



Review Article

Disseminated Histoplasmosis as AIDS-presentation. Case Report and Comprehensive Review of Current Literature

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Abstract. Progressive disseminated histoplasmosis (PDH) is an AIDS-defining illness with a high lethality rate if not promptly treated. The wide range of its possible clinical manifestations represents the main barrier to diagnosis in non-endemic countries. Here we present a case of PDH with haemophagocytic syndrome in a newly diagnosed HIV patient and a comprehensive review of disseminated histoplasmosis focused on epidemiology, clinical features, diagnostic tools and treatment options in HIV-infected patients.

Keywords: Disseminated histoplasmosis, HIV, AIDS, *Histoplasma capsulatum*.

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Introduction. Histoplasmosis is a mycosis resulting from the inhalation of the spores of the dimorphic fungus *Histoplasma spp*, which is a member of the family *Ajellomycetaceae*, a fungal group whose members may all cause systemic disease in otherwise healthy humans (*Blastomyces*, *Coccidioides*, *Paracoccidioides*).¹ Although only occasionally reported in the pre-HIV-infection era, it became a public health issue after the AIDS pandemic, being listed by Center for Diseases Control and Prevention (CDC) among the AIDS-defining illnesses in 1987.²

In the immunocompetent host, exposure to *Histoplasma* microconidia is usually responsible for a symptomless presentation or flu-like syndrome, as the spores are contained by alveolar macrophages and subsequently cleared by the activation of the adaptive immunity, especially the Th1 response. In the immunocompromised host, due to Th1 to Th2 shift, the pathogen can invade the bloodstream and spread to other organs and tissues, leading to progressive disseminated histoplasmosis (PDH), a fatal disease in untreated patients.^{3,4}

The PDH incidence in HIV patients peaked in 1992, and then gradually declined following the introduction of combined antiretroviral therapy (cART). It is currently a health issue especially in countries where cART is not widely available. In Europe, it is only sporadically reported,⁵ mainly in migrants from endemic areas.⁶

In the last years, in Italy, we observed an increase in the proportion of AIDS cases in migrants: they account now for more than 1/3 of the new HIV-infection diagnosis and, among them, the 68% comes from Africa and Latin America.⁷

We believe that it is important to raise the awareness of histoplasmosis also at our latitude, given the recent increase of the migratory fluxes from endemic countries and the high risk of fatal outcome associated with late diagnosis and treatment.

Here we will present a case of a newly diagnosed AIDS patient, previously suspected to have lymphoma with haemophagocytic syndrome. Both microbiological and histological examinations revealed disseminated histoplasmosis. Furthermore, we provide a comprehensive review of the current literature on histoplasmosis in HIV-infected patients focusing on epidemiological, clinical, diagnostic features and treatment options.

Case-Report. In February 2016, a 19-year-old woman coming from Ivory Coast was admitted to a peripheral hospital of our city for persistent fever. After she resulted positive for a screening HIV-Ab test, she was referred to the Infectious and Tropical Diseases Department. At admission she had deep asthenia, chills, fever (max 38.8°C), and tachycardia (110 bpm); examination revealed mono-lateral tonsillar hypertrophy and submandibular lymphadenopathy. Laboratory investigations showed pancytopenia (haemoglobin 8.7 g/dL - normal value 12.0-16.0 g/dL, white blood cells 3,050/ μ L -n.v. 4.00-10.80 $\times 10^3$ / μ L, platelets 80,000/ μ L - n.v. 130-400 $\times 10^3$ / μ L), increased lactate dehydrogenase (5,201 U/L - n.v. 136-234 U/L), increased levels of C Reactive Protein (63.3 mg/L - n.v. < 5.0 mg/L), and high ferritin and triglycerides serum levels (> 40,000 ng/mL and 440 mg/dL - n.v. 20-120 ng/mL and <150 mg/dL respectively). Baseline lymphocytes CD4+ T-cell count was 19 cell/ μ L and HIV-RNA was 1,787,000 cp/mL. In addition, the patient

