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Original article

EVALUATION OF AN ENZYME IMMUNOASSAY TECHNIQUE ON DETECTING URINARY *HISTOPLASMA CAPSULATUM* ANTIGEN IN THE DIAGNOSIS OF DISSEMINATED HISTOPLASMOSIS IN ARGENTINA

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Abstract

Introduction: Histoplasmosis is a systemic mycosis of universal distribution, highly endemic in the Americas. It is caused by a dimorphic fungus *Histoplasma capsulatum* var. *capsulatum*. It affects both immunocompetent and immunocompromised individuals where progressive and disseminated forms are observed. A very important risk factor is HIV infection/AIDS, with a mortality rate of 20-40% in Latin America. The diagnosis of this mycosis is made by conventional and molecular methods or by antigen and antibody detection.

Methods: In this retrospective, longitudinal and analytical study, carried out over a period of 2 years, the sensitivity (S) and specificity (E) of a commercial kit for the detection of *Histoplasma* antigen by EIA technique (HC-Ag) was evaluated in 50 patients with AIDS-associated histoplasmosis. In addition, its performance was compared with that of other diagnostic techniques routinely used in our laboratory.

Results: HC-Ag had a S of 94%, E 96%, positive likelihood coefficient (CVP): 20.68 and negative likelihood coefficient (CVN): 0.06. The delay time of the results was 4 days, similar to that of antibody detection and n-PCR and much less than that of blood cultures. The combination of methods improved S to 100%; with similar values in E.

Conclusion: The HC-Ag method demonstrated its usefulness in the diagnosis of progressive disseminated histoplasmosis and the combination of methods is a good option to increase sensitivity and decrease the time to reach the diagnosis of certainty. This allows improving the strategy in the management of the disease and decreasing its case-fatality rate.

Key words: *Histoplasma*, histoplasmosis, blood culture, HIV infections, Latin America, risk factors

Resumen

Evaluación de una técnica de inmunoensayo enzimático para la detección del antígeno urinario de Histoplasma capsulatum ***en el diagnóstico de histoplasmosis diseminada en Argentina***

Introducción: La histoplasmosis es una micosis sistémica de distribución universal, altamente endémica en las Américas. Es causada por un hongo dimórfico: *Histoplasma capsulatum* var. *capsulatum*. Afecta tanto a inmunocompetentes como a inmunocomprometidos, se observan formas progresivas y diseminadas. Un factor de riesgo muy importante es la infección por HIV/sida, con una tasa de mortalidad del 20-40% en América Latina. El diagnóstico de esta micosis se realiza por métodos convencionales y moleculares o por detección de antígenos y anticuerpos.

Métodos: En este estudio retrospectivo, longitudinal y analítico, realizado en un periodo de 2 años, se evaluó la sensibilidad (S) y especificidad (E) de un kit comercial

para la detección de antígeno de *Histoplasma* por técnica de EIA (HC-Ag) en 50 pacientes con histoplasmosis asociada a sida. Además, se comparó su rendimiento con el de otras técnicas diagnósticas utilizadas habitualmente en nuestro laboratorio.

Resultados: HC-Ag tuvo una S del 94%, E del 96%, coeficiente de verosimilitud positiva (CVP) de 20.68 y coeficiente de verosimilitud negativa (CVN) de 0.06. El tiempo de demora de los resultados fue de 4 días, similar al de la detección de anticuerpos y n-PCR y mucho menor que el de los hemocultivos. La combinación de métodos mejoró la S a 100%; con valores similares en E.

Conclusión: El método HC-Ag demostró su utilidad en el diagnóstico de histoplasmosis diseminada progresiva y la combinación de métodos es una buena opción para aumentar la sensibilidad y disminuir el tiempo para llegar al diagnóstico de certeza. Esto permite mejorar la estrategia en el manejo de la enfermedad y reducir su tasa de letalidad.

Palabras clave: *Histoplasma*, hemocultivo, infecciones por HIV, América Latina, factores de riesgo, reacción en cadena de la polimerasa

KEY POINTS

- The HC-Ag method proved its usefulness in the diagnosis of progressive and disseminated HP, and it can be stated that its use and its complementation with conventional and molecular biology methods in mycology laboratories is essential to know the real incidence and prevalence of this pathology, reduce diagnosis times, improve targeted treatment and propose follow-up, prophylaxis and prevention strategies, in order to reduce the lethality rate in HIV+ patients or those with other immunosuppressive pathologies.
- This is the first study published in Argentina where a commercial EIA HGM 201 with monoclonal antibodies is used and compared with other methods, and we believe that allows us to know the usefulness of this technique so that its implementation can be considered in other institutions in Argentina and Latin America as proposed in the goals set in Manaus for 2025[27].

