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Diagnostic Methods for Histoplasmosis: Focus on Endemic Countries with Variable Infrastructure Levels

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Abstract Diagnosis of histoplasmosis remains challenging in resource-limited regions where HIV/AIDS is epidemic and histoplasmosis is endemic. Early and rapid detection of histoplasmosis is essential to preventing morbidity and mortality, yet few diagnostic options are available in low-resource areas of the world. The aim of this review is to provide an overview of the current status of the diagnosis of histoplasmosis, including an update on recent developments and utilization of new technologies. We discuss the specific diagnostic challenges faced in endemic regions, emphasizing the need for greater availability and standardization of rapid diagnostics for this endemic and neglected disease. While significant progress has been made in the development of new methods, clinical utility must be established by means of formal and extensive clinical studies.

Keywords Histoplasmosis · Diagnosis · Progressive disseminated histoplasmosis (PDH) · HIV · AIDS · *Histoplasma capsulatum*, Endemic countries

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Introduction

Histoplasmosis infections occur throughout the world where the causative fungal agent *Histoplasma capsulatum* is endemic. *H. capsulatum* thrives in guano-enriched soils in humid environments, particularly in caves and river valleys. The majority of reported human infections occur in the Americas, although the habitat of *H. capsulatum* includes Africa and pockets of Southeast Asia and Australia. In healthy persons, infections with histoplasmosis are ordinarily self-limiting, and may present with few symptoms or pass unnoticed, while respiratory and other flu-like symptoms are experienced in 5 % of cases [1, 2]. In individuals who are immunocompromised, histoplasmosis becomes progressive, and infection spreads rapidly from the lungs to other organs. This condition is known as progressive disseminated histoplasmosis (PDH), and it is deadly in persons with AIDS if treatment is not given promptly [3], with mortality rates between 22 % and 48 % when the disease is diagnosed late [4, 5].

Histoplasmosis is often the first presentation of AIDS in endemic regions, and PDH is an AIDS-defining illness that is seen with high frequency in persons whose CD4 blood count falls to ≤150 cells/μl [2]. Because clinical symptoms of PDH (weight loss, fever, cough, malaise, and others) greatly overlap with those caused by *Mycobacterium* spp., PDH is often misdiagnosed. In endemic regions where late diagnosis of AIDS is common, laboratories may have few resources by which to provide rapid, accurate analyses of specimens to confirm disease etiology. Although the global prevalence of HIV has steadied since 2001, transmission of the disease has increased among low- and middle-income countries, accounting for 95 % of all new cases [6]. Regions of particular concern are the Americas, the Caribbean, Sub-Saharan Africa, and Southeast Asia, where HIV is epidemic, histoplasmosis is endemic, and resources are limited [7]. In this review we will

address laboratory capacity to diagnose histoplasmosis and PDH in endemic regions with variable infrastructure and resource challenges. Late diagnosis of PDH is frequent and fatal, and we will describe recent developments in diagnosis that are rapid and inexpensive, and that may be implemented as needed to reduce histoplasmosis-associated morbidity and mortality.

Laboratory Diagnostics

Although there is a broad range of diagnostic tests to detect infection with *H. capsulatum*, in resource-challenged regions, only the most conventional methods, if any, are available (Table 1). Diagnosis of histoplasmosis is traditionally accomplished by direct preparations and histopathology using special staining methods as well as by isolation of the fungus in culture, which is considered the "gold standard" for diagnosis. Immunological tests to detect antibodies and/or antigens are also valuable, and more recently, molecular techniques for identification of *H. capsulatum* are playing an increasingly significant role in clinical diagnosis, offering the distinct advantages of greater speed, sensitivity, and specificity. Both direct and indirect tests offer varying ranges of sensitivity and specificity depending upon the methodology, clinical form of the disease, and immune status of the host.

