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Diagnostic Value of Culture and Serological Tests in the Diagnosis of Histoplasmosis in HIV and non-HIV Colombian Patients

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Abstract. We determined the value of culture and serological tests used to diagnose histoplasmosis. The medical records of 391 histoplasmosis patients were analyzed. Diagnosis of the mycosis was assessed by culture, complement fixation, and immunodiffusion tests; 310 patients (79.5%) were male, and 184 patients (47.1%) were infected with human immunodeficiency virus (HIV). Positivity value for cultures was 35.7% (74/207), reactivity of serological tests was 95.2% (160/168), and a combination of both methodologies was 16.9% (35/207) for non-HIV patients. Positivity value for cultures was 75.0% (138/184), reactivity of serological tests was 92.4% (85/92), and a combination of both methodologies was 26.0% (48/184) for HIV/acquired immunodeficiency syndrome (AIDS) patients; 48.1% (102/212) of extrapulmonary samples from HIV/AIDS patients yielded positive cultures compared with 23.1% (49/212) in non-HIV patients. Lymphocyte counts made for 33.1% (61/184) of HIV/AIDS patients showed a trend to low CD4+ numbers and higher proportion of positive cultures. These results indicate that culture is the most reliable fungal diagnostic method for HIV/AIDS patients, and contrary to what is generally believed, serological assays are useful for diagnosing histoplasmosis in these patients.

INTRODUCTION

Histoplasmosis is a systemic mycosis endemic to the Americas, where it is widely distributed. Its etiological agent is the thermally dimorphic fungus *Histoplasma capsulatum*, and its natural habitat is the soil, especially when contaminated with bird and bat droppings.[1,2] This fungal infection is acquired by inhalation of infectious propagules (microconidia), which on reaching the lungs, undergo a transformation to virulent yeast cells, thus initiating the infectious process.[1] Although the lung is considered to be the primary target, this fungus can disseminate to virtually any organ, especially bone marrow, skin, brain, adrenal glands, and the gastrointestinal tract.[3,4] Clinical manifestations of the disease depend on the degree of exposure, frequency of infectious contacts, and size of inocula as well as age and immune status of the host.[5] The infection is asymptomatic or self-limiting in 90–95% of immunocompetent individuals, where it may remain in a latent stage and only reactivate when a disequilibrium of the immune system occurs. In adult patients with pulmonary structural lesions, the infection may progress to a chronic infection, leading to a cavitary process.[6,7] By contrast, in immunocompromised patients, the infection frequently results in a progressive disseminated and severe disease.[3,4]

In acquired immunodeficiency syndrome (AIDS) patients, the progressive disseminated form is the most frequent clinical presentation, and its course is usually acute or subacute. It is seldom chronic but may be lethal if not treated promptly.[8,9] In human immunodeficiency virus (HIV) patients, particularly those patients living in endemic areas, the mycosis may be the result of recent infection, reactivation of a latent infection, or reinfection. Nowadays, despite the increased use of highly active antiretroviral therapy (HAART), histoplasmosis remains an AIDS-defining illness.[10–13]

The diagnosis of histoplasmosis is based on fungal isolation by culture or microscopic examination of the respiratory tract, biopsies, and body fluid specimens; however, laboratory assays only yield positive results in approximately 50% of cases.[3,5,14,15] Although culture is generally considered to be highly specific, it has several notable limitations, including low sensitivity, a need for invasive procedures in many cases, and delayed fungal growth (approximately 2–6 weeks).[3,5,16] Although serological tests for antibody detection (particularly immunodiffusion and complement fixation) are useful, they often give negative results for immunosuppressed patients; furthermore, elevated antibody titers may persist for several years after the initial infection, thus complicating their interpretation.[3,16] The above results lead us to hypothesize that both culture and serological methodologies (when used to diagnose histoplasmosis) are valuable laboratory tools for diagnosing, despite the differences exhibited in HIV/AIDS and non-HIV patients populations with the mycosis.