Histoplasmosis (HP) is a cosmopolitan mycosis, endemic in the American continent, but with cases in the five continents. It is caused by the dimorphic fungus *Histoplasma capsulatum* var. *capsulatum*[1]. The areas of highest incidence lie along the valleys of the Ohio and Missouri-Mississippi rivers in North America, in several countries of Central America as Guatemala, El Salvador and Panama[1,2] and in the basins of the Orinoco, Magdalena, Amazonas, San Francisco, Paraná and La Plata rivers in South America[3,4]. In Argentina, the main endemic area is the geographic region known as the Pampas and it is estimated that there are approximately seven million individuals infected with *H. capsulatum*[5]. HIV/AIDS associated histoplasmosis presents a prevalence of 2.5-5%[6-8] and it is the second potentially fatal systemic fungal infection in frequency[8].

Histoplasmosis can affect both immunocompetent and immunocompromised individuals. Clinical manifestations depend on the infecting strain[9, 10], the fungal load and the immune status of the patient[6, 7]. A high risk of developing disseminated and progressive histoplasmosis exists in HIV/AIDS patients[11]. In Latin America the fatality rate ranges from 20 to 40%[2,12,13], higher than that of the United States, which ranges from 4 to 8%[14, 15] and it is an AIDS marker disease in 30 to 75% of cases[7, 8, 16-18].

Diverse studies have estimated that the incidence of histoplasmosis in Latin America could be 1.5 cases per 100 HIV+ per year; which would add about 22 000 cases and produce about 9000 deaths annually, this would be higher than annual deaths from tuberculosis[19, 20]. Anyway, the real prevalence in our region remains unknown because it is not a communicable disease, and on the other hand in many countries diagnosis is deficient due to the lack of diagnostic tools[3, 12, 19].

Laboratory diagnosis of histoplasmosis is made through different techniques that can be classified into: (a) Conventional, (b) Molecular and (c) Immunological methods.

Conventional methods include microscopic observation and culture of various clinical samples. When patients present mucocutaneous manifestations, the diagnosis can be quickly performed by microscopic observation of *H. cap-*

sulatum yeasts in Giemsa stained smears from scarifications or biopsies of such lesions; this technique has a variable sensitivity of 9-43% depending on the kind of lesion, the time of evolution and the operator expertise[21, 22]. If the patients have no skin or mucocutaneous lesions culturing different clinical samples including lysis-centrifugation blood culture can be made. This technique can take up to 21 days for fungal growth and has about 70% sensitivity in HIV positive patients[21, 23].

Molecular methods are based on different PCR techniques and have high sensitivity and specificity[24-26]. However, its main disadvantage is that there are still no validated and easily accessible commercial kits and reproducibility of home techniques is highly variable among laboratories and there is a lack of consensus for their application[27].

Antibody detection is an accessible immunological method but depends on the clinical form and the immunological status of the host. In AIDS associated histoplasmosis less than 30% of patients present detectable specific antibodies[23]. In contrast, detection of *H. capsulatum* galactomannan antigens in these cases is much more efficient and also allows monitoring the effectiveness of treatment[28,29]. The first methods for detecting *Histoplasma* antigens by enzyme immunoassays (EIA) were described in 1986[30]. This technique was later adapted to using in urine and serum samples in 1994[31], and currently there are commercial ELISA kits that use monoclonal antibodies such as HGM 101 (IMMY®, Norman, OK, USA)[32-34]. The latter gave very good results in a recent study carried out in Latin America, with very high sensitivity and specificity [35]. However, in our country it is still not used as a routine test due to its high cost. For this reason, its performance as a diagnostic method is unknown yet.

The objective of this study was to evaluate the sensitivity (S) and specificity (E) of the HGM201 kit for detecting *H. capsulatum* urinary antigen (HC-Ag) by EIA, to establish its usefulness in the early diagnosis of AIDS associated HP, and to compare its results with those of blood culture (BC), antibody detection (Ac -ID) and a PCR in blood (Hcp100 nPCR). Clinical and demographic characteristics of the studied patients were also analyzed.

Materials and methods

Type of study

A retrospective longitudinal and analytical study was conducted between January 2019 and December 2020.

Demographic and clinical data as well as the results of diagnosis of proven disseminated and progressive histoplasmosis (PPDH) in HIV/AIDS patients were compared with those of HIV+ patients with other pathologies and a group of healthy volunteers, at the Mycology Unit of Infectious Diseases, F.J. Muñiz Hospital, Buenos Aires.

Inclusion criteria

- HIV + patients over 18 years old with PPDH
- HIV + patients over 18 with other infectious pathologies contemplated in the differential diagnosis, which were treated at the Mycology Unit of F.J. Muñiz Hospital in the mentioned period.
- Healthy volunteers (no apparent disease).

All patients were required to reside in the Rio de la Plata endemic area of Argentina.

Exclusion criteria

- Patients under 18 years' old
- Patients who were not residents of the endemic area
- Patients without characteristic symptoms or clear clinical follow-up
- Patients that had proven coinfections with other etiological agents considered in this study, or treatment confirmed with amphotericin B, itraconazole or fluconazole pre-clinical sampling.

