

Detection of *Histoplasma capsulatum* Antigen in Panamanian Patients with Disseminated Histoplasmosis and AIDS[∇]

Maria Eugenia Gutierrez,^{1*} Alfredo Canton,¹ Patricia Connolly,²
Robert Zarnowski,³ and L. Joseph Wheat²

Department of Infectious Diseases, Arnulfo Arias Madrid Hospital, Panama City, Panama¹; MiraVista Diagnostics, Indianapolis, Indiana²; and Department of Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin³

Received 3 September 2007/Returned for modification 15 October 2007/Accepted 7 February 2008

Histoplasmosis is a common endemic mycosis in the Americas, often causing severe disease in patients with AIDS. Antigen detection has become an important method for rapid diagnosis of histoplasmosis in the United States but not in Central or South America. Isolates from patients in the United States are predominantly found to be class 2 isolates when typed using the nuclear gene *YPS3*, while isolates from Latin America are predominantly typed as class 5 or class 6. Whether infection with these Latin American genotypes produces positive results in the *Histoplasma* antigen assay has not been reported. In this study, we have compared the sensitivity of antigen detection for AIDS patients from Panama who had progressive disseminated histoplasmosis to that for those in the United States. Antigenuria was detected in the MVista *Histoplasma* antigen enzyme immunoassay (EIA) in 95.2% of Panamanian cases versus 100% of U.S. cases. Antigenemia was detected in 94.7% of the Panamanian cases versus 92% of the U.S. cases. Two clinical isolates from Panama were typed using *YPS3* and were found to be restriction fragment length polymorphism class 6. We conclude that the MVista *Histoplasma* antigen EIA is a sensitive method for diagnosis of histoplasmosis in Panama.

Progressive disseminated histoplasmosis (PDH) is a common and serious opportunistic disease among patients with AIDS in the United States and Latin America. In Panama, PDH occurred in 8% of hospitalized patients from 1997 to 2003 and was fatal in 12% of cases (6). The clinical picture was similar to that reported elsewhere and usually included fever, weight loss, and respiratory symptoms, often in association with diarrhea, as well as hepatosplenomegaly, hepatic enzyme elevation, and pancytopenia. Skin lesions were noted in 17% of the Panamanian cases (6), lower than the observed rate for cases from Brazil (66%) but higher than that for cases from the United States (~3%) (7).

Disease in patients in Mexico and Latin America has been described as being caused by isolates of *Histoplasma capsulatum* var. *capsulatum* that exhibit distinct genetic profiles in comparison to those from the United States (3, 8, 9). Keath et al. (9) first described typing based upon restriction fragment length polymorphism (RFLP) in the *YPS3* gene. They showed that Panamanian strains were typed as *YPS3* class 3, 5, or 6, while the North American strains were predominantly class 2. Later, Kasuga et al. used the DNA sequence substitution rates in four independent protein-coding genes to identify at least eight different genotypes, or clades (8). North American class 2 was the predominant genotype in the United States and Latin American group A in Latin America. Latin American group B was found only in Columbia and Argentina. Based on only four isolates from Panama included in the study by Kasuga et al.,

Panamanian strains included Latin American group A and a lone lineage, designated H81.

The diagnosis of PDH relies on demonstration of the organism in clinical specimens or detection of antigen in body fluids (2). Histopathology may be falsely negative in up to 50% of patients, caused by sampling error, paucity of organisms, or inexperience of the pathologist. Culture may require several weeks to isolate and identify the organism and may be falsely negative for 15% of patients (13). Antigenuria can be detected in 95% of cases in patients with AIDS in the United States (4, 13) but has not been evaluated in Latin America. Whether genetic differences in Latin American isolates would affect the sensitivity for diagnosis by antigen detection is unknown. The prevalence of PDH in AIDS patients from Panama offered the opportunity to address this question.

MATERIALS AND METHODS

Case definition. The definition included the presence of AIDS based upon a serologic test positive for human immunodeficiency virus infection, a CD4 cell count below 200 cells/ μ l and/or a previous AIDS defining condition according to CDC classification, and PDH proven by culture or histopathology.

Methods. We collected samples of serum and urine from Panamanian patients with AIDS and clinical suspicion of PDH who were admitted to the AIDS ward of the Arnulfo Arias Madrid Hospital in Panama City, Panama, from December 2005 through November 2006. All of the samples were taken before the start of antifungal therapy, referred to as baseline, and then stored frozen at -20° C. Samples were shipped in batches to MiraVista Diagnostics on three occasions during the course of the study, according to International Air Transport Association regulations.

We included 21 patients with PDH in the study. The study was approved by the institution's review board and followed guidelines for clinical research. All patients read and signed an informed-consent form before the samples were taken. The investigators also completed a case report form for each patient with demographic and clinically relevant data.