Direct Examination and Histopathology. In clinical specimens such as sputum, bronchoalveolar lavage fluid, blood, bone marrow, biopsy specimens of oral, cutaneous, and gastrointestinal lesions, adrenal glands, or liver and spleen, *H. capsulatum* can be detected by direct microscopic examination. Giemsa and Wright's stains can be used to detect yeast cells of *H. capsulatum* in blood or bone marrow smears. Histopathologic stains such as Gomori methenamine-silver (GMS), periodic acid-Schiff (PAS), and hematoxylin and eosin (H&E) are also useful, as they reveal the small (2- to 4-μm) oval budding yeasts often found within macrophages or free in the tissues, enabling a presumptive diagnosis of histoplasmosis. Expertise is necessary to differentiate these yeasts from other pathogens that resemble *H. capsulatum* yeast such as *Candida glabrata, Pneumocystis jirovecii,*

Table 1 Availability of laboratory diagnostic tools to detect *H. capsulatum* infection, by region and country (partial list) Histopathology is available in all included countries. Reference numbers are given for laboratory developed assays

Region	Country	Culture	CF	ID	Ab ELISA	Ag ELISA	PCR	Other
North America	Mexico	E	—	L to E	[51]	—	[51]	Capillary tube precipitin (CTP) [50, 51]
	United States	E	CA	E, CA	CA	CA	[31, 49]	CA; AccuProbe® and others
Central America	El Salvador	L	—	—	—	[26]	—	—
	Guatemala	E	—	—	—	[26]	—	—
	Honduras	L	—	—	—	[26]	—	—
	Panama	L to E	—	U	—	—	—	—
Caribbean	Cuba	E	—	E	[57, 58]	—	—	—
	Jamaica	E	—	—	—	—	—	CA; yeast ID kit [60]
	Puerto Rico*	E	CA	CA	CA	CA	—	—
South America	Argentina	E	E	E	—	—	[41, 78]	Immunoblot [77]
	Brazil	E	E	E	[73]	—	[75, 76]	Western Blot [72]
	Colombia	E	E	E	—	[26]	[35]	—
	Ecuador	L to E	U	L to E	—	—	—	Fecal Examination [70]
	French Guiana	E	—	L to E	—	[26]	[34, 39]	—
	Suriname	E	—	—	—	[26]	—	—
	Venezuela	E	E	U	U	—	—	—
Africa	Cameroon	—	—	—	—	—	—	—
	Chad	—	—	—	—	—	—	—
	Kenya	—	—	—	—	—	—	—
Asia	China	L to E	—	—	—	—	[88••]	—
	India	L to E	—	—	—	—	—	—
	Thailand	L to E	L to E	—	—	—	–	—

*Puerto Rico is a U.S. Protectorate

Abbreviations: L = limited availability; E = extensive availability; CA = commercially available; U = unknown

Cryptococcus neoformans, *Talaromyces marneffei* (formerly *Penicillium marneffei)*, *Toxoplasma gondii*, and *Leishmania donovani* [8•, 9, 10•].

Cultures. Definitive diagnosis is still based on the isolation and identification of *H. capsulatum* from clinical and biological specimens. Culture of clinical specimens has long been considered the gold standard assay to detect *H. capsulatum*. In patients suspected to have PDH, bone marrow, blood, and biopsied tissue may be collected and incubated at 37ºC in nutrient-rich media such as blood agar or brain-heart infusion (BHI) agar with cysteine [8•]. *H. capsulatum* grows slowly, and it may take up to 12 weeks before a positive microscopic identification can be made [8•] through detection of typical budding yeast. After transfer to 25 °C incubation on routine fungal media, cream to brown cottony colonies composed of hyaline septate hyphae with macro- and/or microconidia will develop. Conversion from mold to yeast phase using rich media is used for confirmation of *H. capsulatum* in some laboratories. Alternatively, culture isolates may be identified using the AccuProbe® (Gen-Probe®, San Diego, CA) DNA hybridization system or DNA-based detection methods. The AccuProbe® system may be very costly for routine use outside of the United States [8•].

Immunodiagnostic Tests. Immune-based methods for antibody and antigen detection are useful not only for diagnosis but also for monitoring the patient's course. The two traditional methods for antibody detection are complement fixation (CF) and immunodiffusion (ID) [8•, 11, 12]. These tests are generally performed in larger reference laboratories due to their complexity, particularly with the CF test that requires continuous sources of fresh biological reagents. The ID test qualitatively measures precipitating antibodies to the H and M glycoprotein antigens (H and M precipitin lines or bands), and the presence of these bands is highly suggestive of active *Histoplasma* infection. Sensitivity of ID varies between 70 % and 95 %, and specificity is 100 %. The CF is a quantitative test measuring antibodies to yeast and histoplasmin (mycelial) antigens. CF sensitivity is reported between 72 % and 95 %, depending upon the antigen used, but cross-reactions in patients with other fungal infections are more common [8•, 12, 13], so that the specificity of CF is 70 %–80 %; lower than that of ID [8•, 9]. There are several commercial sources for these reagents (reviewed in [8•, 12]), which include mycelial-phase culture filtrates containing *H. capsulatum* H and M antigens and positive-control sera containing antibodies against these antigens [8•, 12].