Patients and healthy volunteers

- *Patients with PPDH*. Fifty HIV+ patients were included in this group. A positive case of PPDH was defined as an individual with positive culture and/or histopathological findings of intracellular yeasts and granuloma consistent with this fungal infection and by Gomori-Grocott stains [parameter according to recommendations of the EORCT/MSG Consensus Group[36].
- *HIV + patients with other infectious pathologies*. Fifty-one patients were included: 3 pneumocystosis, 12 cryptococcosis, 1 leishmaniosis, 2 candidemias, 6 tuberculosis, 3 paracoccidioi-

domycosis, 24 patients with T CD4+ lymphocyte counts <200 cells/µl and different pathologies; with no characteristics signs or clinical symptoms, nor epidemiological history or other diagnostic elements of mycosis or tuberculosis (18 with impregnation syndrome and fever, 2 pemphigus vulgaris and 4 febrile neutropenia).

• *Healthy volunteers*. 15 individuals older than 18 years old without apparent diseases.

All demographic and clinical-epidemiological data of the patients were recorded and stored in a database for further analysis.

The diagnosis of HIV infection was determined by EIA serological test and confirmation by the real-time PCR technique to determine the viral load.

The Research Ethics Committee, Muñiz hospital, reviewed and approved the conduct of this study

Clinical samples

The following samples from all individuals meeting the inclusion criteria were analyzed:

urine, EDTA anticoagulated peripheral blood, serum and pre-treated blood samples for lysis-centrifugation blood culture.

Tzanck cytodiagnosis was performed in scrapings in all patients who had skin or mucosal lesions.

Samples were taken on the first day and ten days after medical consultation taking into account that no patient had been previously treated with amphotericin B or itraconazole.

Urine samples were stored at -15 °C, serum and whole blood treated with EDTA at 4 °C until the time of their processing. Blood cultures and smears for cytodiagnosis were processed immediately after being collected.

Conventional diagnostic methods

a. Blood culture by lysis-centrifugation (BC). Peripheral blood samples pretreated with 5% saponin and sodium polyanethol sulfonate were cultured on Sabouraud agar and Brain-Heart Infusion agar at 28 °C and 37 °C, according to the technique described by Bianchi et al.[37].

b. Tzanck cytodiagnosis. Scarification with a sterile scalpel was performed in 50 patients who presented skin and mucosal lesions. It was possible to detect the presence of typical elements of the yeast phase of *H. capsulatum* and other etiological agents after Giemsa stain[38, 39].

Immunological methods

a. *Serological tests*. Detection of circulating anti *H. capsulatum* antibodies was performed by agar gel immunodiffusion techniques and counter-immunoelectrophoresis with secondary immunodiffusion in agarose gel (Ac-ID)[40], in serum samples from the 3 groups of patients. The antigen used was an aqueous extract of the yeast phase of *H. capsulatum*[41].

b. *Antigen detection* (HC-Ag). The presence of *H. capsulatum* galactomannan antigen was determined in urine samples of all patients and healthy volunteers with commercial EIA using monoclonal antibodies HGM 201 (IMMY®, Norman, OK, USA). The used cutoff value was 0.2 ng/ml.

Molecular methods

A nested PCR designed by Bialek et al. and modified by Toranzo et al. was performed in whole blood samples anticoagulated with EDTA, from patients and healthy volunteers.

The primers used make it possible to amplify a DNA sequence that codes for the 100 kDa protein involved in the process of infection and survival of the fungus within the host cell[21,25].

The external primers Hc I (5'-GCG TTC CGA GCC TTC CAC CTC ACC-3 ') and Hc II (5'-ATG TCC CAT CGG GCG CCG TGT AGT-3') delimit a sequence of 391 bp. The internal primers Hc III (5'-GAC ATC TAG TCG CGG CCA GGT TCA-3 ') and Hc IV (5'-AGGAGA GAA CTG TAT CGG TGG CTT G-3') delimit a 210 bp sequence.

Statistical analysis

Sensitivity (S), specificity (E), positive likelihood coefficients (CVP), negative likelihood coefficients (CVN) and accuracy with their respective 95% confidence intervals (CI) were evaluated for each of the diagnostic methods used. The latter were: blood culture, antibody detection, PCR in blood and determination of urinary antigen. Statistical parameters were evaluated individually and by combining them using contingency tables. The cases of PPDH were used as reference.

A ROC curve was built to identify one cutoff value according to the urinary antigen study results.

The variables normality was determined with the modified Shapiro-Wilk test, and the homogeneity of variances with the F and the Levene tests. We also analyzed the test of Kruskal-Wallis and Wilcoxon signed-rank test to see if there were any significant differences in the response time (RT), which was defined as the average time between a clinical sample was taken and the result report was emitted by the lab. The programs InfoStat version 2018[42], EPIDAT 4.2 (Anón sf) and SPSS Statistics 20 were used. It was considered a p value <0.05 as statistically significant.