For comparison, paired serum and urine specimens collected at enrollment or week 1 or 2 of treatment from 65 AIDS patients in the United States with PDH

* Corresponding author. Present address: Jose Domingo De Obaldía Hospital, P.O. Box 0426-00283 David, Chiriquí, Panama. Phone: (507) 6617-6054. E-mail: ginnygp@cwpanama.net.

[∇] Published ahead of print on 20 February 2008.

who participated in a fluconazole treatment study (11) were also tested. The basis for diagnosis was positive culture or histopathology in 92% of cases and positive antigen in urine in 8% of cases. These specimens were obtained from 1991 and 1992 and had been stored frozen since original collection for a study conducted by the AIDS Clinical Trials Group, in accordance with institutional guidelines for clinical research.

Genotyping of isolates using the *YPS3* gene. The isolates were identified based on both the size of the PCR-amplified *YPS3* gene (3) and RFLP pattern analysis of the *HaeIII*-digested *YPS3* locus. Briefly, DNA from *H. capsulatum* isolates was obtained with a MasterPure yeast DNA purification kit (Epicenter, Madison, WI). The primers used to amplify the *YPS3* locus were Yps3F (5'-ATGCTGAACATCAAATCGATCTC-3') and Yps3R (5'-TTATGCCTCGCAGTGTATTAAGC-3') (3). PCR was performed using the high-fidelity polymerase Triplmaster (Eppendorf, Westbury, NY) with buffer components provided by the supplier. The amplification parameters were as follows: an initial denaturation step at 95°C for 5 min, followed by 25 cycles which consisted of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 1 min. PCR products were separated on a 2% agarose gel, and their sizes were determined using a 50-bp DNA ladder (NEB, Ipswich, MA). The sizes of the amplified *YPS3* sequences were compared with those for appropriate RFLP class-defined control strains [UCLA531S, RFLP class 1; G217B (ATCC 26032), RFLP class 2; G186B (ATCC 26030), RFLP class 3; JHMA(H502), RFLP class 5; and CFLA(H248), RFLP class 6]. In addition, PCR products were digested with *HaeIII* according to the manufacturer's recommendations (Boehringer, Mannheim, Germany), and digestion products were analyzed after separation on a 2% agarose gel. The unique RFLP patterns were compared with those obtained from the control strains listed above.

MVista *Histoplasma* antigen EIA. This method was reported previously (4) and will be briefly described. The test is a sandwich enzyme immunoassay (EIA) using microplates coated with polyclonal rabbit anti-*Histoplasma* antibodies. Patient specimens and galactomannan calibrators and controls are incubated in the precoated plates, permitting binding of antigen to the capture antibody. Bound antigen is then detected with a biotinylated detector antibody. The microplates are read spectrophotometrically, and optical densities that are more than three times that of the negative control are classified as positive; antigen is quantitated in ng/ml by extrapolation from a calibration curve. Specimens with optical densities that exceed the cutoff for the assay but that are less than the 0.6-ng/ml standard are reported as positive, <0.6 ng/ml, and those with results exceeding the 39-ng/ml standard are reported as positive, >39 ng/ml; all others are read directly from the curve. All of the specimens were tested in a single batch. Testing was performed at MiraVista Diagnostics, Indianapolis, IN.

Statistical analysis. The respective proportion of patients with positive antigenuria or antigenemia among patients from Panama was compared to that from the United States by using Fisher's exact test. Quantitative antigen results in serum or urine from both patient groups were compared using the Mann-Whitney rank sum test.

RESULTS

Clinical and laboratory findings. Twenty-one patients with newly diagnosed cases of PDH were enrolled in the study over 12 months in 2005 and 2006. Our patients' median age was 36.7 years (range, 24 to 60), and 90% of them were male (19/21). The most common findings were fever, weight loss, hepatosplenomegaly, hematological alterations, and diarrhea. The diagnosis of histoplasmosis was confirmed by blood culture in 12 cases and by bone marrow culture in 4, and 5 had positive results in both. Four patients died in the first 6 weeks of treatment, at days 5, 6, 14, and 35. Genotyping using *YPS3* was performed on two clinical isolates from Panama, and both were typed as class 6.

