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HISTOPLASMOSE DISSEMINADA EM CENTRO DE REFERÊNCIA PARA AIDS NO EXTREMO SUL DO BRASIL

Rio Grande, 2021

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Rio Grande, como requisito parcial à obtenção do título de Doutora em Ciências da Saúde.
Área de concentração: Medicina investigativa.

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Histoplasmose disseminada em centro de referência para aids no extremo sul do Brasil

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“Comovo-me em excesso, por natureza e por ofício. Acho medonho alguém viver sem paixões.”

Graciliano Ramos (1892- 1953)

RESUMO

A histoplasmose, causada pelo fungo dimórfico *Histoplasma capsulatum*, pode assumir caráter disseminado no paciente imunodeprimido. Mais de 90% dos pacientes com histoplasmose disseminada têm infecção pelo vírus da imunodeficiência humana. A mortalidade é elevada nesta população, devido a inespecificidade clínica associada a métodos laboratoriais pouco sensíveis, o que culmina com atraso diagnóstico e progressão da doença. Desta forma, esta tese teve como objetivo determinar a frequência da histoplasmose disseminada em pacientes com infecção pelo HIV atendidos em um hospital que é referência para o tratamento do HIV/aids, em Rio Grande, Rio Grande do Sul, extremo sul do Brasil no período entre 2010 e 2019. Dados clínicos, laboratoriais, terapêuticos, desfecho e coinfecções foram avaliados. Um total de 31 pacientes foram incluídos no estudo, correspondendo a uma taxa média de incidência de 12 casos/1.000 pacientes hospitalizados com aids. No entanto, essa taxa oscilou consideravelmente, passando de 8/1.000 pacientes hospitalizados com aids nos primeiros anos do estudo, para 24/1.000 após ações buscando aumento da suspeita clínica e implementação da pesquisa de antigenúria para *H. capsulatum*, correspondendo a um incremento em 300% no diagnóstico a partir de 2017. A taxa de mortalidade foi de 35% e a coinfecção com tuberculose ocorreu em 29% dos pacientes. Outros dois artigos abordam casos de coinfecções atípicas. Reportado o primeiro caso da coinfecção histoplasmose disseminada e *Mycobacterium avium* em um paciente vivo no Brasil. Juntamente é apresentada uma revisão da literatura sobre essa coinfecção, incluindo sete artigos científicos descrevendo 15 casos no mundo. O segundo artigo relata um caso de coinfecção histoplasmose disseminada e covid-19 em uma paciente com aids, sendo o primeiro relato desta coinfecção publicado no Brasil e o segundo no mundo, e com um desfecho positivo. Portanto, a histoplasmose deve ser inserida de forma sistemática na investigação das coinfecções no paciente com aids. Reforça-se ainda a importância da disponibilidade de métodos diagnósticos mais sensíveis juntamente ao aumento da suspeição para contribuir com o desfecho positivo de pacientes com esta infecção fúngica sistêmica.

PALAVRAS-CHAVE: *Histoplasma capsulatum.* Coinfecção. Imunodepressão.

ABSTRACT

Histoplasmosis, caused by the dimorphic fungus *Histoplasma capsulatum*, can assume a disseminated character in immunocompromised patients. Indeed, more than 90% of patients who present disseminated histoplasmosis are infected with the human immunodeficiency virus. Mortality is high in this population, due to nonspecificity symptoms associated with low sensitivity laboratory methods, which culminates with diagnosis delay and disease progression. Thus, this thesis aimed to determine the frequency of disseminated histoplasmosis in patients with HIV infection attended at a reference hospital for HIV/AIDS patients, in Rio Grande, Rio Grande do Sul, southern Brazil in the period between 2010 and 2019. Clinical, laboratory, therapeutic, outcome and coinfection data were acquired. A total of 31 patients were included in the study, corresponding to an average incidence rate of 12 cases/1,000 patients hospitalized with AIDS. However, this rate fluctuated considerably, from 8/1,000 patients hospitalized with AIDS in the first years of the study, to 24/1,000 after actions seeking to increase clinical suspicion and implementation of antigenuria research for H. capsulatum, corresponding to an increase in 300% at diagnosis from 2017 onwards. The mortality rate was 35% and co-infection with tuberculosis occurred in 29% of patients. Two other articles address cases of atypical coinfections. The first case of disseminated histoplasmosis and *Mycobacterium avium* coinfection in a live patient in Brazil is reported. Additionally, a review of the literature regarding this coinfection was performed, found seven scientific articles reporting 15 cases in the world. The second article reports a case of disseminated histoplasmosis and covid-19 coinfection in a patient with aids, being the first case on this coinfection published in Brazil and the second in the world, highlighting the positive outcome of the case. Therefore, histoplasmosis must be inserted in a systematic way in the investigation of coinfections in aids patients. We reinforce the importance of the availability of diagnostic tests with high sensitivity together with an increase in the suspicion for early diagnosis, in view of to contribute to a positive outcome of patients with systemic fungal infection.