Results

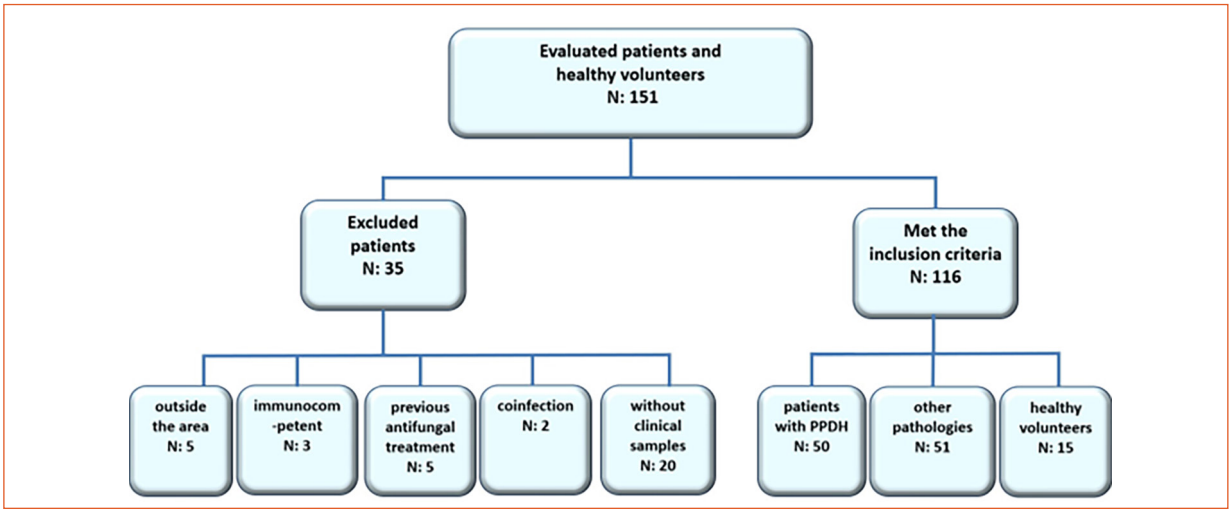
During the study period (24 months) 136 potentially eligible patients diagnosed with PPDH or other pathologies were evaluated. On the other hand, 35 cases were excluded from the analysis: 5 because of coming from outside the area of the Rio de la Plata, 3 immunocompetent, 5 with previous antifungal treatment, 2 had confirmed coinfection with *Cryptococcus neoformans*, and 20 patients in which clinical samples required for this study could not been analyzed. In the end, 116 cases were included. One hundred and one patients met the inclusion criteria: 50 cases were PPDH, and 51 cases suffered from other pathologies. Then, 15 apparently healthy volunteers were added (Fig. 1). Out of the 116 cases, 83 (72%) were men and 33 (28%) women, median age was 42 years (interquartile range- IQR: 33-48 years old). From these apparently healthy volunteers and patients, a total of 514 clinical samples were processed: 116 urine, 116 peripheral blood samples, 116 sera, 116 blood cultures and 50 mucosal or skin lesion scrapings analyzed by Tzanck cytodiagnosis.

Demographic and clinical features of HIV+ patients with PPDH

Within the 50 patients with PPDH, 37 were men (74%) and 13 women (26%) with a median age of 40 years (IQR: 34-48 years old). The 100% had CD4+ lymphocyte count <200 cells/µl, (median 25 cells/µl; ICR: 12-49 cells/µl). In 34% (17/50) of the cases, disseminated histoplasmosis was the AIDS marker disease.

Definitive diagnosis was made by the growth of the fungus in blood cultures in 20 cases (40%), by the visualization of *Histoplasma capsulatum* yeasts in the Giemsa stain of mucocutaneous scarification sample in 15 cases (30%) and in other 15 cases (30%) both techniques were positive simultaneously. Regarding tegumentary manifestations, 33 patients with PPDH presented injuries that were mainly ulcerated skin papules or molluscum-like lesions. Of these, 67% (22/33) were cutaneous and 33% (11/33) mucosal. Most of the skin lesions were found on the thorax and face (90%) and predominated over injuries in the extremities 10% ($\chi^2 = 14.73$; p = 0.0001), whereas the 100% of the mucosal lesions were oral. No yeasts were observed in scarification samples of 3 (9.1%) patients.

Figure 1 | Inclusion criteria flow chart



At the time of consultation, 74% (37/50) of the patients had a pathological chest X-ray. Abnormalities were miliary 70% (n = 26), interstitial infiltrate 16% (n = 6), bilateral nodules 11% (n = 4) and peripheral pulmonary nodules 3% (n = 1). The miliary pattern appeared in a significantly greater proportion than the other lesions (χ^2 = 5.44; p = 0 .02).