Enzyme immunoassays (EIAs) are also used in reference laboratories for detection of antibodies to *H. capsulatum*, although most of these are laboratory developed, or "in-house" tests with varying degrees of efficacy. The Western blot protocol has identified four different *Histoplasma* antigens of 91, 83, 70, and 38 kDa that react with sera from patients with histoplasmosis [14]. Using purified and deglycosylated histoplasmin, sensitivity of 100 % was reported in acute disease, 90 % in chronic disease, 89 % in disseminated infection in individuals without HIV infection, and 86 % in disseminated disease in the setting of HIV infection [15]. An EIA for detection of IgG, A, and M antibodies is commercially available (Focus Diagnostics, Cypress, CA), as well as a latex agglutination test (Immuno-Mycologics, Inc., Norman, OK) for detection of IgM antibody. While useful as ancillary diagnostic tools, serological tests may not discriminate active disease from exposure. Furthermore, humoral responses to *H. capsulatum* infection take between two and six weeks to develop [2], and may be entirely absent in persons with AIDS.

During infection with *H. capsulatum*, antigen can be released by the fungal cells and detected in body fluids such as serum (blood), pleural fluid, bronchoalveolar lavage fluid, cerebrospinal fluid, and urine. Antigen detection is particularly useful for diagnosis of PDH in persons living with AIDS who often lack sufficient immune response to the fungus [9, 12, 16]. The current method detects *H. capsulatum* cell wall polysaccharides in patient serum, urine, and CSF using a sandwich EIA format [17]. The first of these assays, developed and evaluated by Durkin et al. [18], is available at the U.S. MiraVista Diagnostics (Indianapolis, IN) laboratory. While this test shows high sensitivity in detecting antigenuria in PDH patients [19•], the assay cross-reacts with other dimorphic fungal pathogens, and reported specificity is low, between 10 % and 31 % [19•, 20, 21]. A comparison of this test with another commercially available EIA antigen test reported discrepant results [22]. Similar EIA assays to detect *Histoplasma capsulatum* antigenuria in immunocompromised patients have been described [23••, 24–26], but are not commercially available. As existing immunological assays generally use crude or uncharacterized antigens and polyclonal antibodies, one challenge will be to improve upon these methods by using purified and/or recombinant antigens and monoclonal antibodies while retaining high sensitivity and specificity.

Nucleic Acid Detection in Clinical Materials. There are no commercially available systems for detection of *H. capsulatum* DNA in human clinical samples. The number of in-house molecular tests developed and evaluated for the detection of *H. capsulatum* is growing, and the first steps toward multicenter evaluation are underway [27, 28••].

Molecular assays to detect *H. capsulatum* in human specimens can be pan-fungal [29–31] or organism-specific [32–36]. Pan-fungal assays utilize the non-translated ribosomal internal transcribed spacer (ITS) gene regions [37] to discriminate between fungal species. For nearly two decades, ITS targets have been used to detect and identify fungal yeasts in culture and specimens [8•, 9, 27], and these loci were recently recommended as the universal DNA barcode marker for fungi [38••]. Molecular

ITS pan-fungal assays have been utilized to detect *H. capsulatum* DNA in formalin-fixed paraffin-embedded (FFPE) tissues [31] in various assays, including a TaqMan® quantitative PCR (*q*PCR) (Applied Biosystems, Carlsbad, CA) [39, 40] and a multiplex *q*PCR that concurrently detects closely related pathogenic yeasts [36]. Sensitivities of these *q*PCR assays in detecting *H. capsulatum* DNA in human specimens were 70 % [40], 86 % [36] and 95 % [39], and specificities ranged between 96 % [39] and 100 % [40].

