



Histoplasmosis in a Brazilian center: clinical forms and laboratory tests

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Summary

Histoplasmosis, caused by the dimorphic fungus *Histoplasma capsulatum*, is endemic in many regions of the Americas, Asia and Africa. It has a wide spectrum of clinical manifestations, from asymptomatic infection to severe disseminated disease. A retrospective study was carried out to describe the clinical forms and assess the clinical significance of the laboratory diagnostic tests of patients with histoplasmosis during the period of July 1987 to December 2003 at Instituto de Pesquisa Clínica Evandro Chagas/ FIOCRUZ, RJ, Brazil. Seventy-four patients were included. Forty-nine percent of the cases (n=36) occurred in HIV positive patients who presented with disseminated disease. The remaining 38 cases were classified in different clinical forms. *Histoplasma capsulatum* was isolated from 69.5% of the clinical specimens sent to culture. Immunodiffusion and immunoblot were positive in 72.6 % and 100% of the performed tests, respectively. Histopathologic findings suggestive of *H. capsulatum* were found in 63.2% of the performed exams. Serology had a lower proportion of positivity amongst AIDS patients, when compared with HIV negative patients ($X^2 = 6.65$; $p < 0.008$). Statistical differences between AIDS and non-AIDS patients were not observed with culture and histopathology. The specific role of each test varies according to the clinical form. Physicians need to know the value and limitations of the available diagnostic tests, but before that, they have to think about histoplasmosis and consider this clinical entity in their differential diagnosis.

Key words

Histoplasmosis, *Histoplasma capsulatum*, Diagnosis, AIDS

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Histoplasmosis: presentaciones clínicas y pruebas de laboratorio en un centro brasileño

Resumen

La histoplasmosis, causada por el hongo dimórfico *Histoplasma capsulatum*, es endémica en muchas regiones de las Américas, Asia y África. Presenta un amplio espectro de manifestaciones clínicas, desde infección asintomática hasta enfermedad diseminada severa. En el presente trabajo, se realizó un estudio retrospectivo para describir las formas clínicas y evaluar el significado de los exámenes diagnósticos de pacientes con histoplasmosis acompañados durante el periodo de julio de 1987 hasta diciembre de 2003 en el Instituto de Pesquisa Clínica Evandro Chagas / FIOCRUZ, RJ, Brasil. Setenta y cuatro pacientes fueron incluidos, cuarenta y nueve por ciento de los cuales (n=36) eran pacientes VIH positivo que presentaron enfermedad diseminada. Los otros 38 casos fueron clasificados de acuerdo a diferentes formas clínicas. *Histoplasma capsulatum* fue aislado en el 69,5% de los especímenes clínicos enviados para cultivo. La inmunodifusión y el inmunoblot fueron positivos en el 72,6% y 100% de los exámenes realizados, respectivamente. Hallazgos histopatológicos sugestivos de *H. capsulatum* fueron observados en el 63,2% de los exámenes realizados. Las pruebas serológicas presentaron menor positividad en los pacientes con sida, cuando se compararon con los pacientes VIH negativo ($\chi^2 = 6,65$; $p < 0,008$). No se observó diferencia estadística entre los pacientes con y sin sida en relación al cultivo y a la histopatología. El papel efectivo de cada examen varía de acuerdo con la forma clínica. Es necesario que los médicos conozcan el valor y las limitaciones de los exámenes disponibles pero, antes que eso, deben pensar en la histoplasmosis e incluirla en el diagnóstico diferencial de sus pacientes.

Palabras clave Histoplasmosis, *Histoplasma capsulatum*, Diagnóstico, Sida

Histoplasmosis, caused by the dimorphic fungus *Histoplasma capsulatum*, is endemic in many regions of the Americas, Asia, and Africa. Its prevalence has been estimated by histoplasmin skin test. The largest concentration of positive skin reactors is found in the central area of the United States [3]. In Brazil, the positive skin reactors prevalence ranges from 2.6 to 93.2% [19,8] depending on the geographic region. In Rio de Janeiro, located in the southern region, there are areas considered to be endemic or even hyper-endemic [25,29].