KEYWORDS: *Histoplasma capsulatum.* Coinfection. Immunosuppression.

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LISTA DE ABREVIATURAS E SIGLAS

ABCL – Anfotericina B em complexo lipídico

ABDC – Anfotericina B em dispersão coloidal

AmB – Anfotericina B

AMPc – Adenosina-monofosfato-cíclico

BHI – Brain-heart infusion

CBP – Calcium Binding Protein

CDC – Center for Disease Control and Prevention

CEPAS – Comitê de Ética e Pesquisa na Área da Saúde

d - AmB – Anfotericina B descoxicolato

EBSERH –Empresa Brasileira de Serviços Hospitalares

ELISA – Enzyme-Linked Immunosorbent Assay

EUA – Estados Unidos da América

FAMED – Faculdade de Medicina

FC – Fixação do complemento

FURG – Universidade Federal do Rio Grande

HD – Histoplasmose disseminada

H. capsulatum – *Histoplasma capsulatum*

HIV – Vírus da imunodeficiência humana

HE – Hematoxilina-eosina

HPA – Antígeno glicoproteico do *Histoplasma capsulatum*

HU – Hospital Universitário

ID – Imunodifusão dupla

IFN-γ – Interferon gama

IL 12 – Interleucina 12

ITC – Itraconazol

L-AmB – Anfotericina B lipossomal

LAMP – Loop-mediated isothermal amplification

LFA – Lateral flow assay

LT CD4+ – Linfócito T CD4+

LT CD8+ – Linfócito T CD8+

MLST – Multilocus sequence typing

NK – Natural killer

OMS – Organização Mundial da Saúde

OPAS – Organização Pan-Americana para a Saúde

PAS – Ácido periódico de Schiff

PAHO – Pan American Health Organization

PCR – Polymerase chain reaction

PVHA – Pessoa vivendo com HIV/aids

RNA – Ácido ribonucleico

SINAN – Sistema de informação de agravos de notificação

SNC – Sistema nervoso central

TARV – Terapia antirretroviral

TLR2 – Receptor do tipo Toll 2

TNF- α – fator de necrose tumoral alfa

WHO – World Health Organization

LISTA DE SÍMBOLOS

Kg – Quilograma

mg – Miligrama

nm – Nanômetro

μm – Micrômetro

SUMÁRIO

1 INTRODUÇÃO

A histoplasmose é uma micose primariamente pulmonar causada pelo *Histoplasma capsulatum* (*H. capsulatum*), um fungo dimórfico cujo habitat se caracteriza por solo contendo fezes de aves e morcegos. Essa matéria orgânica, com elevado teor de nitrogênio, propicia substrato para o fungo, o qual pode persistir no ambiente por longos períodos após a contaminação inicial (LACAZ, 2002; FOCACCIA, 2021). A infecção humana se dá por via respiratória, onde os microconídios do *H. capsulatum* penetram após inalação e ao chegarem aos alvéolos são fagocitados, multiplicando-se no interior dos macrófagos alveolares. Disseminam-se através dos vasos linfáticos formando um complexo pulmonar semelhante ao Complexo de Gohn da tuberculose. A partir deste momento pode ocorrer a disseminação hematogênica para qualquer órgão. A doença pode ocorrer nas formas pulmonar (aguda e crônica) e disseminada (AIDÉ, 2009; FOCACCIA, 2021).

