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Detection of *Histoplasma capsulatum* DNA in peripheral blood from a patient with ocular histoplasmosis syndrome

JOSÉ M. HERNÁNDEZ*, CESAR O. MUÑOZ-CADAVID†, DIANA L. HERNÁNDEZ‡, CATALINA MONTOYA‡ & ÁNGEL GONZÁLEZ†§

**Medicine School, Universidad Pontificia Bolivariana, Medellín, Colombia, †Medical and Experimental Mycology Group, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia, ‡Ophthalmology Clinic San Diego, Medellín, Colombia and §Microbiology School, Universidad de Antioquia, Medellín, Colombia*

Ocular histoplasmosis syndrome (OHS) is a significant cause of vision loss in young and middle-aged adults. We report here a case of an immunocompetent 37-year-old man who presented fever, malaise, headache, and anterior cervical lymphadenopathy for one week, after which he started to experience a sudden loss in visual acuity of his right eye. Fluorescent angiography and an optical coherent tomography demonstrated the presence of a type II choroidal neo-vascular membrane in the right eye, suggesting a diagnosis of OHS. A peripheral blood sample was tested by nested PCR to detect *Histoplasma capsulatum* using a set of primers known to amplify a DNA sequence coding for a specific 100-kDa protein of this fungus (Hc100-PCR). The blood sample was Hc100-PCR-positive and sequence analysis showed an identity of 97% with the reference sequence. The patient received intravitreal bevacizumab injection and itraconazol therapy, leading to an improvement in media vision acuity. In this case, the molecular test provided evidence linking the ocular lesions with an earlier infection by *H. capsulatum* and demonstrated that the Hc100-nested PCR assay is a valuable tool in the diagnosis of histoplasmosis.

Keywords *Histoplasma capsulatum*, ocular histoplasmosis syndrome, molecular diagnosis

Introduction

Histoplasma capsulatum is the causative agent of histoplasmosis, one of the most important endemic mycosis in the Americas [1]. It is found in most countries of the continents, but is more prevalent in specific regions of the US, such as the Mississippi and Ohio River Valleys [1,2]. High incidence of histoplasmosis has also been observed in Central America (Mexico, Panama, Honduras, Guatemala, and Nicaragua), in the Caribbean (Jamaica, Puerto Rico, Cuba, and Martinique) and in South America (Venezuela, French Guyana, Colombia, Peru, Brazil, and Argentina) [3,4].

The severity of histoplasmosis varies greatly depending on the intensity of exposure to the fungus and on the immune status of the infected individual [1,5]. In patients with immunodeficiency disorders, especially those infected with the human immunodeficiency virus (HIV), histoplasmosis is considered an opportunistic infection [6–8]. In addition, in a high proportion of the cases this fungal infection manifests as a severe disseminated process that often leads to death if not treated promptly [6–8].

Diagnosis of histoplasmosis is usually accomplished by recovery of the etiologic agent in culture and microscopic examination of respiratory tract specimens, biopsies, and body fluid specimens. However, these techniques yield positive results in only approximately 50% of proven cases [1,3,9]. In addition, isolation of the fungus usually takes 2–6 weeks, delaying diagnosis and initiation of therapy. Immunological tests that detect antibodies and/or antigens

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Correspondence: Ángel González, Corporación para Investigaciones Biológicas (CIB) Carrera 72A # 78 B 141; Medellín, Colombia. E-mail: agonzalezm@cib.org.co

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are also of value and may give faster results than cultures, but these procedures have shown variable values of sensitivity and specificity, often yield negative results in immunocompromised patients [10–12], and show cross-reactivity with other fungal agents [3,11,13,14]. In the last decade, molecular approaches have been developed for the detection of *H. capsulatum* DNA in human clinical samples. For example, *H. capsulatum* infection in humans was successfully diagnosed in tissue samples using a nested-PCR assay for the Hcp100 gene, as initially described by Bialek *et al*. [15], which was further validated by Maubon *et al*. [16]. Recently, by using the same molecular marker, Toranzo *et al*. reported 96% specificity in the detection of histoplasmosis in human whole blood samples [17]. Muñoz *et al*. also validated the clinical application of the Hcp100 gene for the diagnosis of histoplasmosis in several human samples finding a sensitivity of 100% and a specificity of 95.2% [18]. However, DNA-based diagnosis for histoplasmosis has not yet been established as a regular diagnostic tool, nor is a *H. capsulatum*-specific PCR assay commercially available.