resulted positive for EBV-DNA (1,297 cp/mL), CMV-DNA (266 cp/mL), and HBV-DNA (> 700,000,000 UI/mL), and for blood stool test. Suspecting a lymphoproliferative disorder evolving in haemophagocytic syndrome, a bone marrow biopsy was performed and steroid treatment was started. Consequently, we performed biopsies of the gut, lateral-cervical lymph node, and tonsil. Microscopical evaluation of hematoxylin and eosin (H&E) sections of the bone marrow showed a prominent lymphohistiocytic infiltrate with budding yeast cells inside macrophages, suggestive of histoplasmosis, which was confirmed by Grocott Methenamine Silver stain (GMS) and periodic acid-Schiff (PAS) stain. Antifungal treatment with liposomal Amphotericin B was started while steroid therapy was gradually reduced. She rapidly became afebrile and, in the following weeks, we observed a slow regression of symptoms. Histological examination of submandibular lymph node, tonsil and colic mucosa also confirmed the diagnosis (**Figures 1, 2 and 3**). *Histoplasma spp* serology was negative, while culture of bone marrow aspirate showed *H. capsulatum* growth. After three weeks of induction therapy, she continued on maintenance therapy with itraconazole, and cART with tenofovir/emtricitabine + dolutegravir was prescribed. The patient was referred to the Outpatient Clinic. She attended at the first follow-up visit ten days after the hospital discharge with improved clinical conditions and blood exams. However in April 2016 she returned to Ivory Coast and was re-linked to care in October 2016. At the

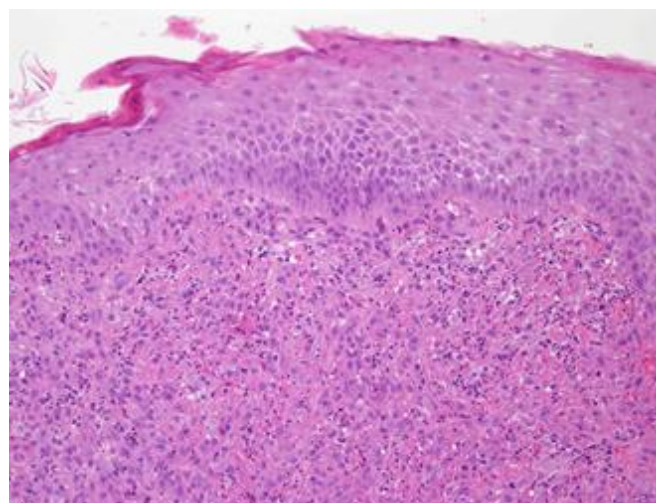


Figure 1. Representative section of the tonsil showing a prominent sub-epithelial histiocytic infiltrate (hematoxylin and eosin staining, 100X).

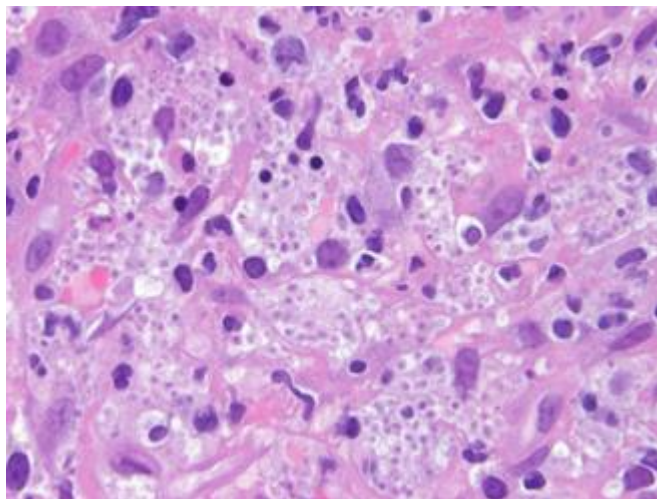


Figure 2. Tonsil. Cytomorphological details, showing foamy histiocytes containing intracytoplasmic yeasts (hematoxylin and eosin staining, 600X).