A histoplasmose disseminada (HD) ocorre nos imunodeprimidos, particularmente na aids, neoplasias hematológicas, transplante de órgãos sólidos, corticoterapia ou com antagonistas do fator de necrose tumoral, e em imunodeficiências monogênicas (KAUFFMAN, 2007; FOCACCIA, 2021; LEE; LAU, 2017). Nestes hospedeiros a doença tende a assumir caráter progressivo e sistêmico (LACAZ, 2002; FOCACCIA, 2021). Desde 1987, mais de 90% dos pacientes diagnosticados com HD têm infecção pelo Vírus da Imunodeficiência Humana (HIV), sendo considerada uma doença definidora de aids (CANO; HAJJEH, 2001). A HD acomete principalmente indivíduos com grave comprometimento imunológico, com linfócito T CD4+ (LT CD4+) geralmente inferior a 200 células/mm^3 (CDC, 1993; WHEAT, 1995).

No início da epidemia de aids, em 1981, a HD foi considerada a infecção oportunista de maior prevalência nestes pacientes (WHEAT; SLAMA; ZECKEL, 1985). Atualmente, a doença continua sendo descrita como de alta prevalência, considerada uma das principais causas de morbidade e mortalidade relacionada a aids nas Américas (ADENIS *et al*., 2018; PASQUALOTTO; TELLES, 2018). Mesmo com uma diminuição progressiva da incidência das infecções oportunistas com o advento da terapia antirretroviral altamente ativa, a taxa de mortalidade entre pacientes com HIV/aids diagnosticados com histoplasmose na América Latina é de cerca de 30% (ADENIS *et al*., 2018; DANTAS *et al.*, 2018).

No Brasil, a histoplasmose é particularmente comum, descrita como a terceira infecção fúngica invasiva mais frequente em pacientes com HIV/aids, sendo responsável por taxa de mortalidade de cerca de 10% (PRADO *et al*., 2009). As manifestações clínicas, os achados laboratoriais e radiológicos iniciais da HD são inespecíficos, semelhantes a outras doenças prevalentes em pacientes com aids, como a tuberculose e a pneumocistose (ADENIS; AZNAR;

COUPIE, 2014; DANTAS *et al*., 2018). Assim, estudos reforçam a hipótese de que a histoplasmose é subdiagnosticada nos pacientes em tratamento de prova para tuberculose e nos casos tratados como tuberculose resistente, sem documentação de teste de sensibilidade (BULMER; BULMER, 2001; NACHER, 2016). A demora na suspeição, dificuldade de acesso aos métodos diagnósticos mais sensíveis e consequentemente o atraso na instituição da terapia antifúngica são as causas da elevada mortalidade associada a HD no Brasil e na América Latina (NACHER, 2016; ADENIS *et al*., 2018).

Devido a não obrigatoriedade da notificação e a dificuldade no diagnóstico, a real influência da HD na morbimortalidade dos pacientes coinfectados com HIV ainda é uma incógnita (LOCKHART *et al*., 2021). Inserido nesta problemática, o município do Rio Grande, situado no sul do RS, apresenta uma taxa de detecção de HIV 3,1 vezes maior do que a do Brasil, e está classificado na pior posição nacional, dentre os municípios com mais de 100 mil habitantes no ranking do índice composto, que avalia a taxa média de detecção juntamente com a taxa de mortalidade e com a variação destas taxas e contagem de células LT CD4+ no momento do diagnóstico (BRASIL, 2020). Nesta linha, a investigação da taxa de incidência e fatores clínicos e epidemiológicos associados a HD em pacientes com infecção pelo HIV/aids acompanhados em um hospital de referência para o tratamento HIV/aids, situado no extremo sul do Brasil, faz-se necessária, justificando o presente estudo.

2 REVISÃO DE LITERATURA

2.1 Definição e histórico da histoplasmose

A histoplasmose foi descrita inicialmente por Samuel Taylor Darling, um patologista norte-americano que trabalhava na Cidade do Panamá, durante a construção do canal do Panamá, em 1905. O nome do agente, *H. capsulatum*, originou-se do seu aspecto microscópico e da sua forma tecidual, que, na opinião de Darling, parecia ter cápsula e sugeria um plasmódio fagocitado num histiócito. Isto ocorreu ao realizar uma necropsia de um homem de 27 anos, negro, natural da Martinica, carpinteiro e que havia falecido de uma doença febril aguda, acompanhada de hepatoesplenomegalia e que inicialmente foi atribuída à tuberculose miliar (HAGAN, 2003). Três anos depois, em 1908, Darling relatou mais dois casos fatais da nova doença, acometendo um paciente chinês que morava há 15 anos no Panamá e um negro natural da Martinica (DARLING, 1909).