Histoplasmosis has a wide spectrum of clinical manifestations, ranging from asymptomatic infection to severe disseminated disease, depending on the inoculum size, the immune status of the host and the virulence of the fungal strain. It was first reported in patients with acquired immune deficiency syndrome in 1982 [21] and, since 1987, extrapulmonary histoplasmosis in an individual with a positive serologic test for human immunodeficiency virus is a case definition of AIDS according to the Centers for Disease Control (CDC) [4]. The severe immune defect in AIDS predisposes to extrapulmonary dissemination. CD4⁺ T cells appear to play the most important role in the development of cellular mediated immunity to *H. capsulatum*. Reactivation of quiescent infection occurs during immunosuppression [14,27].

Clinical diagnosis of histoplasmosis is based upon clinical, radiological and epidemiological aspects. Laboratory tests for the diagnosis of histoplasmosis include culture, fungal detection in stained smears or tissue sections, antibody detection and antigen detection. The specific role of each test varies according to the clinical form, since variations in sensitivity have been associated to different clinical presentations [27]. The gold standard method for diagnosis of histoplasmosis is the isolation of *H. capsulatum* in culture with the observation of the suggestive conidial forms (macro and microconidia) as well as its conversion of mold to yeast phase at 37 °C.

H. capsulatum requires up to four weeks to grow *in vitro*. Fungal staining of tissue sections or body fluid smears show the suggestive yeast forms within macrophages and, occasionally, extracellularly. Special fungus stains such as Gomori's methenamine silver (GMS) and periodic acid-Schiff (PAS) are the ones most commonly used in histological study of mycotic diseases [5,12]. GMS is considered the best technique because "it provides better contrast and often stains fungal cells that are refractory to PAS procedures" [5]. To avoid the limitation posed by the fact that special stains do not allow adequate study of the tissue response to fungal invasion, haematoxylin and eosin (H&E) is recommended as the counter-stain for the GMS procedure [5]. However, experience is required to avoid missing small number of organisms in patients with low fungal burden or misidentification of artifacts or other organisms, such as *Leishmania donovani* and *Toxoplasma gondii* in H&E stained slides or small forms of *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Candida glabrata* and *Pneumocystis carinii*, as *H. capsulatum* [12]. Both culture and fungal staining are time-consuming and lacking in sensitivity [2].

Serological testing is central to the diagnosis of some acute, recent, or chronic infectious diseases. Sometimes serological testing must be relied upon the only diagnostic test that is practical, because the suspect etiologic agent is impossible, difficult, or dangerous to grow in cultures in a routine diagnostic laboratory [6]. In histoplasmosis, the detection of patients' antibody responses offers a more rapid alternative to microbiological means of diagnosis, and the detection of host anti-*H. capsulatum* antibodies by immunodiffusion (ID) and complement fixation (CF) tests is often used [2]. Both the yeast and mycelial phases of the fungus produce a number of exoantigens in culture, the most important and characteristic are the H and M antigens. These two antigens are the primary immunoreactive constituents of histoplasmin (HMN), the

standard diagnostic reagent used in ID and CF for many years [23]. ID tests identify H and M precipitins bands. M precipitins can be detected in patients who were exposed to *H. capsulatum* or who had performed histoplasmin skin test. H precipitins are detected during active infection [12]. Fewer than 20% of patients with proven histoplasmosis show H bands [27].

