection that usually occurs when the CD4+ cell count is <150 cells/mm^3, and its prevalence rates vary widely. The mortality of AIDS-associated histoplasmosis ranges between 20% and 70% in studies carried out mainly in developing countries [21, 22, 24–26]. Clinical manifestations of disseminated histoplasmosis may often mimic other diseases, like TB. Pulmonary involvement is also common, along with systemic symptoms such as fever and weight loss. Skin lesions, hoarseness, gastrointestinal ulceration or strictures, meningitis, and adrenal masses are other clinical manifestations often encountered in this condition [2, 3]. Such unspecific clinical presentations and the high lethality observed lead to an urgent need for rapid and accurate diagnostic tools, along with the identification of clinical features that increase the probability of this diagnosis. In our cohort, weight loss, hepatomegaly, pancytopenia, miliary pattern on thorax imaging, lactate dehydrogenase (LDH) >1000 IU/L, and the absence of generalized lymphadenopathy were independently associated with diagnosis of probable/proven histoplasmosis in PLWHA. These findings are in agreement with other South American reports [21, 22, 26]. In a descriptive study of 25 years of experience in French Guiana, including 200 cases, there were many similarities regarding laboratory findings with our study. In particular, increased LDH, aminotransferases, alkaline phosphatase, ferritin, and thrombocytopenia were more frequent in patients with histoplasmosis in both these large studies. In contrast, the study from French Guiana identified a majority (55.3%) of patients presenting with enlarged lymph nodes [27]. As already postulated as the reason for increased frequency of skin lesions in a Brazilian case series, genetic differences in fungal strains may influence enlargement of lymph nodes [11, 22]. Incomplete records in clinical notes may also have accounted for such differences.

Laboratory diagnosis of histoplasmosis is often difficult and overlooked. Available tests include culture, microscopy, histopathology, antibody detection, antigen detection, and molecular assays. Culture can take up to 4 weeks to reveal growth.

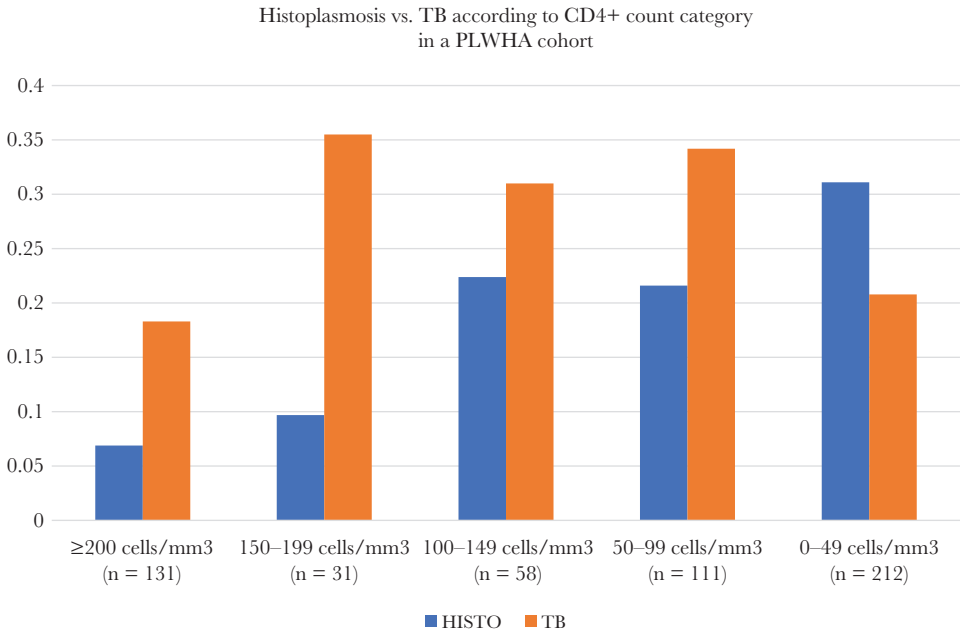

Figure 2. Comparative frequency of histoplasmosis and tuberculosis in a cohort of PLWHA in Brazil. Abbreviations: HISTO, histoplasmosis; PLWHA, people living with HIV/AIDS; TB, tuberculosis.