The established treatment was amphotericin B deoxycholate and itraconazole, depending on the magnitude of the symptoms considered by the treating physician and the general condition of the patients. In some cases, they received both drugs. Patients who were hospitalized at the time of histoplasmosis diagnosis were initially treated with amphotericin B deoxycholate at 0.7 mg/kg/day, amphotericin B lipid complex 5 mg/kg /day, or liposomal amphotericin B 3 mg/kg/day for 7, or 14 days depending on their clinical status. Only in cases of bone marrow involvement, intestinal obstruction, or meningitis, treatment with amphotericin B was prolonged. In all cases, itraconazole 400 mg/day was indicated after amphotericin B. Treatment with itraconazole at the mentioned dose was maintained for 3 or 6 months depending on the evolution. After this period, they continued with the same medication but with 200 mg/day (secondary prophylaxis) until obtaining two LTCD4+ counts > 150 cells/µl with an undetectable HIV viral load.

The fatality rate in this group of patients was 14% (7/50).

Demographic and clinical characteristics of HIV+ patients with other pathologies

HIV+ patients with other diseases were 38 men (75%) and 13 women (25%), with a median age of 42 years (IQR 32-48 years old). The 97% had CD4+ counts < 200 cells/µl (median 68 cells/µl; IQR: 36-86 cells/µl). Eleven patients had skin lesions and 6 mucosal ones. The former were located on the trunk and face (82%), significantly predominating over the limb lesions which were only 18% (χ^2 = 4.45; p = 0. 035), regarding the mucosal lesions the 100% of them were of nasopharyngeal location. Only in 10 lesions nonspecific inflammation was observed, in two *Paracoccidiodes* sp. yeasts, two viral syncitia, 1 *Cryptococcus* sp. yeasts, 1 *Malassezia* sp. and 1 *Molluscum contagiosum*.

The fatality rate in this group of patients was 26% (13/51), the percentage was higher than in the group of patients with PPDH, although without significant differences (χ^2 = 2.101; p = 0.147)

Sensitivity (S), specificity (E), positive likelihood coefficients (CVP), negative likelihood coefficients (CVN), accuracy and response time of the different methods employed for the diagnosis of PPDH.

The results of S, E, CVP, CVN and accuracy of each of the methods can be observed and compared in Table 1.

The RT of the various methods was 19 days for the BC, 4 days HC-Ag, 5 days for Hcp100 nPCR and 5 days for Ac-ID. Significant differences were observed in RT as the statistical H calculated by the Kruskal-Wallis test

Tabla 1 | Evaluation of the analytical parameters of each of the methods used in this study. E, S and accuracy values are percentages

	HC-Ag	Hcp100 nPCR	BC	Ac-ID	HC-Ag + Hcp100 nPCR + Ac-ID
S	94.0 (86.4-100.0)	82.0 (70.3-93.0)	70.0 (56.3-83.7)	26.0 (12.8-39.2)	100.0 (99.0-100.0)
E	95.5 (89.7-100.0)	97.0 (92.8-100.0)	100.0 (99.2-100.0)	100 (99.2-100.0)	92.4 (85.3-99.6)
CVP	20.68 (6.83-62.61)	27.06 (6.87-106.59)	(*)	(*)	6.60 (3.73-11.68)
CVN	0.06 (0.02-0.19)	0.19 (0.10-0.34)	0.30 (0.20-0.46)	0.74 (0.63-0.87)	(*)
Accuracy	94.8 (90.4-99.3)	90.5 (84.8-96.3)	87.1 (80.5 to 93.6)	68.1 (59.2 to 77.0)	95.7 (91.6-99.8)

S: sensitivity, E: specificity, CVP and CVN: positive and negative likelihood coefficient, HC-Ag: determination of urinary H. capsulatum antigen by EIA, BC: blood culture, Ac-ID: detection of antibodies, Hcp100 nPCR: nested PCR in whole blood, HC-Ag + Hcp100 nPCR + Ac-ID: is the combination of the HC-Ag, Hcp100 nPCR and Ac-ID methods. Values in parentheses correspond to 95% CIs. (*) There are no results because the calculation cannot be performed

was 320.97 ($p < 0.05$). This difference in RT is mainly due to the blood culture as when compared with HC-Ag, Hcp100 nPCR and Ac-ID. By the Wilcoxon signed rank test were obtained the following values of the statistic Z: 9.44; 9.37 and 9.40 ($p < 0.05$) in the 3 comparisons of means (Fig. 2).