An alternative approach to immunodiagnosis is the detection of anti-*H. capsulatum* antibody in serum by enzyme immunoassays or Western blot assay (WB) [1,11,13,15,16,24,30,31]. Data from previous immunoassay studies, which incorporated deglycosylated antigens for antibody detection, has confirmed that the use of such antigens improves test specificity. It has been demonstrated that WB analysis using NaIO₃-treated H and M antigens is a highly sensitive method for detecting antibodies in serum from persons with early acute pulmonary histoplasmosis [15]. The advantages of the WB test are identification of some cases of early infection, before seroconversion can be detected by CF and ID, a high degree of disease specificity, and applicability to serum specimens with anti-complementary activity. More recently, a highly sensitive and specific ELISA using the same deglycosylated antigens has been reported [10].

During active infection, antigens are released into the tissues and enter body fluids adjacent to the sites of infection, providing a basis for diagnosis by antigen detection [26]. Antigen detection is most useful in patients with disseminated infection and also in the early phase of acute histoplasmosis. The sensitivity of antigen detection is greater in urine than serum [26]. Molecular methods are being evaluated, but have yet to be compared with culture [26]. The polymerase chain reaction assay might be a powerful and rapid diagnostic tool for the diagnosis of histoplasmosis [18].

A retrospective study to describe the clinical forms and assess the clinical significance of the laboratory diagnostic tests of patients with histoplasmosis at Instituto de Pesquisa Clínica Evandro Chagas/FIOCRUZ, RJ, Brazil was carried out.

Materials and methods

Patients. A review of the clinical registration forms from the Instituto de Pesquisa Clínica Evandro Chagas (IPEC) was performed to identify patients with the diagnosis of histoplasmosis during the period of July 1987 to December 2003. The diagnosis was based on one of the following criteria: (1) isolation of *H. capsulatum* from biological material; (2) seropositivity for anti-*H. capsulatum* antibodies (ID or WB tests) associated with clinical and/or radiological findings suggestive of histoplasmosis with or without a positive epidemiological history; or (3) histopathologic findings revealing organisms consistent with *H. capsulatum* associated with clinical and radiological findings suggestive of histoplasmosis with or without a positive epidemiological history.

The cases were clinically classified into nine forms [7,9,12,20]: (1) acute pulmonary – abrupt onset, fever, headache, non-productive cough, aching or constricting substernal discomfort, pleuritic pain; scattered patchy pneumonic infiltrates and hilar adenopathy on chest radiograph; and a positive epidemiological history of recent exposure; (2) chronic pulmonary – malaise, fatigue, low grade fever, mucoid sputum, chest pain, weight loss; reticulonodular and fibrotic lesions associated with cavitation on chest radiograph; and the presence of chronic obstructive pulmonary lung disease (not obligatory); (3) acute disseminated – preceded by the pulmonary acute form in

half of the cases; fever; hepatosplenomegaly; anemia, leukopenia, thrombocytopenia; (4) subacute disseminated – mild fever, weight loss, malaise; hepatosplenomegaly, focal lesions in various organs, most often gastrointestinal tract, adrenal gland, central nervous system, mucous membrane, skin; interstitial pneumonia (1/3 of cases); (5) chronic disseminated – protracted course; intermittent symptoms; low grade fever, fatigue, weight loss; one or more lesions presenting as oropharyngeal, gastrointestinal, adrenal gland and CNS (meningitis) involvement; lungs are seldom involved; (6) disseminated in AIDS patients – fever, cough; anemia, leukopenia, thrombocytopenia; hepatosplenomegaly; pulmonary involvement with diffuse interstitial infiltrate on chest radiograph; latter, multiple organs involvement; (7) granulomatous mediastinitis – large caseous lymph nodes in the mediastinum; (8) cutaneous – localized cutaneous infection with no obvious evidence of disseminated disease. Cutaneous lesions are either, primary from direct inoculation or secondary from hematogenous dissemination; (9) non-defined – clinical and serological suspected cases who could not be classified in none of the preceding forms.

Data were analyzed in SPSS 11.0. Proportions were tested with χ^2 test and differences were considered significant at 0.05 level.