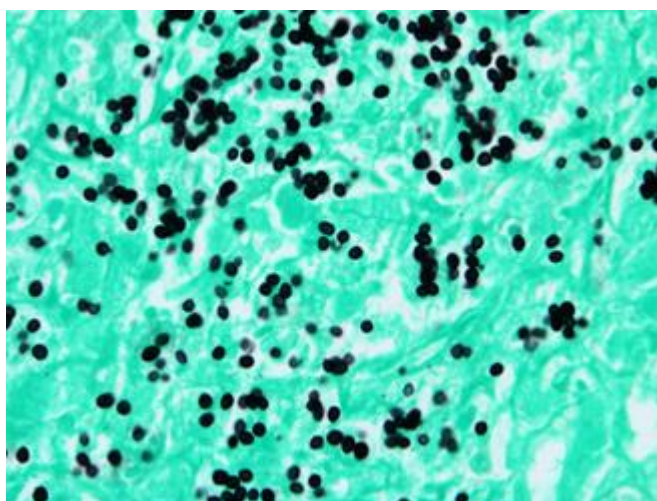


Figure 3. Tonsil. Grocott's methenamine silver (GMS) stain highlighting intracytoplasmic fungal elements. (GMS staining, 600X).

time of the visit lymphocytes CD4+ T count was 29 cell/ μ L, HIV-RNA was 153,300 cp/mL and HBV DNA was 212,600,000 UI/mL. During her stay in Ivory Coast, she interrupted both antiretroviral therapy and histoplasmosis maintenance therapy; moreover, at the moment of the new visit she was pregnant and after proper counseling, she decided to carry on the pregnancy. Only antiretroviral therapy with tenofovir/emtricitabine + atazanavir/ritonavir was re-started and a close follow-up was scheduled. After three weeks she was admitted to the Intensive Care Unit (ICU) for internal abortion, complicated by septic shock. She was treated with wide spectrum antibiotic therapy (meropenem and vancomycin) without any microbiological isolation and she went through several transfusions and mechanical ventilation. Once stabilized, she

was transferred to our Department, she was febrile, pancytopenic and with a skin lesions on her chest, suggestive of histoplasmosis reactivation so that antifungal treatment with liposomal Amphotericin B was reintroduced. Bone marrow biopsy confirmed the suspect. The same day antiretroviral therapy with tenofovir/emtricitabine + dolutegravir was reintroduced. In the following weeks, a slow progressive improvement of the conditions was observed, therefore she was transferred to a facility with proper social support and health assistance. At the time of the last visit in December 2017, the patient was asymptomatic and fully adherent with cART and histoplasmosis prophylaxis. Last CD4+ T-cell count was 150 cell/ μ L and a low-level viral load was detected for HIV and HBV (33 copies/mL and 95 UI/mL respectively).

Epidemiology. *Histoplasma capsulatum* is recognized as a pathogen with worldwide distribution, with many cases registered outside the historically known endemic areas of the Americas.^{8,9} Many countries of the American continent are considered highly endemic as revealed by outbreak reports and skin reactivity studies. In the United States, histoplasmin sensitivity ranges from 60% to 90% in Ohio and in the Mississippi river valley, while in Latin America the prevalence of *H. capsulatum* infection is reported as 50% in some Panama's areas, reaching 93% in specific places of Brazil as in Ilha do Governador, Rio de Janeiro.¹⁰ *H. capsulatum* is also endemic in Asia, where autochthonous cases have been reported since the seventies.¹¹ *H. capsulatum* var. *duboisii* has been isolated in Central and West areas of Africa and in Madagascar.¹² Sporadic cases of histoplasmosis have been reported in Europe, mainly in immigrants or travelers returning from endemic areas. Reports of autochthonous cases in Europe suggest the possible endemic presence in some European areas, such as the south of France and the Po river valley in Italy.^{13,14} Since the HIV/AIDS pandemic spread out, many cases of histoplasmosis have been reported in HIV-infected patients in locations with few previously reported cases. In endemic areas, such as Latin America, PDH is one of the most common causes of AIDS-related deaths, however, it remains underestimated due to misdiagnosis and lack of first choice

Key points:

- *Histoplasma spp.* is endemic in both North and South America and in Asia. *Histoplasma capsulatum var. duboisii* is mainly reported in central and South Africa and in Madagascar.
- Even if its incidence is under-reported, histoplasmosis represents one of the most common causes of AIDS-related deaths by a fungal infection.
- Histoplasmosis is only occasionally reported in Europe, especially in migrants or travelers from endemic areas; only a few autochthonous cases have been reported.