Em 1912, o parasitologista brasileiro Henrique Rocha Lima identificou a natureza fúngica do *H. capsulatum* ao comparar os parasitas presentes nas amostras de tecidos dos pacientes de Darling com amostras de pacientes com leishmaniose (FERNÁNDEZ ANDREU *et al*., 1990). Seu primeiro isolamento em meio de cultivo com demonstração de seu caráter dimórfico, em 1934, foi feito pelo patologista americano William de Monbreun, e no mesmo ano foi relatado o primeiro caso da doença em paciente vivo (FERNÁNDEZ ANDREU *et al*., 1990; HAGAN, 2003).

Na década de 1920, O *H. capsulatum* foi o causador da famosa "Maldição de Tutancâmon" que vitimou arqueólogos que descobriram a tumba do faraó. Por muitos anos a exploração da tumba esteve associada a uma maldição, pelo adoecimento de muitos exploradores. Com a inalação de um grande inóculo, os arqueólogos desenvolveram um quadro de histoplasmose pulmonar aguda. O diagnóstico da histoplasmose foi confirmado através da identificação do *H. capsulatum* em necrópsias pulmonares (WALDRON, 1985).

No Brasil, em 1939, Almeida e Lacaz isolaram pela primeira vez *o H. capsulatum* no país a partir do cultivo de uma biópsia com suspeita de cromomicose. E, em 1941, os mesmos autores relataram o isolamento fúngico em amostra de escarro de um paciente com tuberculose (WANKE; LAZERA, 2004). No mesmo ano, Vilela e Pará relataram o primeiro caso fatal de histoplasmose disseminada no Brasil, diagnosticada por exame histológico do fígado de uma criança de três anos de idade, que residia em Minas Gerais com suspeita de febre amarela (LACAZ, 2002).

Em 1945, Parsons e Zarafonetis descreveram sete pacientes com histoplasmose e revisaram 71 casos de HD, ressaltando seus aspectos clínicos (GOODWIN *et al*., 1980).

Emmons, em 1948, isolou o fungo pela primeira vez, em amostras de solo de um galinheiro e em 1958 foi reconhecida a importância das fezes de morcegos ao serem descobertos casos agudos de histoplasmose em pessoas que haviam permanecido por algum tempo em grutas ou em outros locais habitados por esses animais (EMMONS, 1958). Em meados de 1955, a anfotericina B, produzida naturalmente pelo actinomiceto *Streptomyces nodosus* foi isolada, e em 1965 foi o primeiro agente antifúngico a ser aprovado pela *Food and Drug Administration* dos Estados Unidos da América, para o tratamento de micoses sistêmicas (FILIPPIN e SOUZA, 2006).

No início da década de 1980, Wheat identificou a HD como a principal infecção oportunista em pacientes com aids, até então esta manifestação clínica estava associada a outras condições de imunossupressão como leucoses, uso de quimioterápicos pós-transplante, corticoterapia sistêmica e imunodeficiências congênitas (WHEAT; SLAMA; ZECKEL, 1985). Em 1987 o *Centers for Disease Control and Prevention* (CDC) incluiu a histoplasmose extrapulmonar como critério de definição de aids em pacientes com infecção pelo HIV (CDC, 1987).

No Brasil, em 1988, Severo e Rocha relataram o primeiro caso de HD em um paciente com aids no país. Nesta mesma década, foram introduzidos os antifúngicos azólicos sistêmicos, e já no início da década de 1990 foram aprovadas as formulações lipídicas da anfotericina B (FILIPPIN; SOUZA, 2006).

Em março de 2019, na cidade de Manaus, ocorreu o "The II Regional Meeting on Histoplasmosis in the Americas", que reuniu especialistas de vários países e onde foi elaborado um documento com as recomendações para diagnóstico e tratamento da HD (CÁCERES *et al*., 2019a). A partir deste documento, foi elaborado o primeiro consenso de diagnóstico e tratamento da HD entre as pessoas vivendo com HIV/aids, publicado em maio de 2020 (PAHO/WHO, 2020).