Genetic loci of both the M and H antigens of *H. capsulatum* have been used as targets in assays to confirm the presence of the organism. The M antigen PCR was 100 % sensitive and specific in amplifying *H. capsulatum* DNA extracted from clinical isolates [33], and the H antigen PCR was 100 % sensitive and 95 % specific in detecting *H. capsulatum* DNA in clinical specimens from patients with proven infections [41].

The most frequently utilized molecular target for specific detection of *H. capsulatum* is a genetic locus of a unique 100 kDa protein, *Hcp100* [8•, 9, 27]. Bialek et al. originally reported the utility of this target in detecting *H. capsulatum* DNA in FFPE tissues using nested PCR [42]. Other authors have described the use of nested PCR targeting *Hcp100* in a variety of human specimens [47, 48], reporting sensitivities of 100 % and specificity values between 94 % and 100 %. A *q*PCR developed to target *Hcp100* reported 89 % sensitivity and 100 % specificity in detecting *H. capsulatum* in DNA extracts of FFPE tissue [43]. Lastly, a non-PCR-based loop-mediated isothermal amplification (LAMP) molecular assay recently utilized to detect *Hcp100* in clinical isolates and DNA extracted from urine of patients with proven histoplasmosis [44•] reported 100 % sensitivity and specificity in detecting DNA extracted from isolates and 67 % sensitivity in detecting *Histoplasma* DNA in urine [44•]. While this method shows great promise as an inexpensive molecular tool to detect histoplasmosis, further evaluation with clinical specimens is necessary.

In order to address the wide disparity in sensitivity and specificity among the in-house molecular assays as mentioned above, seven protocols (conventional, nested and *q*PCR) targeting different *H. capsulatum* DNA regions were compared in a multicenter inter-laboratory study [28••]. The protocols tested in the study were highly reproducible, with an average sensitivity of 86 % and specificity of 100 % reported. The authors concluded that *q*PCR appeared to be a promising tool for efficient detection of *H. capsulatum* in clinical samples [28••]. While there has been a sizable amount of effort and resources invested in developing molecular techniques, a consensus on standardization, along with validation from large prospective studies, is necessary to enable widespread adoption of these assays.

Endemic regions and Resources

The Americas

North America

Despite the fact that *H. capsulatum* is highly endemic in areas of the central U.S. and Canada surrounding the Mississippi and Ohio Rivers, public health reporting of these infections is required in only 13 of 50 U.S. states. Laboratory infrastructure and capacity in most of North America is well-supported, yet individual states and large metropolitan areas vary with regard to available budget and expertise. Areas where PDH is diagnosed late overlap with those where there is a greater prevalence of late-stage diagnosis of AIDS, and patients in these regions are of low socioeconomic status [45]. Antigen detection EIA is commonly utilized to diagnose PDH [8•, 19•, 46], and confirmation is achieved through identification of yeasts via culture or examination of extrapulmonary tissue [47]. Several large reference centers perform CF and ID serological tests as well as molecular identification [8•, 31, 48, 49]. No DNA-based tests to detect *H. capsulatum* from human specimens have been approved by the U.S. Food and Drug Administration (FDA) for diagnostic use.

In Mexico, histoplasmosis is commonly acquired in bat-infested locations, and outbreaks occur among persons exposed to guano [50, 51]. The few published studies of PDH in Mexico indicate that clinical diagnosis is supported with conventional laboratory tests, including a capillary tube precipitation test (CTP) performed at the National Institute of Diagnosis and Epidemiological Reference (InDRE), the nation's public health agency [50–52].

Central America

The countries of Central America are highly endemic for *H. capsulatum*, and early histoplasmin skin test sensitivity studies have estimated exposure rates of roughly 50 % in Panama and between 23 %–81 % in Guatemala [53, 54]. Rates of HIV prevalence in Central America are high, with estimates ranging from 0.2 % of the population in Nicaragua at the lower end to 1.5 % in Guatemala at the upper end [7]. In one study, PDH was diagnosed in nearly 8 % of hospitalized patients in Panama between 1997 and 2003 [55], and clinical diagnoses were confirmed by specimen cultures, histopathology, and biopsy. Urine and sera from these patients were later tested using the MiraVista EIA assay, resulting in 95 % sensitivity for antigen detection in both specimen types [18, 55]. Although these data showed promising efficacy of an antigen test in Central America, the MiraVista assay is only available in the U.S.