Antigen levels were similar in patients from Panama and the United States (Fig. 1). The urine antigen test was positive in 20 of 21 (95.2%) of Panamanian cases versus 65 of 65 (100%) of U.S. cases ($P = 0.244$). The median urinary antigen level was also similar between Panamanian and U.S. cases, 18.8 ng/ml (range, none detected to >39.0 ng/ml; 95% confidence interval [CI], 8.4 to 40.0), versus 37.0 ng/ml (range, 0.71 to >39.0 ng/ml;

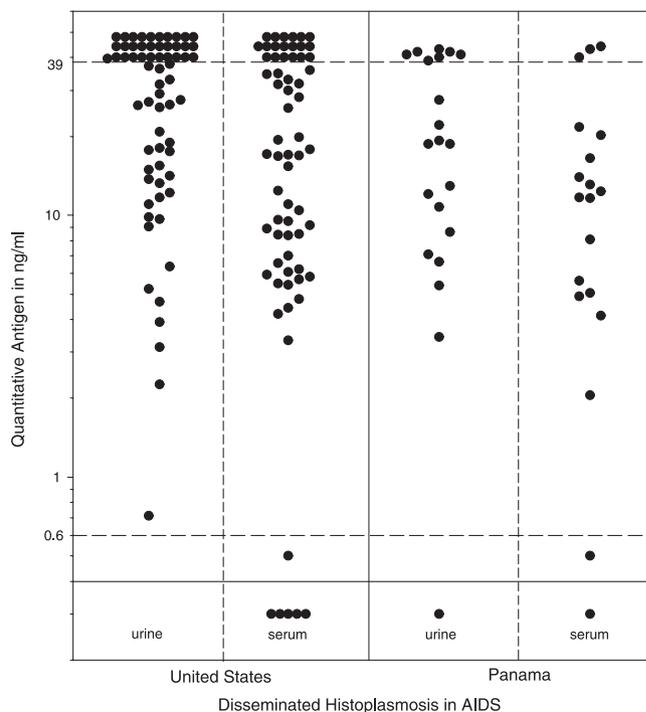


FIG. 1. Urine and serum antigen in patients with AIDS and PDH. The solid horizontal line represents the cutoff for positivity and the dashed lines the lowest (0.6 ng/ml) or highest (39 ng/ml) calibrator. Each point represents a single case.

95% CI, 26.0 to 40.0) ($P = 0.088$). Serum antigen was positive in 18 of 19 (94.7%) Panamanian cases versus 60 of 65 (92%) U.S. cases ($P = 1.0$). The median serum antigen level was 11.7 ng/ml (range, none detected to >39.0 ng/ml; 95% CI, 4.9 to 20.0) in the Panamanian group versus 17.0 ng/ml (range, none detected to >39 ng/ml; 95% CI, 9.2 to 31.7) in the U.S. cases ($P = 0.155$).

False-negative results with serum and urine occurred for one Panamanian patient. He was a 32-year-old with miliary infiltrates on chest radiograph, diarrhea, and vomiting, suggesting gastrointestinal involvement, and a positive blood culture for *H. capsulatum*.

DISCUSSION

The purpose of this study was to determine if the MVista *Histoplasma* antigen EIA could be used for diagnosis of PDH in patients with AIDS from Panama. The rationale for addressing this issue was that *H. capsulatum* strains from Panama were genetically different from those from the United States (3, 8, 9) and thus might also be antigenically different, affecting the sensitivity for diagnosis by antigen detection. Antigen was detected in specimens from 95% of the Panamanian patients, at levels comparable to that for U.S. patients. Of note is that the samples used for the comparison from the U.S. cases were stored frozen for more than 15 years and the Panamanian specimens were stored frozen for 6 to 12 months before testing. We have not observed a reduction in antigen concentration in frozen specimens, as shown by the similar individual levels and proportion that were positive in the U.S. specimens compared to the initial testing of those samples (11). The two

Panamanian isolates that were typed were *YPS3* RFLP class 6, belonging to Latin American group A. (8). In a separate study, we have also shown that antigen was detected in the tissues of mice infected with *YPS3* RFLP class 5 or class 6, Latin American group A isolates (1, 5). These findings establish that the antigens produced by the most common Latin American genotypes of *H. capsulatum* are detected in the MVista *Histoplasma* antigen EIA.

We previously noted the sensitivity for diagnosis of PDH in AIDS patients from Panama to be 67% for blood cultures, 51% for bone marrow cultures, and only 24% for histopathology of bone marrow biopsy (6). The low sensitivity of nonculture methods and delay in diagnosis by culture supports the value of the antigen test for diagnosis of PDH in Panamanian patients. Of note is that specimens from patients with other endemic mycoses are cross-reactive in the MVista *Histoplasma* antigen EIA (10, 12). However, cross-reactivity does not reduce the usefulness of the test for rapid diagnosis, since treatment decisions and regimens are similar and in most cases the causative organism can be suspected based on epidemiological or clinical findings or proven by histopathology, culture, or serology.

In summary, antigenemia and antigenuria were present in similar proportions of patients from the United States and Panama, showing the potential use of the MVista *Histoplasma* antigen EIA for diagnosis of PDH in Panama and probably other Latin American countries.