2.2 Agente etiológico e taxonomia

A espécie *H. capsulatum* pertence ao Reino *Fungi*, Filo *Ascomycota*, Subfilo *Ascomytina*, Classe *Ascomycetes*, Ordem *Onygenales*, Família *Onygenaceae*, Gênero *Histoplasma* (FERNÁNDEZ ANDREU *et al*., 1990). A espécie é composta por três variedades: *H. capsulatum* var. *capsulatum*, a forma mais prevalente e estudada; o *H. capsulatum* var. *duboisii*, agente etiológico da histoplasmose africana, predominante no

continente africano; e *H. capsulatum* var. *farciminosum*, a qual não foi descrita parasitando o homem, relatada apenas como patógeno de cavalos e mulas (LACAZ, 2002).

Estudos voltados para a caracterização, tanto fenotípica quanto genotípica, de diferentes isolados de *H. capsulatum* demonstram uma grande variabilidade genética. Kasuga e colaboradores (2003) usaram a tipagem de sequência multilocus (MLST) e encontraram pelo menos sete espécies filogenéticas dentro de *H. capsulatum.* Teixeira e colaboradores (2016) expandiram o MLST sobre um conjunto maior de isolados e identificaram dois grupos nos Estados Unidos que provavelmente representavam espécies únicas, e quatro populações na América Latina que provavelmente representavam espécies separadas.

Em 2017, pela primeira vez o sequenciamento do genoma completo foi aplicado para avaliação de diferentes cepas do gênero *Histoplasma*, e quatro espécies foram identificadas: *H. capsulatum* sensu stricto identificada como restrita a isolados oriundos do Panamá, o local original da descoberta. *H. mississippiense* e *H. ohiense* representaram os isolados da América do Norte, sem limites geográficos definitivos, e *H. suramericanum* representou os isolados da América do Sul (SEPÚLVEDA *et al.*, 2017). Embora os dados que apoiam a divisão do *H. capsulatum* nas Américas em quatro espécies distintas sejam convincentes, as designações das espécies não seguem estritamente as regras do Código Internacional de Nomenclatura Botânica e são atualmente consideradas inválidas (LOCKHART *et al.*, 2021).

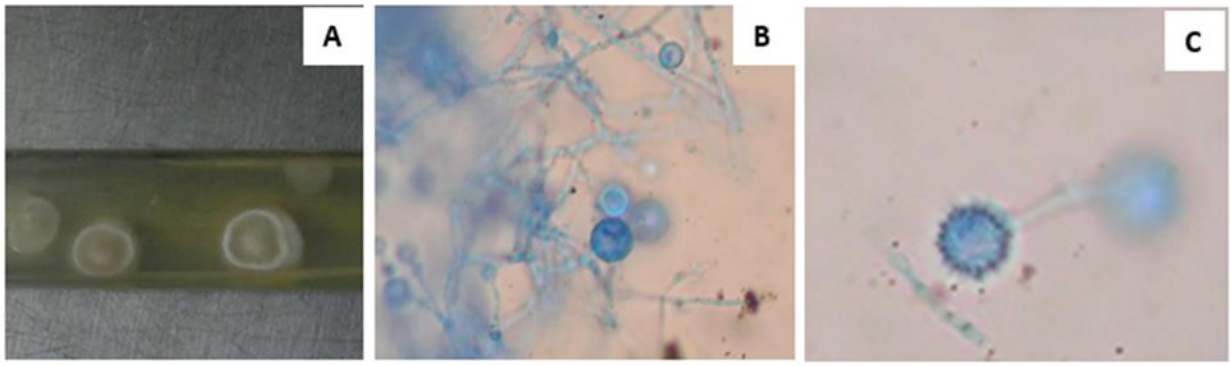
2.3 Aspectos morfológicos

H. capsulatum é um fungo termicamente dimórfico. A forma filamentosa ou micelial cresce em temperatura ambiente, abaixo de 35°C, e a forma leveduriforme, desenvolve-se a 37°C ou mais. A fase filamentosa na avaliação microscópica apresenta dois tipos de conídios, sendo eles: 1) macroconídios ovóides, de 8-14 μm de diâmetro, de paredes grossas, sem septos, com múltiplas protrusões na superfície conferindo um aspecto tuberculado; e 2) microconídios lisos, também ovais, de 2 a 5 μm e de paredes finas (figura 1). Esta última é a forma infectante (LACAZ, 2002).