antifungal therapy.¹⁵ Fungal infections were estimated to be responsible of 47% of over 1,500,000 AIDS-related death in 2013, and, among them, *Histoplasma* was the third responsible pathogen, after *Pneumocystis jiroveci* and *Cryptococcus neoformans*.¹⁶ Some authors recently reported an increase in the diagnosis of histoplasmosis in AIDS-patients in Latin America.¹⁷ In some areas, the situation appears dramatic: histoplasmosis is one of the first causes of death in HIV-infected patients in French Guiana, with a lethality of more than 50%; in Fortaleza, Brazil, almost a half (43%) of hospitalized HIV-infected patients had PDH.⁶ In other known endemic areas, such as Africa, there is a lack of data about the incidence of PDH. A recent study estimated that targeting opportunistic fungal infections through diagnostic and therapeutic tools access could be a crucial factor in reducing AIDS-related deaths, saving more than a million lives over five years.¹⁶

In non-endemic areas as in Europe, most PDH cases are reported in HIV-infected migrants. From 1984 to 2004, 72 patients with HIV-associated histoplasmosis were reported in Europe, mostly observed in Italy; among them, 7 cases were autochthonous.¹⁸

Pathogenesis. *H. capsulatum* is a dimorphic fungus that displays different morphologies depending on environmental conditions: the mold in the soil and the budding yeast in the mammalian host. *H. capsulatum* infection is usually acquired through inhalation of microconidia aerosolized from environmental sites hosting the fungus. The saprophytic form seems to grow best in soils with a high nitrogen content associated with the presence of birds and bats guano. Some activities, such as demolition and construction works, archaeological and speleological works or poultry farming, have been associated with an increased risk of infection because they can lead to microconidia exposure. Infection with the yeast form via tissue transplant, laboratory accident or needle sharing has also been described.

After inhalation, the fungus reaches the alveolar space where it finds favorable temperature; there it turns into the pathogenic yeast form and begins his intracellular life in alveolar macrophages.¹⁹

In the absence of immune-compromising conditions, acute infection resolves with the development of cell-mediated immunity. An antigen-specific CD4+ T lymphocyte-mediated response leads to the formation of granulomas; this immune activation can contain the fungus and protect against reinfection, but it is not able to eradicate the pathogen. The development of specific cell-mediated immune response results in a delayed-type hypersensitivity reaction that can be induced by intradermal injection of fungal antigens (histoplasmin skin test).²⁰ In healthy individuals, the primary infection is usually asymptomatic or mild-symptomatic, resulting in a self-limiting and non-specific febrile syndrome with respiratory involvement. *H. capsulatum* establishes a long-lasting quiescent infection that can reactivate in case of immune system weakening, such as in advanced HIV infection, chemotherapy, immunosuppressive therapy for transplants or autoimmune diseases. Host-pathogen balance plays a crucial role in infection course; when cell-mediated immunity is compromised the fungus moves from the primary site to the whole body through the blood and lymph stream or within cells as macrophages but also dendritic cells and neutrophils, leading to disseminated disease.²¹ The main affected organs are liver, spleen, gastrointestinal tract, and bone marrow. In addition to the immune status of the host, other factors potentially involved in the evolution of *Histoplasma* infection are the number of organisms inhaled and the strains virulence.²²

Clinical Features. Histoplasmosis has a very heterogeneous clinical presentation, ranging from mild and self-limiting respiratory syndromes to disseminated forms with high lethality rate, depending on immune system conditions.¹⁹ In most cases, the infection is completely asymptomatic or may be associated with a non-

Key points:

- *H. capsulatum* infection is acquired through the inhalation of the fungus microconidia aerosolized from soils contaminated by birds' or bats' guano.
- After inhalation, the pathogenic yeast form infects alveolar macrophages; in the immunocompetent host, the infection is contained by the development of cell-mediated immunity, with the formation of granulomas. In immunocompromised hosts, the fungus can spread through the blood or lymph stream leading to disseminated histoplasmosis.

specific syndrome characterized by fever, chills, cough and chest pain. Rheumatologic manifestations including arthritis, arthralgia, and *erythema nodosum* have also been described in a small percentage of cases.^{23,24} In endemic areas, peculiar forms of histoplasmosis have been described, such as ocular histoplasmosis with chorioretinal involvement and “histoplasmoma,” a slow enlarging lung nodule.^{25,26}