Laboratory methods. Clinical specimens – sputum, bronchoalveolar lavage, bone marrow aspiration, biopsies, cerebrospinal fluid – were submitted for direct examination with KOH 10% and culture on Sabouraud agar and/or staining with H&E and GMS techniques. Blood samples were cultured on brain heart infusion agar (BHI). Cultures were incubated for 4 to 6 weeks at room temperature to assure isolation of fungi. The observation of white to brownish filamentous colonies on the slants, and microscopic examination of slide cultures revealing septate hyaline hyphae, with globose macroconidia, and microconidia presuntively identify the isolates as *H. capsulatum*. Conversion to oval yeast cells with single budding confirmed the identity of the strain as *H. capsulatum*. Fungal stained slides exhibiting intracellular ovoid yeasts, measuring 3 to 5 μ m in diameter with narrow-based budding were considered compatible with *H. capsulatum*.

Standard diagnostic reagent used in ID was histoplasmin, whose primary constituents are the H and M antigens, exoantigens produced in culture by *H. capsulatum*. Seropositivity was determined by the presence of precipitins against M and/or H antigens. Western blot test was performed with NaIO₃-treated H and M antigens.

Results

Seventy-four patients were included. Age ranged from 15 to 74 years, with a median of 36 years. Male sex (82.4%) and white race (63.5%) predominated.

Clinical forms. Forty-nine percent of the cases (n=36) occurred in HIV positive patients who presented with disseminated disease. In almost one third of these patients (n=11) histoplasmosis was considered an AIDS defining infection. The remaining 38 cases were classified in the following clinical forms: acute pulmonary: 10; chronic pulmonary: 9; acute disseminated: 1; subacute disseminated: 4; chronic disseminated: 5; mediastinitis: 5; cutaneous: 2; non-defined: 2. In HIV negative patients, pulmonary forms, acute (n=10) and chronic (n=9), represented half of the cases (Table 1).

Laboratory tests. Table 1 correlates culture, serology and histopathology results to the clinical forms. *H. capsulatum* was isolated from 69.5% of the clinical

specimens sent to culture. ID and WB were positive in 72.6 % and 100% of the performed tests, respectively. Histopathologic findings suggestive of *H. capsulatum* were found in 63.2% of the performed exams. Serology had a lower proportion of positivity amongst AIDS patients, when compared with histoplasmosis HIV negative patients ($X^2 = 6.65$; $p < 0.008$). Statistical differences between AIDS and non-AIDS patients were not observed with culture and histopathology.

The agreement and disagreement between the results obtained from culture and from immunodiffusion tests (ID) on different clinical forms are shown on Table 2. Results agreed in 52.2% of the cases in non-AIDS patients. In the remaining, only ID was positive in 39.1% and only culture in 8.7%. In AIDS patients culture and ID were both positive in 38.2% of the cases, only the ID was positive in 23.5% of the patients and only the culture in 35.3%.

The same analysis was performed between results from culture and histopathology. Both tests were performed in eight non-HIV infected patients: in five (62.5%) both culture and histopathology were positive. In two patients only histopathology showed yeast-like forms suggestive of *H. capsulatum*, and in one of them both tests were negative. Among the 23 AIDS patients in whom both tests were performed, in 13 (56.5%) both were positive; in three (13%) both were negative; in four (17.5%) just the

culture was positive and in three (13%) only the histopathology was suggestive of *H. capsulatum*.

Observations on clinical forms. All cases of acute pulmonary form had a compatible epidemiological history and clinical findings suggestive of histoplasmosis. All but one of them had positive ID titers. The one with negative ID had a positive WB.