A transição da fase filamentosa para a fase leveduriforme é essencial para o desencadeamento da doença após a infecção pelo fungo (WOODS, 2003). A 37°C, o fungo passa por alterações genéticas, bioquímicas e fenotípicas que resultam na formação de uma célula leveduriforme uninucleada, oval, também com 2-5 μm, de paredes finas que se reproduzem por brotamentos e são observadas microscopicamente nos tecidos do hospedeiro e in vitro em meios de cultura (figura 2) (LACAZ, 2002). *H. capsulatum* não possui cápsula, mas

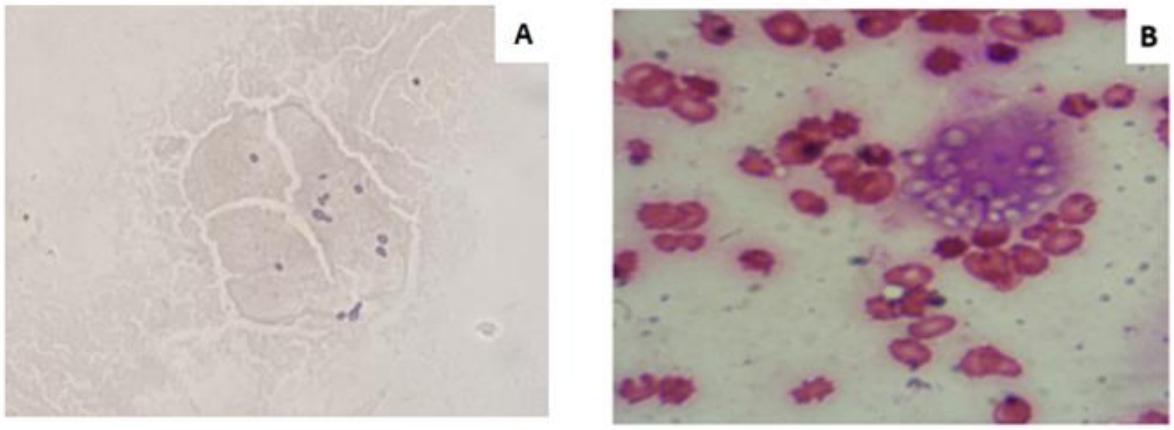
em tecidos observa-se uma zona clara ao redor, que foi confundida com uma cápsula por Darling na primeira descrição do agente (KAUFFMAN, 2007).

FIGURA 1 - *Histoplasma capsulatum* (Fase filamentosa). (A) Cultivo da fase filamentosa. (B) e (C) Aspectos microscópicos da fase filamentosa com micro e macroconídios característicos (Aumento de 40X)



Fonte: Imagens do arquivo do Laboratório de Micologia FAMED-FURG

FIGURA 2 - *Histoplasma capsulatum* (Fase leveduriforme). (A) e (B) Aspectos microscópicos da fase leveduriforme com leveduras unibrotantes corados pela Prata e Giemsa, respectivamente (Aumento de 40X)



Fonte: Imagens do arquivo do Laboratório de Micologia FAMED-FURG

A parede celular de *H. capsulatum* consiste principalmente de α-1,3 glucana, β-1,3 glucana e quitina. Em comparação com outros fungos patógenos dimórficos, o conteúdo de quitina é maior no *H. capsulatum*. No entanto, existem diferenças na composição de glucanas nas duas fases: α-1,3 glucana é o principal componente da fase leveduriforme, enquanto na filamentosa o conteúdo de β-1,3 glucana é maior. Altos níveis de α-1,3 glucana têm sido associados com maior virulência de cepas (RAPPLEYE; EISSENBER; GOLDMAN, 2007).

Outro componente importante, embora seja encontrado em menor quantidade, é uma galactomanana localizada na camada mais externa da parede celular, que é considerada o principal polissacarídeo antigênico do *H. capsulatum*. Outras galactomananas encontradas em

outros agentes fúngicos são responsáveis por reações cruzadas em testes sorológicos com *Blastomyces dermatitidis* e *Paracoccidioides brasiliensis* e reação cruzada com antígeno galactomanana do *Aspergillus* spp. em paciente com imunodepressão severa (XAVIER *et al*., 2009; FERNÁNDEZ ANDREU *et al.*, 2011; RIVIÈRE *et al*., 2012).