Presumed ocular histoplasmosis syndrome (POHS) is characterized by chorioretinal scars in the peripheral retina, peripapillary choroiditis, and maculopathy with disciform lesion with hemorragic, subjacent retinal detachment, choroidal neovascularization without uveitis signs, which leads to severe loss of central vision. Infrequently, ocular histoplasmosis is established by the isolation of *H. capsulatum* in cultures of samples of the vitrous or other tissues, as well as by PCR techniques [19,20]. Although substantial epidemiologic and laboratory evidence links the ocular disease with this fungal pathogen, some experts are in disagreement whether or not *H. capsulatum* is a causative agent in all cases of OHS, and prefer to refer to the syndrome as POHS [21]. Interestingly, although some cases of OHS have also been reported in non-endemic areas, the geographic distribution of the majority of OHS cases show that they have occurred in both residents and visitors to endemic areas with high prevalence of histoplasmosis [22,23]. In particular, POHS has been associated with individuals who have certain histocompatibility antigens, including HLA-B7 and HLA-DRw2, which may increase the risk of developing POHS [20,24].

We report here a patient with clinical features of OHS who was Hc100-PCR-positive for *H. capsulatum* in a peripheral blood sample and recovered from the syndrome after a combined therapy of intravitreal bevacizumab and itraconazol over the course of one year.

Case report

An immunocompetent 37-year-old man, who was an employee in a mycobacteriology research laboratory, indicated that prior to consultation with an ophthalmologist he had been experiencing fever, malaise, headache, and anterior cervical lymphadenopathy for one week. While he indicated gradual improvement of his symptoms, he experienced a sudden loss of visual acuity of his right eye.

Fluorescent angiography and optical coherent tomography showed the presence of a type II choroidal neovascular membrane in the right eye (Fig. 1 and Fig. 2). On the basis of these results, the ophthalmologist indicated a presumptive diagnosis of OHS. Conventional laboratory tests such as immunodiffusion and complement fixation tests for antibodies against *H. capsulatum* were negative at the beginning of infection. However, the tests became weakly positive after eight weeks with a titer of 1:8 using two histoplasma antigens: histoplasmin and whole yeast.

A peripheral blood sample was tested by nested PCR for *H. capsulatum* using a set of primers that specifically amplify a DNA sequence coding for a specific 100-kDa protein of the fungus (Hc100-PCR) as described elsewhere [18]. The results were compared to a positive control sample of *H. capsulatum* DNA and with various negative peripheral blood samples from healthy individuals (Fig. 3). Additionally, the PCR products were subjected to DNA sequencing which confirmed the amplified product had an identity of over 97%. The patient then received intravitreal bevacizumab injection and itraconazol therapy (200 mg, three times daily for three days and then 200 mg once or twice daily) for one year. Median vision improved from 20/100 to 20/60 after eight weeks of treatment.

In summary, we present a case of OHS with unusual manifestations, and the use of a *H. capsulatum*-specific molecular test to provide evidence linking the ocular lesions with an earlier infection by *H. capsulatum*.

Discussion

OHS is a significant cause of vision loss in young and middle-aged adults. Despite the fact that *H. capsulatum* has not been clearly demonstrated to play a direct role in OHS, some epidemiological studies appear to implicate the fungus in this syndrome.

Along the same lines, researchers from the department of Ophthalmology and Visual Science at the Washington University School of Medicine reviewed OHS cases from different regions of Europe in which *H. capsulatum* isolation had been unsuccessful. They suggested that these patients must have been previously exposed to either *H. capsulatum* or a related microorganism as the etiologic agent of this ocular disease [25]. Moreover, after a study of a series of patients from a non-endemic region of Brazil who exhibited clinical features similar to POHS (including negative antibody serum tests for histoplasmosis, negative medical and laboratory tests for toxoplasmosis, syphilis