One patient with sub-acute disseminated form was a 20-year old non-smoking young man. He had a one-year history of low fever, cough and scarce pulmonary infiltrates on chest x-ray. He had two episodes of hemoptysis. Several acid-fast stains and culture of sputum were repeatedly negative and tuberculin skin test was negative. Histoplasmosis was diagnosed based on clinical findings that also included weight loss and splenomegaly, and an ID seropositivity of 1:32, with H and M precipitins bands. ID for *Paracoccidioides brasiliensis* and *Aspergillus* sp were negative. *H. capsulatum* was not isolated from sputum nor observed on direct microscopy of stained clinical specimens. Itraconazole was started and symptoms rapidly subsided. Seven months later he presented daily low-grade fever and cavitation in the apical segment of the upper right lobe on chest x-ray. A diagnosis of tuberculosis was made based on acid-fast bacilli on smear of sputum and culture positive for *Mycobacterium tuberculosis*. Itraconazole was stopped and he received rifampin, isoniazid and

Table 1. Positive results of the diagnostic tests in the different clinical forms of histoplasmosis.

Clinical form (n)	Culture		ID		Immunoblot		Histopathology	
	+/n	(%)	+/n	(%)	+/n	(%)	+/n	(%)
Non-AIDS								
Acute pulmonary (10)	0/2	(0)	9/10	(90%)	1/1*	(100%)		
Chronic pulmonary (9)	7/9	(77.8%)	9/9	(100%)			2/2	(100%)
Acute disseminated (1)	1/1	(100%)	1/1	(100%)	1/1	(100%)		
Subacute disseminated (4)	1/4	(25%)	4/4	(100%)			0/1	(0)
Chronic disseminated (5)	3/5	(60%)	5/5	(100%)			4/4	(100%)
Mediastinitis (5)			3/5	(60%)	2/2*	(100%)	0/5	(0)
Cutaneous (2)	2/2	(100%)	0/2	(0)			1/1	(100%)
Non defined (2)	0/1	(0)	2/2	(100%)			0/1	(0)
Total non-AIDS (38)	14/24	(58.3%)	33/38	(86.8%)	4/4	(100%)	7/14	(50%)
AIDS (36)								
Disseminated (36)	27/35	(77%)	20/35	(57%)			17/24	(71%)
Total (74)	41/59	(69.5%)	53/73	(72.6%)	4/4	(100%)	24/38	(63.2%)

ID = immunodiffusion; n = sample number tested; + = positive; * = negative ID

Table 2. Agreement among the "gold standard" culture methodology and immunodiffusion test.

Clinical form (n)	ID + / Culture +	ID + / Culture -	ID - / Culture +	ID - / Culture -
Non-AIDS				
Acute pulmonary (2)		2		
Chronic pulmonary (9)	7	2		
Acute disseminated (1)	1			
Subacute disseminated (3)	1	2		
Chronic disseminated (5)	3	2		
Mediastinitis (0)				
Cutaneous (2)			2	
Non defined (1)		1		
Total non-AIDS (23)	12 (52.2%)	9 (39.1%)	2 (8.7%)	
AIDS				
Disseminated (34)	13 (38.2%)	8 (23.5%)	12 (35.3%)	1 (3%)

n = number of cases submitted to both exams; + positive result; - negative result

pyrazinamide for six months. Two months afterwards, fever returned accompanied by hemoptysis, ID test for *Aspergillus fumigatus* became positive and a pulmonary fungus ball (aspergilloma) was diagnosed on chest x-ray. Itraconazole was re-started. A surgical resection was performed and *Aspergillus fumigatus* was isolated from the resected fungus ball. Six months after the surgery ID for *Aspergillus* became negative. ID for *H. capsulatum* remained positive with low titers.

One case of chronic pulmonary histoplasmosis was a 20-year old non-smoking young woman. She had a history of chest pain and a chest radiograph revealing a cavitation at the superior segment of the right lower lobe. Acid-fast stains of sputum were negative and tuberculin skin test was negative. She was started on empirical anti-tuberculous therapy. Six months later, pulmonary image worsened. At that time, the diagnosis of histoplasmosis was made by the isolation of *H. capsulatum* from bronchoalveolar lavage (BAL). ID titer was 1:32, with M precipitin band.