The increase in the number of patients with progressive disseminated hitstoplasmosis (PDH) being diagnosed at the Corporación para Investigaciones Biológicas (CIB) laboratories during the last years and the regular presence of HIV comorbidity in a sizeable population of patients prompted us to search for the accuracy of the methods used for the diagnosis of this fungal disease. Thus, in the present study, which was carried out over a period of 20 years (1987–2007), we evaluated patients diagnosed with histoplasmosis through the use of conventional methods (mainly culture and serological assays). The main objectives were to determine the diagnostic value of these conventional laboratory assays and compare the results obtained for HIV/AIDS and non-HIV patients with histoplasmosis.

METHODOLOGY

Patients. In total, 391 cases of histoplasmosis diagnosed from 1987 to 2007 at the Medical and Experimental Mycology

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Unit of the CIB in Medellín, Colombia were studied. Clinical and laboratory records of the patients with the mycosis were analyzed and stored in an Excel database (Microsoft, Redmond, WA). The database included demographic characteristics, date of diagnosis, HIV status, and clinical sample origin. Of 391 patients with histoplasmosis, 184 patients were HIV-positive (confirmed by laboratory test); CD4+ lymphocyte counts were determined for only 61 of the patients in this group.

Laboratory data. Definition of histoplasmosis was based on the presence of a compatible clinical record and at least one of the following mycological test parameters[3,17]: (1) isolation of the fungal pathogen in culture and/or (2) detection of specific antibodies against *H. capsulatum* in serum or cerebrospinal fluid (CSF). Cultures were assessed using different clinical specimens, including bronchoalveolar lavage fluids (BALs), bronchial lavages (BLs), sputum, whole blood, bone marrow, peritoneal fluid, CSF, biopsies, and exudates among others. A positive culture obtained from a different clinical of lung was considered as a criterion of dissemination. Serological assays included agar gel immunodiffusion (ID), which was considered reactive when bands M, H, or both were present, and complement fixation (CF), where titers ≥ 1:32 with either histoplasmin (H antigen) or whole yeast cells (Y antigen) were considered to be positive. In certain cases, CF titers of 1:8 and 1:16 were also accepted for the diagnosis of the mycosis. In addition, the number and results of serological tests performed on some patients during the clinical follow-up period were also recorded.

Ethical considerations. In the present study, the authors did not interact with the patients or perform any manipulation of their clinical samples; they only worked with some of the clinical, epidemiological, and laboratory data contained in their records.

Statistical analysis. Data were processed with the statistical program SPSS 16.0 (SPSS Inc., Chicago, IL). Statistical analyses were done using χ^2 or Fisher exact test. The Pearson correlation test was used to evaluate an association between CD4+ lymphocyte counts and number of culture isolates or serological test reactivity.

RESULTS

During the study period (1987–2007), 391 patients were diagnosed with histoplasmosis at the Medical and Experimental Mycology Unit of the CIB. Of these patients, 207 patients (52.9%) were ascribed to the non-HIV patient cohort, and 184 patients (47.1%) were placed in the HIV/AIDS-infected cohort. Most of the patients were men, who comprised 71% of the non-HIV–infected individuals and 89.1% of the HIV/AIDS-infected group. The mean ages were 36.3 (SD ± 18.6) and 34.1 (SD ± 9.1) years for the non-HIV– and the HIV/AIDS -infected individuals, respectively. It is noteworthy that increasing numbers of patients were diagnosed with histoplasmosis from 1999 onward (Figure 1).

Histoplasmosis was diagnosed by fungal culture alone in 212 (54.2%) of 391 patients studied, serology in 245 (62.7%) of 391 patients studied, and a combination of both methods in 83 (21.0%) of 391 patients studied. When HIV status was taken into account, we observed that, in the infected patient group, the percentages of positive values for culture, CF, and ID tests were 75% (138/184), 78.7% (74/94), and 92.4% (85/92), respectively, whereas non-HIV patients presented corresponding values of 35.7% (74/207), 86.8% (145/167), and 95.2% (160/168), respectively (Figure 2). The combination of culture and serological methods allowed diagnosis of the mycosis in 48 of 184 HIV/AIDS patients (26.0%) and 35 of 207 non-HIV patients (16.9%).