ROC curve

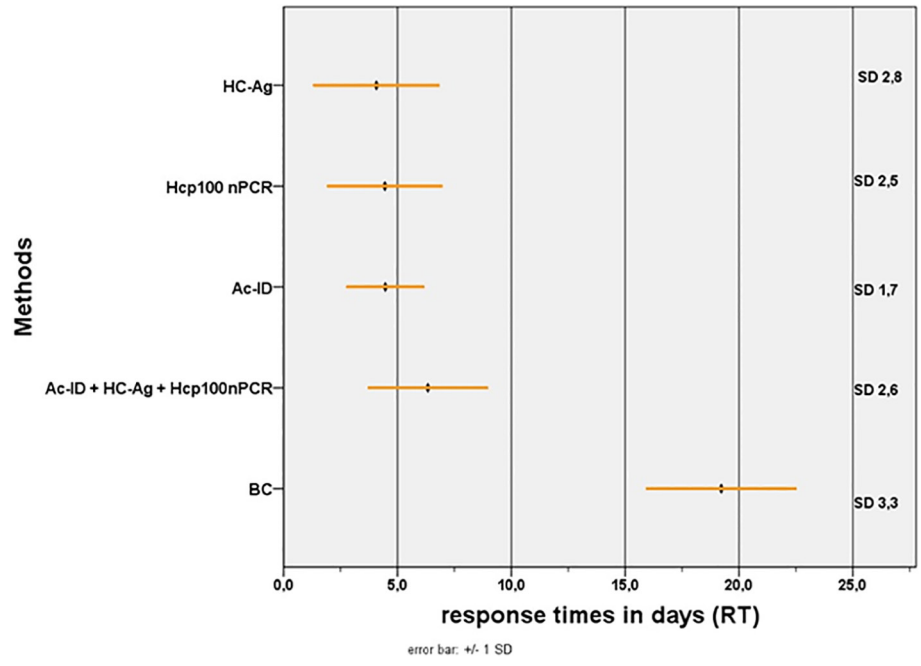
It was calculated one cutoff for the HC-Ag technique by a ROC curve that was of 0.39 ng/ml, with S 94% (86% to 100%) and E 97% (92% to 100%). Area under the curve and other parameters were determined for this new cutoff value and can be seen in Fig. 3.

The statistical parameters of this method for the different cutoff values were compared in Fig. 4 and Table 2.

Clinical characteristics of patients with PPDH and false negative results of HC-Ag

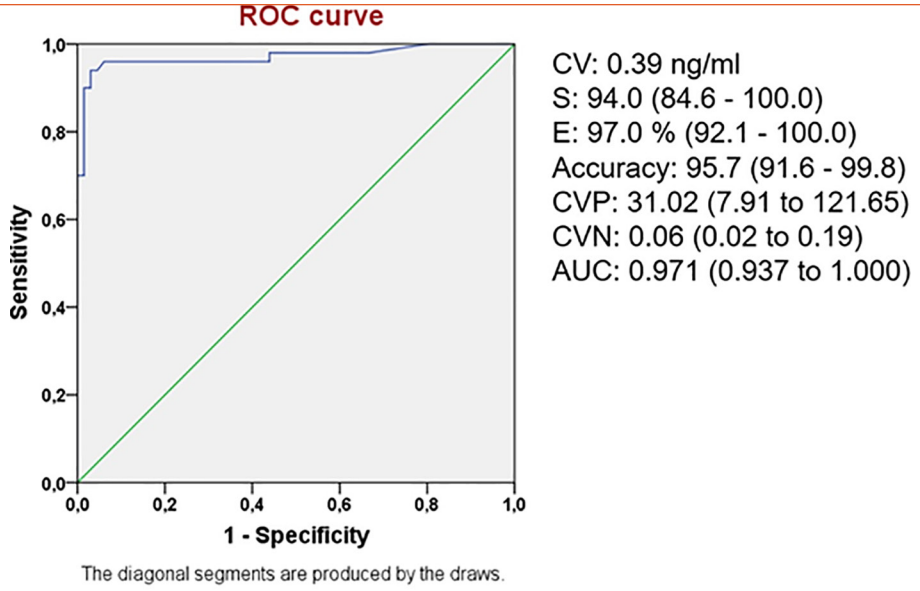
6 percent (3/50) of patients with PPDH had false negative results on the HC-Ag. Diagnosis was confirmed through positive BC, in 100% of these cases; *H. capsulatum* grew in an average of 19 days. In all patients of this group the Hcp100 nPCR was negative (0%) and the Ac-ID determination was positive (100%). Only one had lesions in the oral mucosa, but no intracellular yeasts characteristic of *H. capsulatum* were observed by scraping and subsequent Giemsa stain. Central nervous system histoplasmosis was seen in one patient and *H. capsulatum* was obtained in CSF culture but both HC-Ag determination and Hcp100 nPCR gave negative results for this clinical sample. The median age of this group of patients was 34 years old (IQR: 28-51 years old), all patients were male. All had CD4+ counts < 200 cells/µl and a median of 74.00 cells/µl (IQR: 49.00-77.00 cells/µl). They were initially treated with amphotericin B dc 0.5 mg/kg/day followed by itraconazole 400 mg/day. Lethality rate was 33.3%, higher than that in the group of patients with PPDH with HC-Ag positive results, but without significant differences ($\chi^2 = 0.991$; $p = 0.320$).