In Guatemala, laboratories were reliant on conventional diagnostic methods for detection of PDH until 2010, when

the Asociación de Salud Integral (ASI) began to use a CDC-developed rapid antigen detection ELISA to expedite diagnosis of PDH [26]. Implementation of urine antigen detection resulted in earlier detection and treatment of PDH, and early treatment was associated with reduced patient mortality six months after diagnosis [56]. EIA antigen detection technology is being transferred to the national public health laboratories and regional public hospitals in Honduras and El Salvador.

Caribbean

Reports of histoplasmosis are sporadic throughout the Caribbean islands, and many are travel-related or epidemiologically linked to bat guano exposure in caves. Many Caribbean nations are now considered endemic for histoplasmosis, and rates of persons living with AIDS are high (0.6 %–3.5 %) in these countries, with the exception of Cuba (≤ 0.1 %) [7]. Cuba has reported high endemicity of *H. capsulatum,* and PDH is estimated to affect 4.2 % of AIDS patients [57]. Cuba maintains a robust diagnostic program that includes an antibody ELISA but lacks antigen detection and molecular methods, relying primarily on cytological examinations to detect PDH, as serology has proven insensitive [57, 58].

In all other Caribbean nations, diagnostic and epidemiologic data for PDH are scarce [59]. A single case report of PDH from Jamaica describes the incidental identification of *H. capsulatum* fungemia in one patient using a yeast identification kit as a supplement to blood culture techniques [60]. In Puerto Rico, histopathology and culture were used to diagnose PDH in a patient who tested negative for antigen (MiraVista) and positive via quantitative PCR (performed in the U.S.) [61].

South America

Cases of PDH are well-documented in French Guiana, and are the leading cause of AIDS-defining illness, at 15 cases per 100 patient-years in persons with CD4 cell counts below 100 cells/μl [62••]. The efficacy of various diagnostic techniques has been evaluated, and culture of biopsied material has been determined more sensitive than direct microscopic examination [63]. Both nested PCR [34] and a sensitive TaqMan® *q*PCR [39, 62••] have been developed to supplement clinical and laboratory diagnoses. Antigen detection technology was recently transferred to Cayenne and Suriname in a project led by the French National Institute of Health and Medical Research–French National Agency for Research on AIDS and Viral Hepatitis (INSERM–-ANRS) to improve awareness of PDH in the Guiana Shield and surrounding region and to increase diagnostic capacity [64].

In Venezuela, PDH is the most frequently diagnosed endemic mycosis in persons living with AIDS. Published reports indicate that ID serologic testing is used to supplement biopsy cultures and histopathology [65, 66]. Colombia has a proactive program for diagnosis of PDH and has recently published a national survey of PDH cases in the country [67]. One laboratory uses antigen detection in serum [25] and nested PCR [35] as supplements to diagnose histoplasmosis, and urine antigen detection has been evaluated (manuscript submitted). The value of CF and ID serology as a supplement to culture methods in the diagnosis of PDH has also been described [68].

In Ecuador, clinical cases of PDH in persons with AIDS have been described as having novel clinical features and histological patterns [69]. An earlier study in Ecuador described the diagnostic utility of examination of feces for *H. capsulatum* to diagnose PDH in children [70]. The authors found that due to its rapidity, fecal matter examination was superior to blood culture, and that ID serology was insensitive in these patients [70].

H. capsulatum is widespread in Brazil, with documented hyperendemicity in the extreme northern and southern states [71•]. The clinical presentations and causative agent of histoplasmosis have been well-characterized by mycologists at major Brazilian universities. Several diagnostic methods have been explored, including Western blot [72], antibody ELISA [73], and molecular amplification of diagnostic antigens [33, 74]. Recent literature describes the development of PCR assays utilizing *H. capsulatum* DNA-spiked blood samples [75], and serum and whole blood specimens from PDH patients [76]. Northern Argentina is hyperendemic for *H. capsulatum*; like Brazil, it has a number of tools for PDH detection. Although perhaps not routine in all laboratories, these include the use of serology, immunoblotting techniques [77], and PCR [41, 78]. Research using molecular assays to expedite diagnosis is ongoing [79].