Two patients with chronic pulmonary histoplasmosis had *Aspergillus* sp observed on wet smear and isolated from BAL culture besides *H. capsulatum*. One was *A. flavus* and the other one *A. fumigatus*. They both had positive ID for *H. capsulatum* and *Aspergillus* sp.

The two patients with the cutaneous form were nonimmunocompromised adult men without any other manifestation of active disseminated disease. One presented a verrucous lesion at the right elbow without a history of a local injury. The other one, an army officer, presented with a papulonecrotic lesion on the back of his right hand with a history of crawling through a small bat inhabited tunnel three months earlier.

Except for the AIDS patients, in whom it was difficult to analyze a negative serologic result, in all the other clinical forms antibody levels remained low or fluctuate between negative and low levels, after therapy. Two patients who have had the pulmonary acute form manifested ID titers of 1:1 six years after the acute episode; a third one had titer of 1:2 two years after the acute episode. One patient with the subacute disseminated form, a 30-year old non-smoking man without any epidemiological history, still had a titer of 1:32 one and a half year after the diagnosis, while receiving itraconazole and free of any symptom. Six months later, that is two years after diagnosis, the titer was 1:4. In patients with the chronic pulmonary form, ID titers fluctuate between negative and low levels. In the three cases of mediastinitis with seropositivity by ID, antibody levels were low at diagnosis (1:1; 1:2) and remained low at two, three and five years after diagnosis, in each one of the cases, respectively.

Discussion

Each one of the approaches to the diagnosis of histoplasmosis such as culture, histopathology, measurement of antibody titers or detection of antigens, has limitations. So the best approach to the diagnosis is an appropriate combination of these methodologies. Culture and histopathology have an important role in disseminated histoplasmosis in immunosuppressed patients and in the cutaneous form. Serologic tests are useful for pulmonary forms (acute and chronic), mediastinitis and disseminated forms in immunocompetent patients but have limitations in immunosuppressed patients with disseminated infection. Those observations are in accordance with the pathogenesis of *H. capsulatum* and the immunology of host defense against the fungus [14,27,28].

According to the literature [17,26,27], in acute pulmonary histoplasmosis, culture and histopathology have a low positivity; serology has a positivity of 80-95% and antigen detection is positive in 75-80% of the patients. The sensitivity of antigen detection is lower in serum than in urine; also, levels of antigen in the bronchoalveolar lavage fluid can be much higher than in serum and urine. In chronic pulmonary histoplasmosis, culture is positive in 50-85% of the cases, histopathology has a positivity of 40%, serology has a high positivity of 85-100% and tests for antigen are negative. In mediastinitis, serologic tests for antibodies are positive (~70%) and antigen tests are negative; the histopathology of biopsies performed to exclude malignancy is positive in less than 25% of the cases. In disseminated histoplasmosis in non-immunosuppressed patients, culture is positive in 80-90% of the cases, histopathology in 40-60%, serology in 80-100% and antigen detection in 80%. In disseminated histoplasmosis in AIDS patients, culture has a positivity of 90%, histopathology of 40-90%, serology of 50-70% and antigen detection of 85-95%.

Severo et al. [22], from the state of Rio Grande do Sul, Brazil, reported a 21-year period casuistic of 137 cases. The diagnosis was mostly based on culture and histopathology. Serology wasn't regularly performed. Acute and chronic pulmonary forms were mainly diagnosed by histologic examination of biopsied lung tissue. In the disseminated forms (one acute; 91 chronic: 65 HIV positive, nine immunosuppressed, 17 with no associated condition) the diagnosis was based on histopathology (75 of 81 patients) or culture (42 of 56 patients). The ID performed in 53 patients was positive in 62% of them.