Figure 2 | Analysis of response times in days (RT) of each of the methods used in this study



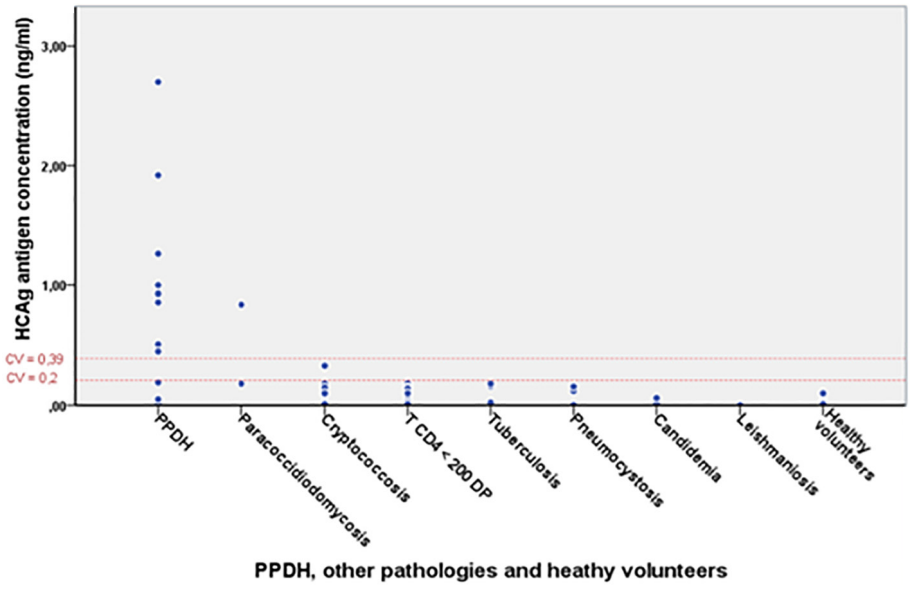
HC-Ag: determination of urinary H. capsulatum antigen by EIA, BC: blood culture, Ac-ID: detection of antibodies, Hcp100 nPCR: nested PCR in whole blood, HC-Ag + Hcp100 nPCR + Ac-ID: is the combination of the HC-Ag, Hcp100 nPCR and Ac-ID methods, SD: standard deviation

Figure 3 | ROC curve and other parameters of the HC-Ag test. E, S and accuracy values are percentages



CV: cutoff values, S: sensitivity, E: specificity, AUC: Area under the curve, CVP and CVN: positive and negative likelihood coefficient. Values between brackets correspond to 95% CIs.

Figure 4 | Urinary antigen concentration in ng/ml (HC-Ag) in patients with PPDH, other pathologies and healthy volunteers



CV: cutoff values, HC-Ag: urinary H. capsulatum *antigen determination by EIA, T CD4+ <200 DP: patients with T CD4+ lymphocyte counts <200 cells/µl and different pathologies; with no characteristic signs or clinical symptoms, nor epidemiological history or other diagnostic elements of mycosis or tuberculosis.*

Clinical characteristics of patients with pathologies other than PPDH and healthy volunteers with false positive results of HC-Ag

Five percent (3/66) of patients without PPDH and healthy volunteers had a positive result of HC-Ag. Two had paracoccidioidomycosis and one cryptococcosis. The Hcp100 nPCR, BC and Ac-ID methods were negative in all these patients. One patient with paracoccidioidomycosis was the only one in this group who presented

Tabla 2 | Comparison of the analytical parameters and the Cohen's Kappa statistic of the HC-Ag method, using the cut-off point of 0.2 ng/ml and 0.39 ng/ml*

	HC-Ag 0.2 ng/ml	HC-Ag 0.39 ng/ml
S	94.0 (86.4-100.0)	94.0 (86.4-100.0)
E	95.5 (89.7-100.0)	97.0 (92.1-100.0)
Accuracy	94.8 (90.4-99.3)	95.7 (91.6-99.8)
CVP	20.68 (6.83-62.61)	31.02 (7.91-121.65)
CVN	0.06 (0.02-0.19)	0.06 (0.02-0.19)

*S: sensitivity, E: specificity, CVP and CVN: positive and negative likelihood coefficient, HC-Ag 0.2 and HC-Ag 0.39: determination of urinary antigen of H. capsulatum by EIA, using cut-off values of 0.2 ng/ml and 0.39 ng/ml. Values between brackets correspond to 95% CIs. *Values in percentages of E, S and Precision.*

mucocutaneous lesions, on the oral mucosa. Multi-budding yeasts compatible with *Paracoccidioides* spp. were found in in Giemsa-stained smears from the scarification. The median age in this group was of 42 years old (IQR: 38-74), all three patients were male. Sixty-six percent had CD4+ counts <200 cells/µl, median 70 cells/µl (IQR: 46-686 cells/µl).

None of the patients in this group died, therefore the lethality rate was 0%.