Africa

H. capsulatum is found throughout Africa, and the former *H. capsulatum* var. *duboisii* is found in the Western and Central Sub-Saharan regions of the continent. The epidemiology of PDH and other forms of histoplasmosis are not well-understood, as the preponderance of cases in the literature refer to African expatriates living in Europe. PDH is largely underdiagnosed in Africa, and reports indicate that it is rarely suspected in patients with wasting and fever [80, 81]. Culture is not readily available, and diagnosis has been accomplished traditionally by microscopic examination of tissues and, more recently, peripheral blood smear [80, 82].

Asia

H. capsulatum is endemic in the Southeastern countries of the Asian continent, and while reports abound of acute histoplasmosis from travelers visiting caves and other remote locations,

the majority of PDH cases originate from Thailand and India. Two studies in Thailand indicate that PDH may actually be acquired more frequently than infections caused by *Talaromyces* (formerly *Penicillium*) *marneffei*, which is hyperendemic in Southeast Asia [83, 84]. Although *H. capsulatum* grows more slowly than *T. marneffei*, and thus appropriate fungal diagnosis may be missed [83, 85], antifungal treatment regimens for both infections are identical. Likewise, in India, many cases of PDH may go unrecognized, as indicated in two recent studies that described the infrequency of bone marrow examination and culture [86, 87].

In China, which has long been considered a non-endemic or low-endemic country, histoplasmosis may be an emerging disease. Between 1990 and 2011, 300 cases of histoplasmosis (257 of which were PDH) were reported in China, and 75 % occurred near the Yangtze River [88••]. *H. capsulatum* was identified by histopathology in the majority of patients (242), while the organism was isolated in culture in only 68 patients, and six of these were confirmed using PCR. The increase in autochthonous *H. capsulatum* infections described here may be artifactual, however, as laboratory diagnostic capacity has increased in the region and higher morbidities are apparent in persons with AIDS [100].

Conclusions

Early and rapid diagnosis of histoplasmosis is essential to preventing morbidity and mortality in immunocompromised persons. Antigen assays that detect histoplasmosis using readily accessible specimens such as urine are available in developed countries, but are unavailable to most areas of the world where *H. capsulatum* is endemic. Regions with the highest burden of PDH may have the least capacity to detect the disease, and may rely on histopathology alone. Furthermore, the majority of PDH cases described in the literature originate from countries with strong laboratory infrastructures (and sometimes low burden of disease), while countries known for high *H. capsulatum* endemicity combined with high HIV burden lack substantive reports of PDH.

Since public health reporting of histoplasmosis cases is not mandatory in most developed countries, the true burden of this disease is not known with certainty, and there has been little incentive for commercial development of rapid assays to detect the disease. Lack of awareness of this disease acts as a further impediment to the development of diagnostics for histoplasmosis. Symptoms of PDH are nonspecific, and differential diagnosis includes tuberculosis and several other diseases that require very different treatments. Epidemiological data collected using histoplasmin skin tests indicate that exposure to *H. capsulatum* is prevalent in many endemic regions where HIV is epidemic, but few cases of PDH are reported. Undiagnosed or misdiagnosed cases of PDH are likely occurring in these regions.

Rapid and reliable detection assays for *H. capsulatum* are desperately needed in endemic areas, as articulated by the authors of many of the studies cited in this paper. Even in laboratories where PCR is available, it is not used routinely, as maintenance and operation of molecular testing is an expensive proposition for any laboratory. A commercial urine antigen detection kit (IMMY) recently approved by the U.S. FDA may facilitate improved diagnosis through the availability of antigen detection at a reasonable cost, but its utility has not yet been determined in clinical settings outside of the U.S.

Progressive disseminated disease caused by *H. capsulatum* is under-recognized and underreported. The high mortality rate and financial burden associated with PDH primarily affect underserved, limited-resource populations of the world. Increased availability and use of rapid, inexpensive diagnostic assays will save lives, and could be used for surveillance of PDH to better understand the burden and impact of this disease on underserved populations.

Notes

The findings and conclusions in this report are those of the authors, and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Compliance with Ethics Guidelines

Conflict of Interest Christina M. Scheel and Beatriz L. Gómez declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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