Table 4. Clinical Predictors of 30-Day Mortality in 123 PLWHA With Probable/Proven Histoplasmosis (Bivariate and Multivariate Analysis)

	Bivariate Analysis			Multivariate Analysis
Variable	30-d Survivors (n = 96)	Death in 30 d (n = 27)	*P* Value	OR (95% CI)
Demographics				
Age, median [IQR], y	40 [34–46]	36 [30–43]	.078	
Female gender, No. (%)	25 (26.0)	8 (29.6)	.710	
HIV disease status				
Receiving antiretroviral treatment at study entry, No. (%)	34 (35.4)	8 (29.6)	.575	
CD4+ count, median [IQR], cells/mm^3	38 [18–84]	49 [8–133]	.948	
CD4+ <50 cells/mm^3, No. (%)	55 (58.5)	11 (52.4)	.608	
Previous history				
Previous diagnosis of histoplasmosis, No. (%)	9 (9.4)	3 (11.1)	.724	
Symptoms and clinical examination				
Weakness, No. (%)	86 (89.6)	25 (92.6)	.642	
Anorexia, No. (%)	73 (76.0)	26 (96.3)	.025	
Night sweats, No (%)	52 (54.2)	17 (63.0)	.416	
Weight loss, No. (%)	87 (90.6)	25 (92.6)	>.999	
Dyspnea, No. (%)	36 (37.5)	17 (63.0)	.018	2.83 (1.19–7.06)
Oral lesions, No. (%)	41 (42.7)	12 (44.4)	.872	
Diarrhea, No. (%)	29 (30.2)	11 (40.7)	.302	
Splenomegaly at clinical examination, No (%)	23 (24.0)	6 (22.2)	.851	
Hepatomegaly at clinical examination, No (%)	33 (34.4)	10 (37.0)	.798	
Skin lesions, No. (%)	37 (38.5)	11 (40.7)	.836	
Imaging				
Miliary pattern on thorax imaging, No. (%)	24 (30.8)	4 (17.4)	.291	
Hepatomegaly on imaging, No. (%)	35 (81.4)	11 (84.6)	>.999	
Splenomegaly on imaging, No. (%)	19 (44.2)	11 (84.6)	.013	
Laboratory abnormalities				
Pancytopenia, No. (%)	52 (54.2)	13 (48.2)	.580	
Lymphocytes ≤500 cells/mm^3	34 (35.8)	15 (57.7)	.044	
LDH >1000 IU/L, No. (%)	31 (38.3)	16 (61.5)	.038	
Ferritin >1000 ng/L, No. (%)	16 (53.3)	5 (55.6)	>.999	
HIV opportunistic diseases				
Coinfection with *P. jirovecii*, No. (%)	9 (9.4)	5 (18.5)	.186	
Coinfection with tuberculosis, No. (%)	15 (15.6)	4 (14.8)	>.999	
Coinfection with CMV, No. (%)	16 (16.7)	9 (33.3)	.057	
Diagnosis of histoplasmosis				
Diagnosis by classic methods, No. (%)	57 (67.9) (n = 84)	21 (84.0) (n = 25)	.116	
Positive direct microscopy, No. (%)	21 (53.9) (n = 39)	7 (77.8) (n = 9)	.270	
Positive culture for *H. capsulatum*, No. (%)	25 (38.5) (n = 65)	10 (41.7) (n = 24)	.784	
Only antigen testing positive, No. (%)	36 (37.5) (n = 96)	6 (22.2) (n = 27)	.139	
Therapy				
Empirical antifungal therapy	77 (80.2) (n = 96)	23 (85.2) (n = 27)	.558	
Histoplasmosis-directed antifungal therapy	53 (68.8) (n = 77)	18 (78.3) (n = 23)	.381	
Use of antifungals before study entry	32 (33.3) (n = 96)	7 (25.9) (n = 27)	.465	

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IQR, interquartile range; LDH, lactate dehydrogenase; OR, odds ratio; PLWHA, people living with HIV/AIDS.