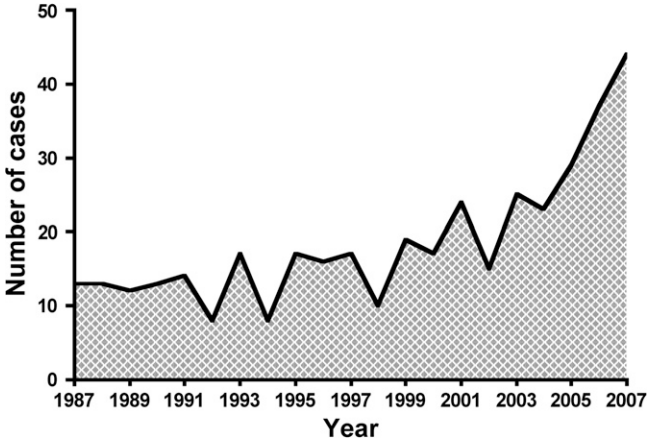

FIGURE 1. Increase in the number of patients with histoplasmosis diagnosed at the CIB during the years 1987–2007.

Culture methods. A total of 212 primary isolates of *H. capsulatum* was obtained by culturing different clinical samples sent from the various hospitals to the CIB Medical and Diagnostic Mycology Laboratory. The numbers of patients in which fungal pathogen was isolated by culture according to the HIV status and the type of clinical specimens used are described in Table 1.

We observed a significantly higher proportion of positive cultures in the HIV/AIDS patients (138/184) than the non-HIV patients (74/207; 75.0% versus 35.7%, respectively; $P < 0.001$) (Figure 2). It is noteworthy that significantly more of the clinical samples assessed by culture ($N = 102$) were from HIV/AIDS patients with the disseminated forms (extrapulmonary samples) of the mycosis than the non-HIV patient population ($N = 49$; 48.1% versus 23.1%, respectively; $P < 0.001$)

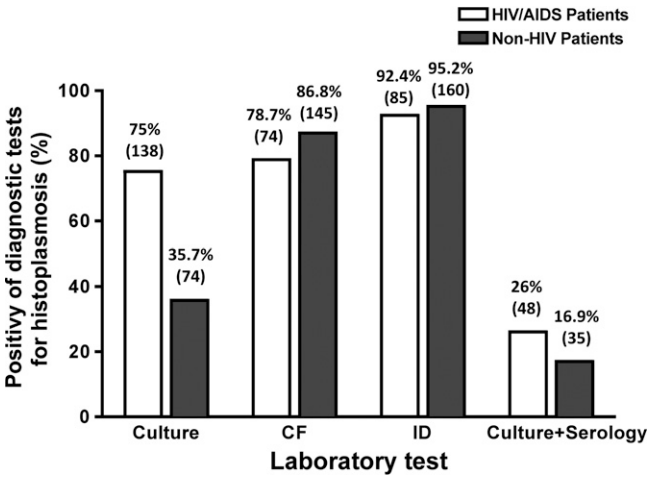

FIGURE 2. Positivity of the laboratory tests (percentage) used to diagnose histoplasmosis in both HIV- and non-HIV–infected individuals.