We have classified and grouped the patients according to the observed clinical manifestations. Although our casuistic was small in the different clinical forms, the results of the laboratory diagnostic tests observed in our study are in accordance with the literature. Exception was observed for histopathologic exams in which our results presented a higher percentage of positivism, approaching that observed by Severo et al. [22]. This fact could be because the exams were carried out on national reference centers for systemic mycoses and histopathology of infectious diseases, where fungal stains are always employed and the pathologists are prepared to recognize fungal infections.

We have decided to describe a few clinical cases to illustrate clinical and laboratory aspects in the course of histoplasmosis and to point out that many times histoplasmosis is not considered at the initial differential diagnoses and months elapse until the correct diagnosis is made.

All in all, the role of each methodology used in the diagnosis of histoplasmosis varies according to the clinical form and physicians need to know the value and limitations of the available diagnostic tests. But, before that, clinicians have to think about histoplasmosis and consider this clinical entity in their differential diagnosis; many times the diagnosis is missed or delayed simply because histoplasmosis was not considered. This is particularly true in developing countries, like Brazil, where tuberculosis, a highly prevalent infection, is always the first, the second and even the third thought.

References

- Brock EG, Reiss E, Pine L, Kaufman L. Effect of periodate oxidation on the detection of antibodies against M antigen of histoplasmin by enzyme immunoassay (EIA) inhibition. *Curr Microbiol* 1983; 10: 177-180.
- Bullock WE. *Histoplasma capsulatum*. In: Mandell GL, Bennett J, Dolin R (Eds.). *Principles and Practice of Infectious Diseases*. New York, Churchill Livingstone, 1995: 2340.
- Cano MV, Hajjeh RA. The epidemiology of histoplasmosis: a review. *Semin Respir Infect* 2001; 16: 109-118.
- Centers DC Revision of the CDC surveillance case definition of acquired immunodeficiency syndrome. *JAMA* 1987; 258: 1143-1154.
- Chandler FW, Kaplan W, Ajello L. A colour atlas of the histopathology of mycotic diseases. London, Wolf Medical Publications, 1980.
- Crump JA, Corder JR, Menshaw NG, Barth Reller L. Development, implementation, and impact of acceptability criteria for serologic tests for infectious diseases. *J Clin Microbiol* 2004; 42: 881-883.
- Faust H, Treadwell P. Cutaneous *Histoplasma capsulatum* in a nonimmunocompromised patient with previously treated cutaneous *Mycobacterium kansasii*. *J Am Acad Dermatol* 1991; 25: 418-422.
- Fava SdC, Netto CF. Epidemiologic surveys of histoplasmin and paracoccidioidin sensitivity in Brazil. *Rev Inst Med Trop Sao Paulo* 1998; 40: 155-164.
- Goodwin RA Jr, Des Prez RM. State of the art: histoplasmosis. *Am Rev Respir Dis* 1978; 117: 929-956.
- Guimaraes AJ, Pizzini CV, De Matos Guedes HL, Albuquerque PC, Peralta JM, Hamilton AJ, Zancopé-Oliveira RM. ELISA for early diagnosis of histoplasmosis. *J Med Microbiol* 2004; 253: 509-514.
- Kumar BV, Medoff G, Kobayashi GS, Sieling WL. Cross-reacting human and rabbit antibodies to antigens of *Histoplasma capsulatum*, *Candida albicans*, and *Saccharomyces cerevisiae*. *Infect Immun* 1985; 48: 806-812.