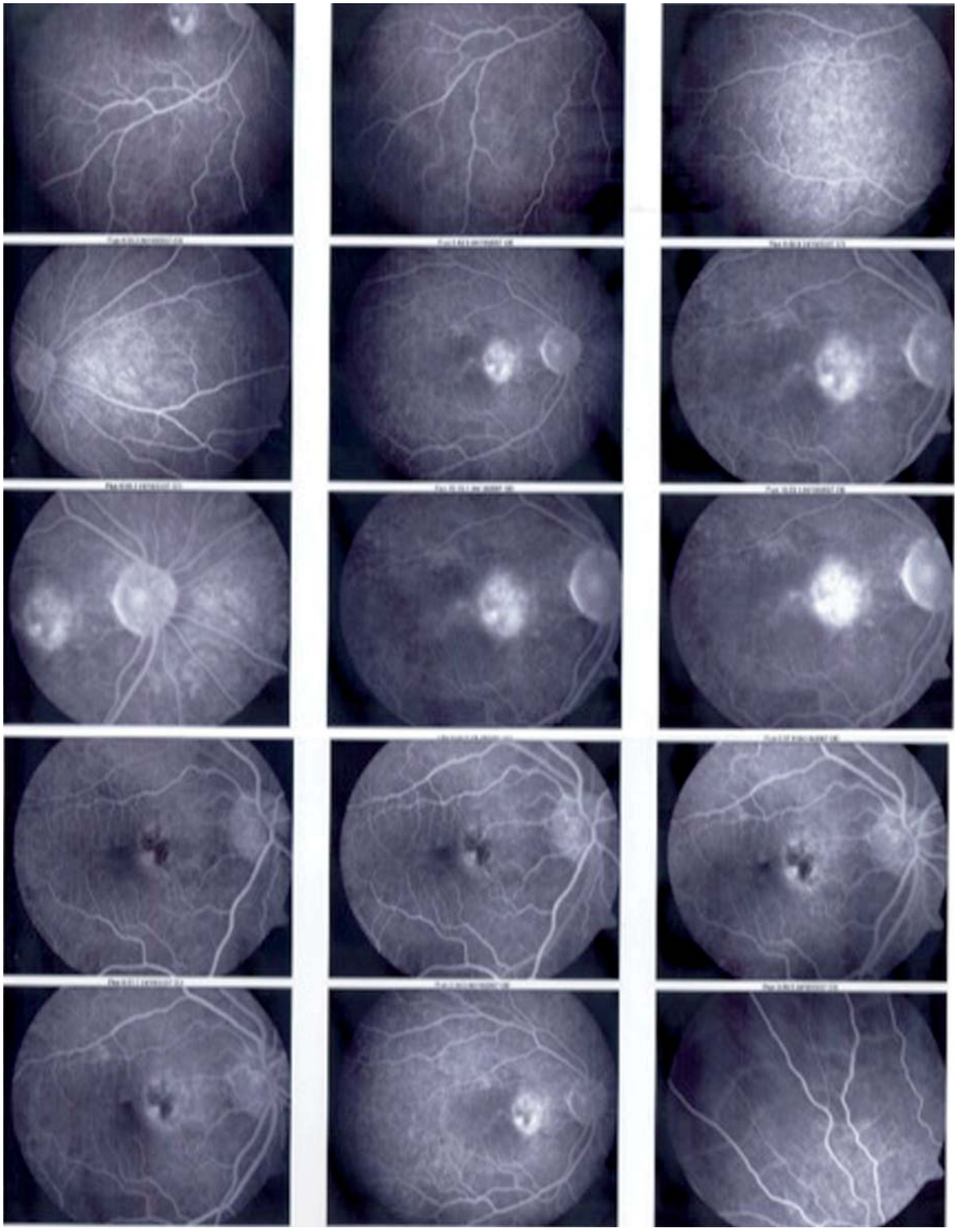

Fig. 1 Fluorescent angiography showing a hypofluorescent zone surrounded by a halo of irregularly-pointed hyperfluorescence, which is more pronounced towards the lesion center; during the angiogram, this hyperfluorescence also showed a halo all around comprising 5 or 6 disc diameters and the foveal area. There was substantial increase in hyperfluorescence towards the end of the angiogram. A slight hyperfluorescence at the nerve level was also observed.