2.4 Ecologia

O crescimento do fungo *H. capsulatum* no seu habitat natural possui estreita relação com fatores ambientais. É influenciado por fatores físico-químicos presentes no ambiente, como umidade, temperatura, e tipo de solo (KAUFFMAN, 2007). No ambiente, o fungo é encontrado em solos com alto teor de nitrogênio e fosfatos, geralmente associados ao acúmulo de guano (fezes) de aves, principalmente galinhas, e morcegos. Essas características foram relatadas pela primeira vez em 1949, por Emmons, e posteriormente por Ajello em 1964. Devido a sua elevada temperatura corporal, as aves não são portadoras do fungo, contudo suas fezes favorecem o crescimento do *H. capsulatum* no ambiente (AJELLO, 1964; KAUFFMAN, 2007; AIDÉ, 2009). Dos vários animais suscetíveis à infecção, o morcego exerce também o papel de disseminador do fungo na natureza, devido ao intenso parasitismo das células de sua mucosa intestinal, que ao apresentar lesões, secretam o fungo nas suas fezes, desempenhando um importante papel na manutenção do ciclo biológico do *H. capsulatum* na natureza (DIAS *et al*., 2011). Assim, cavernas, construções abandonadas, sótãos ou porões de casas, galinheiros, árvores ocas e campos cultiváveis são importantes fontes de infecção deste agente fúngico. Além disso, a própria movimentação do solo proporciona a dispersão dos microconídios pelo ar (LONDERO; WANKE, 1988).

H. capsulatum pode crescer em intervalos de pH entre 5 e 10 e requer oxigênio para sobreviver e desenvolver sua fase filamentosa, multiplicando-se em profundidades de 5 a 15 centímetros da superfície do solo, porém não de maneira homogênea, mas com formação dos chamados microfocos (KAUFFMAN, 2007; FERNÁNDEZ ANDREU *et al*., 2011). Solos com estas características estão localizados preferencialmente em regiões tropicais e subtropicais com temperatura média anual de 22 a 29ºC, umidade relativa de 60-80% e elevado índice pluviométrico, aproximadamente 1.000 mm/ano. Porém, já foram descritos casos autóctones em regiões com clima desfavorável para o crescimento do *H. capsulatum* no ambiente natural (FERNÁNDEZ ANDREU *et al*., 2011).

Adicionalmente, a partir da aerossolização os microconídios são dispersos no ambiente, disseminados pelo vento por vários quilômetros podendo causar infecção mesmo em pessoas que não tiveram contato direto com a fonte de infecção (FERNÁNDEZ ANDREU *et al*., 2011).

Não há transmissão do animal para o homem, portanto a histoplasmose não é considerada uma zoonose, mas sim uma sapronose, considerando que a infecção se dá a partir da vida sapróbia do fungo na natureza (HUBALEK, 2003).

2.5 Imunopatogenicidade

A infecção por *H. capsulatum* ocorre a partir da inalação de fragmentos de hifas e microconídios que devido ao pequeno tamanho e hidrofobicidade são facilmente dispersos na via aérea, chegando aos alvéolos pulmonares. Uma vez no interior do hospedeiro, passam para a fase leveduriforme, responsável pela patogênese desse fungo (WOODS, 2003).

A dose infectiva do inóculo depende em grande parte do estado imunológico do hospedeiro (LÓPEZ, 2006). O processo de transformação para levedura ocorre nos macrófagos alveolares. A temperatura é um fator determinante nessa transição, mas também outros fatores foram identificados, como a presença de cisteína e monofosfato de adenosina cíclico (AMPc) (WOODS, 2003). O calor estimula a transição para a fase leveduriforme pela indução do gene *cdc*2, seguido pelo aumento da transcrição do gene *yps*3, específico da fase leveduriforme (WOODS, 2003), o qual codifica uma proteína ligante de quitina localizada na superfície da parede celular, identificada pela sua liberação em meio de cultura. De fato, ao silenciar este gene, em estudo de virulência realizado por Bohse e Woods (2007) houve significativa diminuição da carga fúngica em modelos animais.

Além destes, foram identificados dois outros genes codificantes de proteínas com importância para a patogenicidade do fungo. O gene *arf*, que participa da transdução de sinais intracelulares, além de ser importante para o crescimento das hifas, também participa da montagem de vesículas e do transporte destas entre organelas; e o gene *ole*, que está envolvido na regulação da fluidez da membrana, sendo expresso em resposta ao aumento da temperatura (25°C-37°C) e na alteração de fase (filamento para levedura) (MUÑOZ *et al*., 2010).