TABLE 1

Number of patients in which *H. capsulatum* was isolated by culture according to the HIV status and type of clinical specimen used

Specimen type	Number of positive cultures in HIV patients	Number of positive cultures in non-HIV patients	Total *n* (%)
Biopsy	59	23	82 (38.7)
BAL	22	9	31 (14.6)
Skin scrapings	14	6	20 (9.4)
Sputum	11	7	18 (8.5)
BL	8	7	15 (7.1)
Exudates	7	5	12 (5.7)
Whole blood	8	1	9 (4.2)
CSF	2	6	8 (3.8)
Bone marrow	2	3	5 (2.4)
Urine	3	0	3 (1.4)
Synovial fluid	1	1	2 (0.9)
Buccal smear	1	2	3 (1.4)
Peritoneal fluid	0	2	2 (0.9)
Pericardial fluid	0	1	1 (0.5)
Lymph node aspirate	0	1	1 (0.5)
Total	138	74	212 (100)

(Figure 3A). The remaining clinical samples were restricted to those samples involving only the lungs (Figure 3A).

Note that 108 (50.9%) of HIV/AIDS patients and 67 (31.6%) of non-HIV individuals had only a single isolate of the fungus by culture, whereas 18 (8.5%), 9 (4.3%), and 3 (1.4%) of the HIV/AIDS patients had two, three, and four fungal isolates from different samples, respectively. By contrast, only four (1.9%) and three (1.4%) of the non-HIV–infected patients had two and three fungal isolates by culture from different samples, respectively (data not shown).

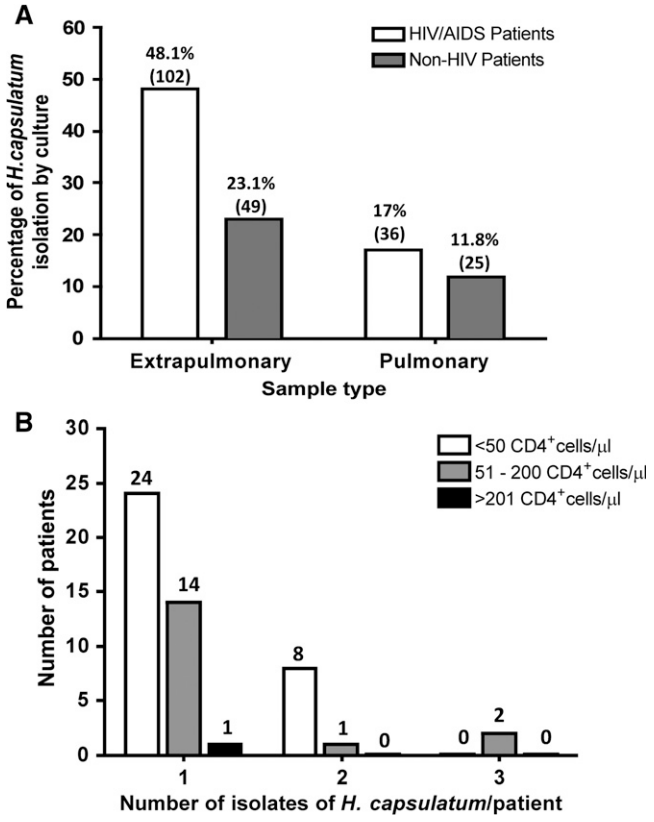

FIGURE 3. (**A**) Percentage of *H. capsulatum* culture isolates from pulmonary or extrapulmonary specimens obtained from HIV- and non-HIV–infected patients. (**B**) Number of *H. capsulatum* culture isolates per patient according to CD4+ lymphocyte counts.

To determine whether an association existed between the CD4+ lymphocyte counts and the number of *H. capsulatum* isolates obtained per patient, we created a 3 by 3 contingency table and analyzed the data using the χ^2 and Pearson statistical correlation tests. A total of 50 patients was grouped according to the number of isolates (one, two, or three cultured isolates per patient) and the CD4+ lymphocyte counts as follows: (1) patients with < 50 CD4+ cells/μL, (2) patients with CD4+ counts between 51 and 200 cells/μL, and (3) patients with > 201 cells/μL. Although we did not observe a significant correlation between these two variables (P = 0.1673), eight (16%) of the patients with CD4+ counts below 200 cells/μL had two culture isolates from different clinical samples (Figure 3B).