In immunocompromised host, a deficit in cellular immunity can lead to fungus dissemination, massive organ involvement and severe systemic disease (PDH). PDH is usually diagnosed in the late stage of HIV infection; however, some rare cases have been reported in other conditions, such as hematologic patients^{27,28} and even in immune-competent individuals living in developing countries.²⁹ Mortality rate reaches 39% in endemic areas, such as Latin America¹⁰ but is even higher in non-endemic areas like Europe, where the disease is often misdiagnosed.⁸ PDH usually presents with persistent fever, deep asthenia, and weight loss; diarrhea and other digestive symptoms are often described since gastrointestinal tract is a frequent site of fungus dissemination. Reticuloendothelial system is the main organ affected in PDH with severity ranging from isolated lymphadenopathy, hepato- and spleno-megaly to hemophagocytic lymphohistiocytosis (HLH). HLH is a life-threatening disease in which a massive immune stimulation results in macrophages activation and hemophagocytosis; it is a rapidly progressive syndrome with non-specific symptoms so that it can mimic many different etiologies: malaria (if patient has a recent history of travel in endemic areas),³⁰ sepsis, hepatic failure, hematologic disorders and malignancies among others.³¹ Fever, splenomegaly, deep pancytopenia, altered liver function with hypertriglyceridemia, hypofibrinogenemia and increased ferritin level are the main clinical diagnostic criteria.³² According to a recent review, 27 cases of HLH secondary to PDH were reported so far, with a mortality rate of 38%.³³ Up to 10% of patients with PDH may develop central nervous system

(CNS) involvement as a primary manifestation or relapsing disease.³⁴ Neurologic dissemination of the pathogen may occur as subacute or chronic meningitis, focal neurologic deficit and encephalitis.^{35,36} Skin involvement in PDH has been reported in up to 25% of AIDS patients.³⁷ Dermatologic findings are not specific: papules, plaques, and nodules are commonly described, usually affecting trunk and face. Mucosal involvement is often reported, especially as painful and infiltrated ulcers in oral mucosa.³⁸ Other less common manifestations of PDH include endocrine syndromes, such as chronic adrenal insufficiency,³⁹ and myositis, of which only 4 cases were reported so far.⁴⁰ In AIDS patients from endemic areas starting cART, a few cases of immune reconstitution inflammatory syndrome (IRIS) revealing PDH have been described: fever, weight loss, and lymphadenitis were the main symptoms in these reports.⁴¹

The recognition of PDH in AIDS may be challenging since most patients have other concomitant opportunistic infections, especially those with CD4+ counts <150 cells/mL: pneumocystosis, cryptococcosis, and mycobacteriosis are the main reported co-infections.⁴²

Diagnostic Tools. There are multiple methods for the diagnosis of histoplasmosis including histopathology or cytology, direct examination using specific fungal stains, cultures, Matrix-Assisted Laser Desorption/Ionization – Time Of Flight (MALDI-TOF), antigen detection, immunization determination, serological tests and molecular biology-based tests. Only hospitals with a dedicated mycology sector in their laboratory allow the most rapid and accurate diagnosis. It is worth noting that managing cultures of *Histoplasma spp.*, as for other dimorphic fungi, requires a biosafety level 3 or 4 with class II or III biological safety cabinets, and laboratory personnel involved with *Histoplasma* cultures should use appropriate procedures to prevent exposure.

Each of the available tests is designed and

Key points:

- In the immunocompetent host histoplasmosis is generally asymptomatic and self-limiting, otherwise, it may be responsible for non-specific symptoms as fever, cough, and chest pain.
- Immunocompromised individuals may develop disseminated histoplasmosis with multiple organ involvements. Clinical presentation is heterogeneous: high fever, asthenia along with gastrointestinal, neurological, and skin manifestation may be present; in untreated patients, mortality reaches the 39%.
- Disseminated histoplasmosis can lead to HLH, a life-threatening and rapidly evolving disease in which a massive immune stimulation results in macrophages activation and hemophagocytosis.
- IRIS after ART initiation has been described in AIDS patients from endemic areas

performed for specific histoplasmosis syndromes depending on the patient condition or level of immunosuppression, but all can be used to complete the diagnostic path. The diagnostic methods not only differ in sensibility and specificity but also in time-to-response, total cost, quality of the information, and have certain limitations that must be recognized if they are to be used correctly.