Microscopy is more rapid but may have low sensitivity and specificity. Histopathological findings of small intracellular budding yeasts using specific stains are diagnostic, although they can be mistaken with other organisms such as *Leishmania* species (also endemic in Brazil). In addition, both microscopy and histopathology require highly trained professionals to make a proper diagnosis [28]. Antibody detection has limited sensitivity in immunocompromised individuals with disseminated disease. Hence, quick, noninvasive, and sensitive diagnostic methods are needed, and so far only antigen detection and polymerase chain reaction (PCR) tests meet these requirements [2]. Unfortunately, PCR is not available in most laboratories in Latin America and the Caribbean. Also, *H. capsulatum* antigen detection is not available in endemic areas for histoplasmosis, including Brazil [29]. Therefore, histoplasmosis is frequently diagnosed as a late-stage disease.

We used a previously validated *Histoplasma* antigen test (*Histoplasma* galactomannan single-monoclonal-antibody

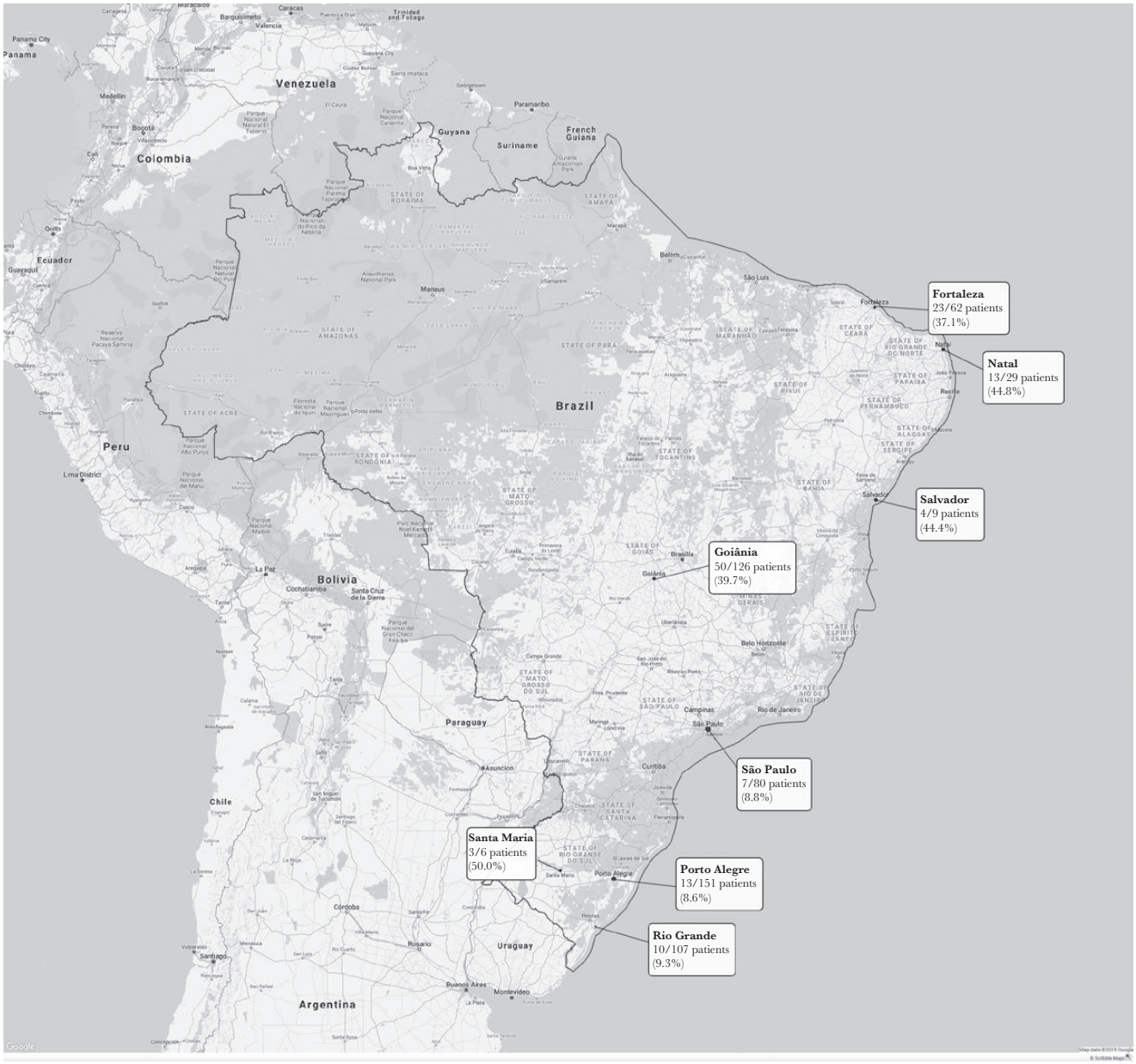

Figure 3. Prevalence of probable/proven histoplasmosis in people living with HIV/AIDS according to city of sample collection.