ACKNOWLEDGMENTS

We disclose that L. J. Wheat and P. A. Connolly are employees of MiraVista Diagnostics and MiraBella Technologies, the company that developed and performs the MVista *Histoplasma* antigen EIA.

REFERENCES

1. Allton, D. R., R. G. Rivard, P. A. Connolly, L. J. Wheat, and D. R. Hospenthal. 2007. Antigen detection of Latin American strains of *Histoplasma* in a murine model. Abstr. 45th Conf. Infect. Dis. Soc. America, abstr. 159.
2. Asciglu, S., J. H. Rex, B. De Pauw, J. E. Bennett, J. Bille, F. Crokaert, D. W. Denning, J. P. Donnelly, J. E. Edwards, Z. Erjavec, D. Fiere, O. Lortholary, J. Maertens, J. F. Meis, T. F. Patterson, J. Ritter, D. Selleslag, P. M. Shah, D. A. Stevens, and T. J. Walsh. 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin. Infect. Dis.* **34**:7–14.
3. Bohse, M. L., and J. P. Woods. 2007. Expression and interstrain variability of the *YPS3* gene of *Histoplasma capsulatum*. *Eukaryot. Cell* **6**:609–615.
4. Connolly, P. A., M. M. Durkin, A. M. LeMonte, E. J. Hackett, and L. J. Wheat. 2007. Detection of *Histoplasma* antigen by a quantitative enzyme immunoassay. *Clin. Vaccine Immunol.* **14**:1587–1591.
5. Durkin, M. M., P. A. Connolly, K. Karimi, E. Wheat, C. Schnizlein-Bick, S. D. Allen, K. Alves, R. P. Tewari, and E. Keath. 2004. Pathogenic differences between North American and Latin American strains of *Histoplasma capsulatum* var. *capsulatum* in experimentally infected mice. *J. Clin. Microbiol.* **42**:4370–4373.
6. Gutierrez, M. E., A. Canton, N. Sosa, E. Puga, and L. Talavera. 2005. Disseminated histoplasmosis in patients with AIDS in Panama: a review of 104 cases. *Clin. Infect. Dis.* **40**:1199–1202.
7. Karimi, K., L. J. Wheat, P. Connolly, G. Cloud, R. Hajjeh, E. Wheat, K. Alves, C. S. Lacaz Cd, and E. Keath. 2002. Differences in histoplasmosis in patients with acquired immunodeficiency syndrome in the United States and Brazil. *J. Infect. Dis.* **186**:1655–1660.
8. Kasuga, T., T. J. White, G. Koenig, J. McEwen, A. Restrepo, E. Castaneda, L. C. Da Silva, E. M. Heins-Vaccari, R. S. De Freitas, R. M. Zancope-Oliveira, Z. Qin, R. Negroni, D. A. Carter, Y. Mikami, M. Tamura, M. L. Taylor, G. F. Miller, N. Poonwan, and J. W. Taylor. 2003. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. *Mol. Ecol.* **12**:3383–3401.
9. Keath, E. J., G. S. Kobayashi, and G. Medoff. 1992. Typing of *Histoplasma capsulatum* by restriction fragment length polymorphisms in a nuclear gene. *J. Clin. Microbiol.* **30**:2104–2107.
10. Kuberski, T., R. Myers, L. J. Wheat, B. M. Kubak, D. Bruckner, and D. Peuges. 2007. Diagnosis of coccidioidomycosis by antigen detection using cross-reaction with a *Histoplasma* antigen. *Clin. Infect. Dis.* **44**:e50–e54.
11. Wheat, J., S. MaWhinney, R. Hafner, D. McKinsey, D. Chen, A. Korzun, K. J. Shakan, P. Johnson, R. Hamill, D. Bamberger, P. Pappas, J. Stansell, S. Koletar, K. Squires, R. A. Larsen, T. Cheung, N. Hyslop, K. K. Lai, D. Schneider, C. Kauffman, M. Saag, W. Dismukes, W. Powderly, et al. 1997. Treatment of histoplasmosis with fluconazole in patients with acquired immunodeficiency syndrome. *Am. J. Med.* **103**:223–232.
12. Wheat, J., H. Wheat, P. Connolly, M. Kleiman, K. Supparatpinyo, K. Nelson, R. Bradsher, and A. Restrepo. 1997. Cross-reactivity in *Histoplasma capsulatum* variety *capsulatum* antigen assays of urine samples from patients with endemic mycoses. *Clin. Infect. Dis.* **24**:1169–1171.
13. Williams, B., M. Fojtasek, P. Connolly-Stringfield, and J. Wheat. 1994. Diagnosis of histoplasmosis by antigen detection during an outbreak in Indianapolis, Ind. *Arch. Pathol. Lab. Med.* **118**:1205–1208.