Histopathology. The diagnosis of histoplasmosis can be obtained through examination of histological or cytological specimens.⁴³ Histological specimens from tissue biopsies of different anatomical sites stained with H&E and special stains such as PAS and GMS can be used. Cytological specimens of bone marrow aspirates stained with Giemsa,⁴⁴ fluids⁴⁵ (ex. bronchoalveolar lavage) or tissues⁴⁶ (lung, lymph nodes, spleen, cutaneous lesions, etc.) can also be utilized.

There are two different forms of *H. capsulatum* causing human histoplasmosis; *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii* but the two are difficult to distinguish.

In H&E stains *H. capsulatum* is characterized by the presence of a clear space or artefactual halo, due to the retraction of basophilic cell cytoplasm from the cell wall. Budding yeasts, usually difficult to identify, are connected to a narrow base, a feature that helps the distinction of *H. capsulatum* from other fungi.^{47,48}

In specimen, it can be highlighted with different staining methods: Romanowsky-type stains, Giemsa, Wright-Giemsa stains, Grocott-Gömöri methenamine–silver (GMS), mucicarmine, periodic acid–Schiff (PAS) stains and Gram stains. GMS, PAS and mucicarmine are the most commonly used. GMS and PAS provide contrast to yeast cells showing black-colored and magenta-colored intracellular or extracellular yeasts respectively; at mucicarmine stain, the yeast forms are barely visible.

H. Capsulatum must be distinguished, in particular, from other fungi such as a small variant of *Blastomyces dermatitidis*, capsule-deficient Cryptococci, endospores of *Coccidioides spp.*, *Pneumocystis jirovecii*, and *Candida glabrata*.^{49,50}

H. capsulatum should also be distinguished from protozoa, like *Leishmania spp* (amastigotes), *Toxoplasma gondii* (bradyzoites) and *Trypanosoma cruzi* (amastigotes).

The patient histological reaction to *H. capsulatum* infection varies according to the severity and phase of the infection and the host's immune system. In the acute phase, subsequent to pulmonary infection, *H. capsulatum* may be seen within alveolar space and in the interstitium, inside macrophages. Usually, there is an associated lymphohistiocytic infiltrate with necrosis and vasculitis. The histopathologic picture resembles lymphomatoid granulomatosis, but scattered small granulomas with small yeasts in the parenchyma should suggest the diagnosis of histoplasmosis.⁵¹

In chronic pulmonary histoplasmosis, the most common host's reaction is a necrotizing granulomatous inflammation with a low number of organisms, resulting in nonviable (culture-negative) yeast. Special stains are needed to detect *Histoplasma* in this setting since it cannot usually be visualized on H&E.

In disseminated histoplasmosis, there is extensive tissue infiltration by organisms, and usually, the host's tissue reaction is poor, and it can be represented by a subtle inflammatory associated with extensive tissue necrosis.

It is important to remember that histoplasmosis can induce a variety of organ-site responses with unfamiliar or unusual histological patterns, and the diagnosis of histoplasmosis can be missed if the clinicians do not provide adequate clinical data.⁵²

In particular, when evaluating tissue lesions from patients with profound immunodepression, histological tissues examination should include special histochemical stains for infectious agents.⁵³

Microbiology. Microscopy: The diagnosis of invasive histoplasmosis can be obtained through

various direct examination methods. The major part of collected specimens can be freshly prepared on a wet mount and examined.^{54,55,56}

Direct microscopic examination of the clinical specimen is a simple but useful method to provide a rapid hint on the possible presence of fungal infection. The limit of microscopy is the low specificity due to the similarity between the different fungal species.^{57,58} *H. capsulatum* can be easily misidentified with *B. dermatitidis*, various *Candida* species, *Cryptococcus gattii*, *Cryptococcus neoformans*, *Talaromyces marneffei*, and endospores of *Coccidioides* species.⁵⁹

Culture methods: *H. capsulatum* is a dimorphic fungus: at temperatures above 30 °C, there is the yeast phase while in the cultures incubated at lower temperatures (25 °C) there is the development of mold phase. The yeast phase allows a fast growth of the isolate, with an average growth time of 5-7 days; the mold phase takes from 4 weeks to up to 12 weeks to grow.

The gold standard for the identification of the pathogen is the culture demonstrating the thermal dimorphism of the fungus from yeast to mold and vice versa.