Serological assays. A total of 262 patients was evaluated by serological assays, of which 245 patients (93.5%) were positive for CF, ID, or both assays when either serum or CSF was assessed.

The ID test was reactive in 85 of 92 (92.4%) HIV/AIDS patients evaluated with the precipitin or band M (present in 82 patients) or both H and M bands (present in 3 patients) (Table 2). By contrast, 160 of 168 (95.2%) non-HIV–infected patients were reactive for the ID test. The precipitin M was seen in 151 patients (9 patients reactive to CSF), whereas 9 patients showed both precipitins H and M (2 patients reactive to CSF) (Table 3). A total of 15 patients in both groups of patients (7 of the patients belonging to HIV/AIDS group) was non-reactive for the ID test (Tables 2 and 3). It is noteworthy that these negative patients in the ID assay were reactive for the CF test or had *H. capsulatum* isolated from their cultures.

Moreover, 94 patients belonging to the HIV-infected group were evaluated by CF test, with 68 (72.3%) of the patients showing CF titers above the 1:32 dilution. However, when CF titers of 1:8 and 1:16 dilutions were considered, six more patients were identified, enhancing the positivity value to 78.7% (Table 2). Twelve patients in this group were non-reactive, and eight patients were anticomplementary (Table 2).

A total of 167 non-HIV patients was evaluated using the CF test, and 122 patients (73%) showed CF titers above the 1:32 dilution. When CF titers of 1:8 and 1:16 dilutions were also taken into account, 23 more patients were diagnosed, enhancing the positivity value to 86.8% (Table 3). Among this group, 21 patients were non-reactive, and 1 patient was

TABLE 2

Results of the serological test performed to detect antibodies against *H. capsulatum* in HIV-infected patients

Serological test	Specimen type (*n*)		Total
	Serum	CSF	
Immunodiffusion			
Band M	82	0	82
Bands H and M	3	0	3
Non-reactive	6	1	7
Total	91	1	92
Complement fixation			
H-Ag and Y-Ag ≥ 1:32	67	1	68
H-Ag and Y-Ag ≤ 1:16	6	0	6
Non-reactive	12	0	12
Anticomplementary	8	0	8
Total	93	1	94

H-Ag = histoplasmin antigen; Y-Ag = yeast antigen.

TABLE 3
Results of the serological test performed to detect antibodies against *H. capsulatum* in non-HIV–infected patients

Serological test	Specimen type (*n*)		Total
	Serum	CSF	
Immunodiffusion			
Band M	142	9	151
Bands H and M	7	2	9
Non-reactive	8	0	8
Total	157	11	168
Complement fixation			
H-Ag and Y-Ag ≥ 1:32	112	10	122
H-Ag and Y-Ag ≤ 1:16	22	1	23
Non-reactive	21	0	21
Anticomplementary	1	0	1
Total	156	11	167

H-Ag = histoplasmin antigen; Y-Ag = yeast antigen.

anticomplementary (Table 3). Furthermore, all patients who were non-reactive or anticomplementary in the CF assay were reactive to the ID test or had *H. capsulatum* isolated from their cultures.

Two antigens were used in the CF assay (i.e., the H antigen or histoplasmin [a fungal mycelial form filtrate] and the Y antigen [whole yeast cells]). It is noteworthy that 69 HIV/AIDS patients (73.4%) were reactive with CF titers above 1:8 dilutions when the H antigen was used compared with 45 HIV/AIDS patients (47.9%) when the Y antigen was used (data not shown). When the non-HIV patient group was assessed by CF test using the H and Y antigens, we observed that 145 patients (86.8%) reacted to the H antigen at CF titers above the 1:8 dilution, whereas only 101 patients (60.5%) were reactive with the Y antigen at this level (data not shown).