sandwich ELISA; Immuno-Mycologics, Norman, OK) which is known to have a better performance than gold standard tests in the diagnosis of histoplasmosis (ie, classical methodological methods). Nevertheless, we observed a lower sensitivity of the antigen test (71.5%) in comparison with a previous study [17]. We believe that the reduced test sensitivity observed in our study could be attributable to previous use of antifungal drugs (which occurred in 39.7% of "proven" histoplasmosis patients). Some of the discordant results between antigen detection tests and classical mycology tests could be justified by the time between sampling for fungal culture and the urinary *Histoplasma* antigen test (average 39 days).

Our cohort study supports these arguments, demonstrating high 30-day mortality (26.9%) in patients with proven disease—that is, confirmed by classic mycological methods, which is concordant with current reports. Antigen detection using the *Histoplasma* galactomannan test improved diagnostic yield in 53.8%. In patients with only a positive antigen test, 30-day mortality was lower (14.3%), though it was not significantly different, than in patients diagnosed with classical methods. Indeed, patients only with a positive antigen test could be false positives, which should lead to lower mortality. However, these were all febrile inpatients individuals with HIV/AIDS, many of whom had very low CD4+ counts. The pretest probability for histoplasmosis is much higher than a false-positive result in these settings. It also should be noted that centers were not notified of antigen results on time to make clinical decisions; therefore, patients were

not treated based on antigen results, something that may prove to substantially decrease mortality in future studies. These findings illustrate the potential benefit of the availability of this diagnostic tool in the management of PLWHA.

Brazil is a continental country and has many disparities in terms of climate, soil composition, and economics. Despite the HIV infection being widespread in the country, some opportunistic diseases still occur in a typically endemic behavior. We observed a huge (>40%) prevalence of probable/proven histoplasmosis among febrile PLWHA in the Central-Northeast region of Brazil, especially in the cities of Fortaleza and Natal. Despite these figures, cities from South, including Porto Alegre and Sao Paulo, had prevalence rates under 10%. These findings should allow for an increase in disease awareness nationwide, with a special emphasis on particular regions of the country.

TB is a huge threat to public health in Brazil. Brazil is 1 of the 20 countries with the highest TB burden in the world [30]. However, as shown in our study, histoplasmosis is an HIV opportunistic infection that has a comparable frequency. The endemicity of histoplasmosis, along with the occurrence of disseminated disease in immunocompromised people, is now a well-defined phenomenon in Central and South America, and it represents a significant threat to PLWHA with low CD4+ counts [5]. We demonstrated that in these low–CD4+ cell count categories, even though histoplasmosis is a major opportunistic infection affecting PLWHA in Brazil, it tended to be more frequent than TB (with the lack of statistical difference being probably due to the limited number of patients in this category included in the trial). This should prompt revisiting of the argument for itraconazole prophylaxis in the region, particularly in the Northern states where the prevalence is very high. Itraconazole has significant drug–drug interactions that should be taken into account, considering the possibility of TB coinfection and the need of rifampin as the basis of TB therapy [31]. Another interesting but speculative approach could be an aggressive screening and preemptive therapy strategy for histoplasmosis in patients with low CD4+ cell counts from areas with high prevalence. This strategy is currently recommended for cyrptococcal infection using cryptococcal antigen detection [32, 33] and may also be reasonable for histoplasmosis. In areas with lower prevalence, this screen-and-treat strategy should be more restricted considering cost-effectiveness, just like what has been done with cryptococcosis [34, 35].

In summary, histoplasmosis is a frequent opportunistic infection in Brazil, and antigen detection could improve the diagnostic capacity of this condition, potentially improving clinical outcomes, including mortality. Knowledge on histoplasmosis epidemiology is absolutely necessary given the high mortality associated with this condition. Access to diagnostic tools and antifungal drugs, including point-of-care technologies and antiretroviral treatment, is urgently needed to decrease disease burden in South America.

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