Isolation from samples from the lower respiratory tract with bronchial and bronchoalveolar lavage (BAL) in cases of chronic pulmonary histoplasmosis has a sensitivity of 60-75%. In the case of disseminated histoplasmosis, isolation through blood culture is the most sensitive method while the sensitivity of bone marrow culture is 75%.^{59,60}

The use of MALDI-TOF technology is a method of direct identification of the colony of both the yeast phase and the mold phase, capable of providing rapid detection of *H. capsulatum* with excellent sensitivity, greatly reducing diagnosis times, but until now only a scarce data have been published so far.⁶¹

Non-culture methods: Many non-culture methods were developed in order to make a correct and rapid diagnosis of histoplasmosis. Other tests such as the research of beta-3-D-glucan or the Platelia test (Bio-Rad Laboratories, Redmond, WA) for *Aspergillus* can cross-react in case of histoplasmosis and are not to be considered specific.⁶²

Antibody detection methods: Most of these assays are based on the ability to search for antibodies to histoplasmin (HMIN). HMIN has

three antigens. It is an extremely specific test (100%), but sensitivity is reduced (70-100%) depending on the type of infection and the patient's level of immune depression.

The complement fixation test is a more sensitive method (94.3%) but less specific (70%) than immunodiffusion; however, use of this test is of little help in immunocompromised or AIDS patients. A Semi-Quantitative Indirect Enzyme Immunoassay (EIA) testing for IgM and IgG against the *Histoplasma* polysaccharide antigen is available by Mira-Vista Diagnostics (Indianapolis, Indiana). It is validated on both serum and CSF; false negative results may result in some progressive or chronic cases, especially in immunocompromised patients. A western-blot test has recently been validated in Brazil, providing sensitive, specific, and faster results.⁶³

Antigen detection: Enzyme immunoassay is a quantitative test which detects the presence of *Histoplasma* polysaccharide with a reported sensibility of up to 95%. The antigen is present in larger quantities in the urine than in the serum and the sensitivity of the test increases in immunocompromised patients and in disseminated histoplasmosis. An indication of response to therapy is the decrease over time of the urinary antigen.^{64,65}

An antigen-capture enzyme-linked immunosorbent assay (ELISA) to detect *H. capsulatum* antigenuria in immunocompromised has been validated and distributed by the CDC. Some other antigen-based tests for *Aspergillus* spp. have been proposed and showed promising accuracy on bronchoalveolar lavage fluid.^{66,67,68}

Molecular tools: The absence of commercially available FDA-approved molecular tests has led to the development of multiple homemade solutions from conventional PCR to semi-nested, nested, real-time, LAMP, and RCA.^{69,70,71,72}

Therapeutic and Preventive Approach.

Guidelines from the CDC, the National Institutes of Health (NIH), and the HIV Medicine Association of the Infectious Diseases Society of America (IDSA) recommend treatment with intravenous (IV) liposomal amphotericin B (3 mg/kg daily) as induction treatment for at least 2 weeks, or up to a clinical improvement and a possibility of oral treatment.⁷³

Amphotericin B lipid complex and Amphotericin B deoxycholate may represent a less

Key points:

- Microscopical examination of histological or cytological specimens with H&E and special stains such as PAS and GMS may lead to the identification of *Histoplasma spp*; the infection can be associated with a lymphohistiocytic infiltrate or a granulomatous response, which is more frequently observed in immunocompetent individuals.
- Serological tests include immunodiffusion (highly specific but low sensitive in immunocompromised patients) and complement fixation (sensitive but less specific due to possible cross reactions).
- Antigen detection tests are accurate but generally non-available in non-endemic countries.
- Identification from culture is still the gold standard for *Histoplasma spp*. diagnosis.

expensive alternative in patients with a low risk of nephropathy;⁷⁴ given the risk of nephrotoxicity and the high number of interaction with other compounds, the patient should be strictly monitored during induction therapy particularly for the renal function and electrolytes balance.

After induction therapy, the treatment should be prolonged with oral itraconazole (200 mg 3 times daily for three days and then 200 mg twice daily for at least 12 months).

Additionally, long-term suppressive therapy with itraconazole (200 mg daily) may be considered in permanently immunosuppressed patients and in patients with recurrent symptoms of PDH. Even though the timing of secondary prophylaxis is still unclear, few data suggest it should be prolonged for one year and until the CD4+ T-cell count reaches 150 cells/mm³ and the patient is on effective cART for at least six months.