- Lacaz CS, Porto E, Martins JEC, Heins-Vaccari EM, Melo NT. *Tratado de Micologia Médica* Lacaz. São Paulo, Sarvier, 2002.
- Maiga YI, Marjolet M. Value and limits of serological methods: electrosyneresis and E.L.I.S.A. for the study of the prevalence of histoplasmosis in Mali. *Bull Soc Pathol Exot Filiales* 1985; 78: 585-593.
- Newman SL. Cell-mediated immunity to *Histoplasma capsulatum*. *Semin Respir Infect* 2001; 16: 102-108.
- Pizzini CV, Zancopé-Oliveira RM, Reiss E, Hajjeh R, Kaufman L, Peralta JM. Evaluation of a western blot test in an outbreak of acute pulmonary histoplasmosis. *Clin Diagn Lab Immunol* 1999; 6: 20-23.
- Raman C, Khardori N, Von Behren LA, Wheat LJ, Tewari RP. Evaluation of an ELISA for the detection of anti-Histoplasma ribosomal and antihistoplasmin antibodies in histoplasmosis. *J Clin Lab Anal* 1990; 4: 199-207.
- Restrepo A. Histoplasmosis: pasos para su diagnóstico. *Med & Lab* 1998; 7: 665-667.
- Rickerts V, Bialek R, Tintelnot K, Jacobi V, Just-Nubling G. Rapid PCR-based diagnosis of disseminated histoplasmosis in an AIDS patient. *Eur J Clin Microbiol Infect Dis* 2002; 21: 821-823.
- Rodrigues CC. Avaliação da infecção por *Histoplasma capsulatum* por meio de reações intradérmicas em moradores da zona urbana e rural do Município de Pratânia (SP) [PhD Thesis]. Botucatu; Faculdade de Medicina de Botucatu/UNESP, 2004.
- Romano C, Castelli A, Laurini L, Massai L. Case report. Primary cutaneous histoplasmosis in an immunosuppressed patient. *Mycoses* 2000; 43: 151-154.
- Sathapatayavongs B, Batteiger BE, Wheat J, Slama TG, Wass JL. Clinical and laboratory features of disseminated histoplasmosis during two large urban outbreaks. *Medicine (Baltimore)* 1983; 62: 263-270.
- Severo LC, Oliveira FM, Irion K, Porto NS, Londero AT. Histoplasmosis in Rio Grande do Sul, Brazil: a 21-year experience. *Rev Inst Med trop S Paulo* 2001; 43: 183-187.
- Standard PG, Kaufman L. Specific immunological test for the rapid identification of members of the genus *Histoplasma*. *J Clin Microbiol* 1976; 3: 191-199.
- Torres M, Diaz H, Herrera T, Sada E. Evaluation of enzyme linked immunosorbent-assay and western blot for diagnosis of histoplasmosis. *Rev Invest Clin* 1993; 45: 155-160.
- Wanke B. Histoplasmosis: Estudo epidemiológico, clínico e experimental [PhD Thesis]. Rio de Janeiro, Universidade Federal do Rio de Janeiro, 1985.
- Wheat LJ. Current diagnosis of histoplasmosis. *Trends Microbiol* 2003; 11: 488-494.
- Wheat LJ, Kauffman CA. Histoplasmosis. *Infect Dis Clin North Am* 2003; 17: 1-19, vii.
- Woods JP, Heinecke EL, Luecke JW, Maldonado E, Ng JZ, Retallack DM, Timmerman MM. Pathogenesis of *Histoplasma capsulatum*. *Semin Respir Infect* 2001; 16: 91-101.
- Zancopé-Oliveira RM. Histoplasmosis: Estudo epidemiológico em área periurbana do município do Rio de Janeiro (RJ) [MSc Thesis]. Rio de Janeiro, Instituto Oswaldo Cruz-FIOCRUZ, 1985.
- Zancopé-Oliveira RM, Bragg SL, Reiss E, Wanke B, Peralta JM. Effects of histoplasmin M antigen chemical and enzymatic deglycosylation on cross-reactivity in the enzyme-linked immunoelectrotransfer blot method. *Clin Diagn Lab Immunol* 1994; 1: 390-393.
- Zimmerman SE, French ML, Kleiman MB, Wheat LJ. Evaluation of an enzyme-linked immunosorbent assay that uses ferrous metal beads for determination of antihistoplasmin immunoglobulins G and M. *J Clin Microbiol* 1990; 28: 59-64.