In addition, we evaluated 48 patients who had more than four serological tests in their follow-up program, and 9 of these patients were HIV-positive. After evaluation, we observed that 21 patients (43.7%) became non-reactive for the CF test, whereas a decrease of antibody titers with values of ≤ 1:16 dilutions was observed in 13 patients (27.1%). In seven (14.6%) of these patients, antibody titers fell but remained in a range between 1:32 and 1:128 dilutions during the clinical follow-up, and in the other seven patients (14.6%), the CF antibody titers remained higher during clinical monitoring (data not shown); 41 (85.4%) of 48 patients were reactive to the ID test, showing the presence of the M precipitin at the end of the follow-up period (data not shown).

We found no significant correlation between CD4+ lymphocyte counts and CF titers or the presence of precipitin M or H.

DISCUSSION

In the present report, we evaluated a total of 391 patients with histoplasmosis diagnosed over a period of 20 years at the Medical and Experimental Mycology Unit of CIB. An increase in the number of patients with this mycosis was observed from 1999 onward. We have observed that this mycosis was more common in males (79.5%), especially males between the ages of 21 and 40 years with or without HIV/AIDS.[17–19] Other studies have reported similar results; an epidemiological survey performed in European countries by the European Confederation of Medical Mycology Working Group found that 75% of histoplasmosis patients were males ages 21–40 years.[19] More recently, Arango and others[17] reported that, in a cohort of Colombian histoplasmosis cases, 77% were males, with a mean age of 38.4 years; an AIDS-related condition was observed in 70.5% of these patients.[17]

It is well-known that HIV/AIDS patients who live in endemic areas are among the groups of individuals at greatest risk of developing histoplasmosis. Thus, the progressive disseminated form has emerged as an important opportunistic infection among Colombian HIV/AIDS patients.[20] In HIV/AIDS patients, histoplasmosis may occur as a result of primary infection (exogenous) or reactivation of a previous infection (endogenous); both have been documented in studies conducted in our country.[2,20,21] In the present study, we observed that 184 (47%) of patients had HIV coinfections, and 138 patients (75%) yielded positive cultures. Similar results were reported in a study performed by Huber and others,[22] which evaluated 200 HIV/AIDS patients with histoplasmosis and found that 76.6% of them were positive based on cultures.[22] It is interesting that, in our study, we observed that 48.1% of HIV/AIDS patients had *H. capsulatum*-positive cultures when extrapulmonary samples were assessed compared with 23.1% of non-HIV–infected individuals. Moreover, 30 (14.2%) and 7 (3.3%) of HIV/AIDS and non-HIV patients, respectively, had more than two *H. capsulatum* isolates (one of them in a different clinical specimen of lung), indicating that, in the former, the disseminated form of the mycosis is the most common clinical presentation. In addition, a mean of 61.4 lymphocytes/μL (range = 3–507 CD4 cells/μL) was detected in 61 patients belonging to the HIV/AIDS group. A correlation between the CD4+ lymphocyte count and the number of *H. capsulatum* isolates per patient was performed in 50 of these individuals. Although we did not find a significant correlation in this analysis, we observed that, in eight of the patients who had two culture isolates, their CD4+ lymphocyte counts were below 50 cells/μL. This result confirms that HIV/AIDS patients tend to be at most risk of exhibiting severe and progressive disease when their CD4 counts are less than 150 per microliter.[3] Among other fungal disorders observed in HIV/AIDS patients, it has been noted that the most common oral lesions (particularly candidiasis) cannot be correlated with CD4+ T-cell counts,[23] an observation that concurs with our results. As additional corroboration of this result, Vail and others[24] reported that, among a cohort of HIV/AIDS patients with recent histoplasmosis, those patients with CD4 counts between 200 and 500 cells/μL gave positive results for lymphoproliferative response and interferon-γ production of 8% and 33%, respectively. Corresponding values for those individuals who had CD4 counts of over 500 cells/μL were 31% and 46%, respectively.[24] We can, therefore, conclude that the immune responsiveness is depressed in patients with CD4 counts below 200 cells/μL, preventing adequate control of fungal burden and allowing it to disseminate.