Discussion

Histoplasmosis is the endemic mycosis of greater impact on public health in Latin America[43], although its true impact is still not known and it is considered a neglected disease by various authors[13, 44]. PPDH is associated with HIV infection in 90 to 95% of cases[4,13] and occurs mainly in patients who are not receiving antiretroviral treatment and with a CD4+ cell count <100 cells/µl[8, 12, 16, 45-47]. Eighty to 95 % of patients may have a favorable evolution with an early diagnosis and adequate treatment[47]. The case fatality rate in different endemic areas of America averages 30%[12], due to several factors that must be considered in order to design strategies that contribute to reducing the impact of this scourge. Among them: unknown actual incidence as it is not a compulsory notification disease, there is little information related to this mycosis and the scarce number of trained professionals. Clinical manifestations are not very specific and often wrongly diagnosed as tuberculosis or other diseases. Treatment is not always accurate because of economic crisis in many countries and consequent lack of access to liposomal amphotericin B or even itraconazole. Low socio-economic status of Latin American patients and their cultural differences from those of other countries contribute to the poor adherence to antiretroviral and antifungal treatment and, finally delay in diagnosis when rapid and simple methods such as urinary antigen detection, which began to be developed in the US in 1986[48], are not available. These issues were analyzed in The Manaos Declaration in 2019[27] where participated 24 countries and whose main objective was to achieve access to rapid diagnostic methods and to treatments with amphotericin B and itraconazole in 100% of Latin American countries by 2025.

Argentina is not the exception to this problem since antifungals are difficult to obtain in most institutions and the HC-Ag kit is too expensive in our country, so only hospitals and mycology reference centers, where there is a high prevalence of histoplasmosis can have access to this method, as is the case in our hospital. Moreover, the situation is more serious because 70% of HIV+ patients are living in the endemic area[8, 47]. Considering that the prevalence of asymptomatic histoplasmosis infections is close to 30% in our country, we can estimate that approximately 41,000 HIV+ individuals could be infected with this fungus and suffer a reactivation with the progression of the HIV/AIDS disease[49, 50]. According to the Official Bulletin, there was an average of 5,800 new annual cases of HIV infection in the period 2011-2019[49]. Therefore, an incidence of 100 cases/year of PPDH and consequently about 20 deaths can be estimated, as the prevalence

of HIV in this endemic area is about 2.5% with a case fatality rate close to 20%[7, 8, 23, 47].

This estimated number may be even higher since there are 17% HIV+ people who are unaware of their diagnosis and there are also some authors who consider that the prevalence of HP in HIV+ is even higher[8, 18, 19,47, 51, 52] and similar to the number of annual deaths caused by tuberculosis in those patients (close to 76 cases in 2019)[53]; and coincident with Adenis et al. publication[20].

In 2007, a HC-Ag urinary kit using polyclonal antibodies developed by the CDC[54] was evaluated first in Guatemala and then in Colombia, on patients with PPDH in HIV+ patients. Its S was 81% in Guatemala[32] and 86% in Colombia[28] and its global E was 95% and 94% respectively. After that, a multicenter study using the HGM, IMMY (with monoclonal antibodies) to evaluate the performance of this kit demonstrated a 98% S with the quantitative assay and 95% in the semiquantitative one, on 589 urine samples from patients with PPDH from Colombia and Guatemala[35]. In our research, the HC-Ag with the same kit had a S of 94% (with both 0.2 ng/ml and 0.39 ng/ml cutoff values) and its E reached 97% when 0.39 ng/ml cutoff value established in this research, was used.

False positive HC-Ag results were observed mainly with other mycoses (all cases of paracoccidiodomycosis and cryptococcosis), as it was seen in the mentioned multicenter study[35]. The cut off value established in our research was useful to reduce false positives and improve E, without changing the other parameters.

The HC-Ag test had the highest S and the lowest CVN compared to the other diagnostic methods. In all proven cases where urinary antigen could not be detected (false negatives), the patients had demonstrable antibodies. Moreover, when different rapid diagnostic methods were combined (HC-Ag, Hcp100 nPCR and Ac-ID) S reached a performance of 100%.

The time to reach diagnosis has a fundamental impact on patient outcome and survival. Samayoa et al., were able to determine that the average survival time of PPDH patients without treatment was 19 days[2].

In this study, 66% of patients with PPDH had mucocutaneous manifestations, which is consistent with data from Latin America[8, 16], and differs from those observed in the US where only 6% of patients present this type of lesions[4]. When lesions are accessible, a diagnosis can be obtained in less than 2 hours by Tzanck cytodiagnosis[55].

However, about 30-40% of patients do not have tegumentary manifestations[43], and in these cases the use of rapid diagnostic methods would be highly recommended since cultures take more than two weeks. When the diagnostic times of the tests were compared by means of the RTs, it was possible to determine that those of HC-Ag, Hcp100 nPCR and Ac-ID were significantly lower than those of BC whose average time was 19 days, coinciding with the average survival time estimated by Samayoa[2].

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