Itraconazole 200 mg daily is also recommended as primary prophylaxis for HIV-infected patients with CD4+ T-cell counts <150 cells/mm³ living in highly endemic areas or with potential occupational exposure to the fungus.⁷⁵

Among other azoles, fluconazole at a dosage of 800 mg daily may be used as an alternative regimen in patients who can't be treated with itraconazole, but showed a lower effectiveness and a higher risk of developing resistance. In addition, the administration of posaconazole and voriconazole seems to be effective but current literature offers only a few experiences in the usage of these antimycotic agents. Echinocandins are not active against *Histoplasma spp* and should not be used.

In case of CNS involvement liposomal amphotericin B at a dosage of 5 mg/kg daily for 4 to 6 weeks should be used as initial therapy, followed by itraconazole at a dose of 200 mg 2 or 3 times daily for at least one year and until cerebrospinal fluid normalization.

The cART should also be started as soon as possible, but in severe forms, it can be delayed since the resolution of the acute phase to prevent the potential development of IRIS.

There is a lack of data regarding the better antiretroviral regimen to administer in these patients; however, a high genetic barrier drugs, as well as unboosted regimens, should be preferred according to possible drug-drug interactions between antiretroviral and antifungal treatment. Protease inhibitor-based regimens may be chosen for their potency and high genetic barrier, but they are not free from interactions because of their boosting need (with ritonavir or cobicistat). The administration of the new class of integrase inhibitors guarantees a low risk of drug-drug interactions and a rapid viral load decline. Nonetheless, among them, raltegravir does not have a high genetic barrier, elvitegravir is boosted by cobicistat, and only a few data are available on dolutegravir, and all these drugs are less available in resource-limited countries.⁷⁶

Lastly, possible drug-drug interactions and adherence to cART should be strictly monitored by physicians.⁷⁷

Conclusions. PDH carries a poor prognosis, especially when the diagnosis is delayed; giving its rarity in non-endemic areas and the variety of its clinical presentation, along with the restricted range of sensitive tests in non-endemic countries it poses a real challenge to clinicians.

We are nowadays experiencing an increase in migratory flows from tropical areas, especially those where cART is not widely available. This phenomenon could raise the probability to encounter AIDS-related diseases previously only anecdotal at our latitude in the daily clinical practice.

In our case, the prompt diagnosis was possible only thanks to direct microscopy identification since serology was negative, due to the deep immunosuppression of the patient.

Prognosis may be favorable when antifungal therapy and cART are promptly administered; the co-administration of steroid treatment may be necessary when associated with HLH or immune-reconstitution syndrome. In our case, the adequate combined treatment (antifungal, antiretroviral and steroidal) allowed a good clinical outcome, also if the relapse after an early interruption of the

secondary prophylaxis highlights the need of a prolonged course of antifungal maintenance therapy.

Early diagnosis remains crucial to guarantee the survival of the patients, and we believe that the improvement of surveillance on this disease represents the best tool to reach this endpoint also in non-endemic countries.

Key points:

- Patients with PDH should be treated with intravenous liposomal amphotericin B (3 mg/kg daily) as induction treatment for at least two weeks, followed by oral itraconazole (200 mg 3 times daily for three days and then 200 mg twice daily for a total of at least 12 months)
- The administration of itraconazole (200 mg daily) may be considered as secondary prophylaxis in permanently immunosuppressed patients, and as primary prophylaxis in permanently immunosuppressed patients living in endemic areas.
- Patients with CNS involvement should be treated with liposomal amphotericin B at a dosage of 5 mg/kg daily for 4 to 6 weeks, followed by itraconazole at a dose of 200 mg 2 or 3 times daily for at least one year and until cerebrospinal fluid normalization.
- cART should be started as soon as possible monitoring the patients for the risk of drug-drug interaction, IRIS occurrence, and adherence to therapy.

TAKE HOME MESSAGES

- **PDH should be considered in immunocompromised patients (particularly in HIV-infected ones) coming from endemic areas presenting with fever and multiple organ dysfunctions**
- **In Europe diagnosis is mainly histological as serological tests are not accurate in immunocompromised patients and antigen detection tests are not generally available**
- **Prognosis is poor, but reversible if treatment is promptly administered**
- **Liposomal amphotericin-B is the treatment of choice for the first two weeks, followed by Itraconazole as prolonged maintenance therapy**

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