Although most laboratory tests for the diagnosis of histoplasmosis seem to be effective, their sensitivities and specificities vary according to the clinical presentation of the disease. Cultures are the most reliable test for the establishment of the fungal diagnosis. However, it may take 3–4 weeks for the fungus to grow, and the sensitivity of cultures is also dependent on the fungal load. Moreover, serological tests are widely considered to be of great value for histoplasmosis diagnosis. Wheat[25,26] reported that serological assays detected 100% of

patients with chronic pulmonary histoplasmosis; 71% of those patients had the disseminated form, and 98% of those patients had self-limited infection, although only 70% of AIDS patients had self-limited infection.[25,26] The poor sensitivity in HIV/AIDS victims can be explained by the immune system deterioration suffered by these patients. In the present study, we observed that 78.7% and 92.4% of HIV/AIDS-infected patients reacted with CF and ID, respectively, whereas non-HIV individuals showed reactivities of 86.8% and 95.2%, respectively. Reactivity to CF was enhanced when titers ≥ 1:8 were considered as positive. Moreover, use of the two antigens (H and Y) allowed more patients with histoplasmosis to be diagnosed. In most cases, those patients who did not react to the H antigen did react to the Y antigen or vice versa. CF assays also allowed follow-up of a group of patients, including nine HIV/AIDS patients, and revealed that 43.7% of them became non-reactive after treatment, whereas 41.7% experienced a decrease in their CF titers relative to the results obtained at diagnosis. However, antibody titers remained reactive for dilutions between 1:8 and 1:128, and 14.6% of the remaining patients showed higher CF titers than observed at diagnosis (data not shown). Contrary to what was expected, we found no significant correlation ($P = 0.1625$) when CD4+ cell counts and the antibody titers were analyzed. HIV/AIDS patients have altered capability to produce normal levels of specific antibodies during microbial infections. However, the results observed in the present study indicate that HIV/AIDS patients are capable of producing specific antibodies against *H. capsulatum*; in addition, it is important to note that most of the patients were living in endemic areas and thus, may have had contact with the fungus before acquiring the HIV infection. If so, these patients might have retained an immunological memory that could have been activated by fungal reinfection. Nevertheless, the serological results observed in this study suggest that these assays are reliable for patients with histoplasmosis, including those patients infected with HIV.

It is important to note that histopathologic analyses are an acceptable means to establish a probable diagnosis of hitoplasmosis; however, in the present study, we did not take into consideration the results of microscopic examinations, because these examinations were only performed in a few patients.

The diagnoses of fungal infections and especially, histoplasmosis are generally an arduous process, particularly in severely immunocompromised patients with non-specific symptoms. In such patients, whole-blood cultures have low sensitivity, the specific humoral immune response is diminished, and furthermore, the general condition of the patients often does not allow invasive procedures to be used to obtain representative samples.[25] Thus, physicians must be cautious in diagnosing histoplasmosis and have an adequate knowledge of its epidemiology and clinical features as well as a broad understanding of the uses and limitations of mycological diagnostic tests.

Major limitations of the present study were its retrospective nature along with the fact that some variables were not analyzed. However, this study is the first study in which the diagnostic value of serological tests was evaluated in a sizeable population of patients with promising results. Despite the limitations of this study, the findings presented here may be an accurate reflection not only of the Latin American situation but also, the situation in North America and Europe, where similar results have been described.[19] Although techniques for antigen level determination or molecular diagnosis are unavailable in most laboratories worldwide, serological assays seem to be important and valuable laboratory tools for not only the initial detection of histoplasmosis but also, monitoring patients. Finally, a combination of different laboratory methodologies as well as the use of two or more antigens in serological tests should be used to obtain a faster and more accurate diagnosis of histoplasmosis. In addition, the results presented in this communication clearly indicate that, contrary to what is generally believed, serological tests are valuable laboratory tools in most patients for the diagnosis of histoplasmosis, even immunocompromised patients.

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