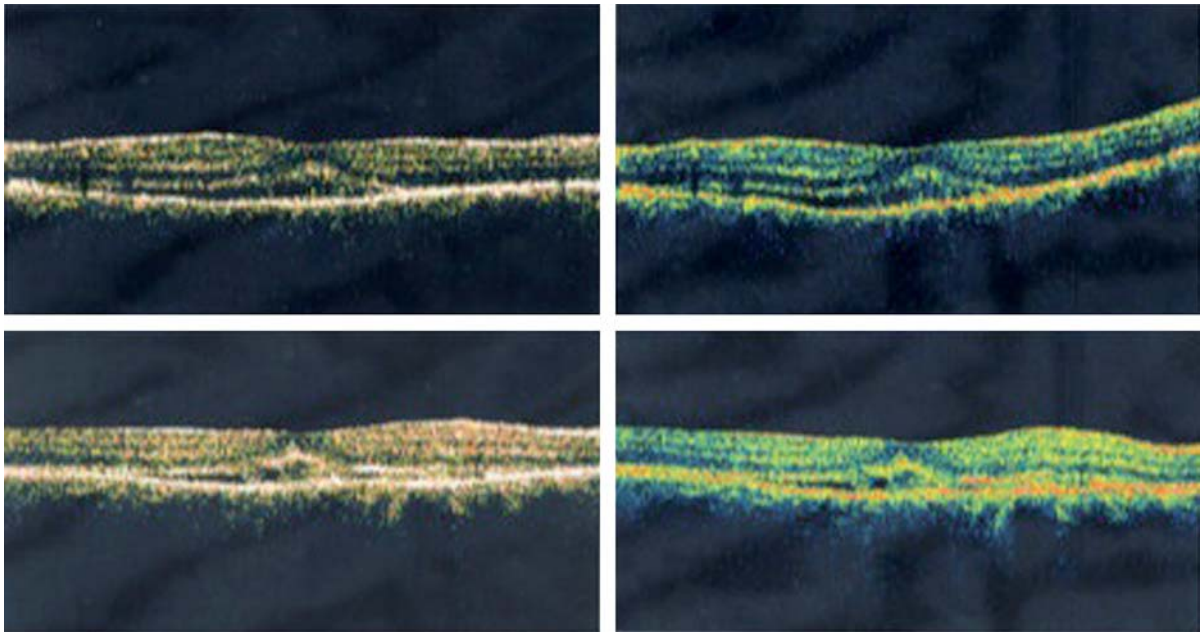

Fig. 2 Ocular tomography. A subfoveal neurosensorial detaching with apparent retinal thickening can be observed; note the presence of pointed hyper-reflective area in the neurosensorial space over the EPR.

and tuberculosis), the authors suggested that another etiologic agent(s) could be associated with these clinical presentations [25].

Other evidence linking OHS to *H. capsulatum* comes from histopathologic and molecular studies published by Scholz *et al*. and Spencer *et al*., who found blastoconidia and *H. capsulatum* DNA, respectively, within the endothelial cells of choroids in patients who presented clinical evidence of OHS [26,27]. Additionally, two animal models of experimental OHS have been developed by Wong *et al*. and Smith *et al*., who infected rabbits and monkeys, respectively, with *H. capsulatum* spores, producing an acute multifocal choroiditis similar to that observed in humans [28–30], thus implicating the development of OHS by *H. capsulatum* infection.

On the other hand, the treatment used for OHS is a combination of intravitreal bevacizumab and itraconazol. The bevacizumab is employed to improve or stabilize the visual acuity in patients with neovascular complications, as in the case of OHS [31], while the itraconazol is used as the first antifungal choice for the treatment of *H. capsulatum* infections. The patient reported in this study received a unique dose of intravitreal bevacizumab and itraconazol for one year. Although it is difficult to say if the visual improvement was due to the use of bevacizumab, the itraconazol, or the combination of drugs, there is circumstantial evidence that could suggest the role played by the itraconazol.

One of the difficulties in demonstrating the involvement of *H. capsulatum* in the development of ocular diseases is the fact that in the early stages of infection, the detection of antibodies, as well as the identification of fungi in clinical samples, has proven to be unsuccessful. For this reason, the development and implementation of new diagnostic tools are needed. In this report, we applied a molecular test (Hcp100-PCR) for the first time using a non-invasive procedure (peripheral blood sample), which allowed us to detect DNA of the microorganism implicated in OHS. It is important to note that this sample was obtained during the

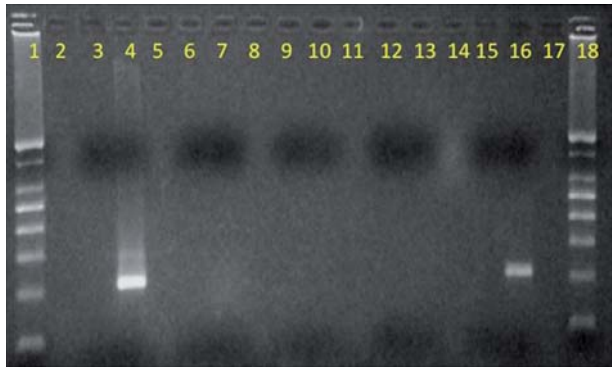

Fig. 3 *Histoplasma capsulatum* DNA detection in peripheral blood sample. Track 1 and 18, molecular weights; lines 2 and 3, negative control; line 4, positive control; lines 5–15, samples from negative patients (as control); line 16, DNA amplification from peripheral blood from the patient with OHS.

earlier course of infection, when the patient was presenting clinical symptoms, with negative serological test results and without any treatment.

In conclusion, we report a case of a patient with OHS from an endemic area in whom we were able to amplify *H. capsulatum*-specific DNA in peripheral blood. Interestingly, the patient showed low titers in the complement fixation assay using two different *Histoplasma* antigens. In addition, the patient improved his median vision acuity after a treatment with intravitreal bevacizumab and itraconazol therapy over the course of one year. Although the above results are not direct evidence that *H. capsulatum* is the cause of the ocular symptoms, in the absence of other findings, it does suggest a relationship. Moreover, the implementation of such non-invasive molecular tests could be a valuable tool for the diagnosis of unusual manifestations of histoplasmosis, especially in endemic areas.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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