A interação entre as leveduras e os macrófagos é o evento chave na patogenia da histoplasmose. Os macrófagos interagem com as leveduras do *H. capsulatum* através de vários receptores, tais como integrinas CD11b/CD18, lactosilcermide, dectinas e receptores do tipo Toll 2 (TLR2) (PEREIRA, 2009). Uma vez fagocitado por macrófagos o fungo pode sobreviver no ambiente intracelular através de vários mecanismos que permitem, em primeiro lugar, modular pH e resistir ao efeito dos radicais tóxicos do oxigênio, bem como driblar os efeitos deletérios de diferentes enzimas (DEEPE, 2000). A presença do *H. capsulatum* em outros fagócitos, como as células epiteliais, é descrita, podendo ser este um mecanismo chave para as infecções persistentes, por atuar como reservatórios alternativos (FERNÁNDEZ ANDREU *et*

al., 2011). Após a fagocitose pelos macrófagos alveolares e neutrófilos, pode ocorrer a disseminação para os linfonodos hilomediastinais e/ou disseminação hematogênica (NOSANCHUK; GRACSER, 2008).

A dectina-1 é um receptor imune inato essencial, o qual reconhece os β-glucanos da parede das células fúngicas, porém alguns fungos, dentre estes o *H. capsulatum*, evoluíram para evitar a detecção por esse receptor. Garfoot e colaboradores (2017) apresentaram dados mostrando que as leveduras desse microrganismo secretam uma β-glucanase (codificada pelo gene *Eng1*) que hidroliza β-glucanos que estão expostos na superfície da célula fúngica. O corte destes açúcares reduz o reconhecimento imunológico através da dectina-1 e subsequentes respostas inflamatórias, aumentando a patogênese do *H. capsulatum* (BROWN, 2016).

Várias proteínas podem participar da patogenia da histoplasmose e são consideradas fatores de virulência deste fungo. Um exemplo, demonstrado *in vitro* e *in vivo*, é uma proteína de ligação de cálcio secretada pelas células fúngicas durante a fase leveduriforme, a *Calcium Binding Protein* (CBP). A realização de experimentos de disrupção gênica, com a deleção do gene CBP1 codificador desta proteína, demonstrou que as leveduras resultantes não cresceram quando privadas de cálcio e não foram capazes de destruir os macrófagos *in vitro* ou se proliferar em um modelo de infecção animal (SEBGHATI; ENGLE; GOLDMAN, 2000).

A produção de pigmentos do tipo melanina como fator de virulência no *H. capsulatum* é apontada, como em outros fungos patogênicos (NOSANCHUK *et al.*, 2002). Este pigmento auxilia na proteção do fungo contra fatores ambientais como extremos de temperatura, e como mecanismo de defesa do sistema imune do hospedeiro, além de interferir reduzindo à sensibilidade à anfotericina (VAN DUIN; CASADEVALL; NOSANCHUK, 2002; ALMEIDA PAES *et al*., 2018).

O principal mecanismo imunológico responsável pela proteção é a resposta mediada por células, enquanto a resposta humoral não parece ter um efeito direto (DEEPE, 2000). Em geral, a imunidade protetora na histoplasmose é complexa e baseia-se na interação coordenada entre macrófagos, células *natural killer* (NK) e leucócitos polimorfonucleares, além da liberação de citocinas, fundamentalmente interferon gama (IFN-γ), fator de necrose tumoral alfa (TNF-α) e interleucina 12 (IL-12) (SEGAL; ELAD, 2006; PEREIRA, 2009).

Deepe (2000) relatou a capacidade dos macrófagos em controlar a infecção por *H. capsulatum*, a partir da interação com os receptores fúngicos, como o CR3 (integrina CD11b/CD18). A resolução da infecção é condicionada pelo desenvolvimento de uma resposta de linfócitos T específicos, cerca de duas semanas após o início da infecção, e desempenha um papel fundamental nos mecanismos de defesa contra *H. capsulatum*. Neste contexto, os

linfócitos T auxiliares ativam os macrófagos, aumentando suas capacidades fungicidas e estimulando a formação de granulomas. Por outro lado, as células NK são capazes de eliminar o fungo extracelularmente e a presença de anticorpos anti *H. capsulatum* aumenta a capacidade fungicida destas células (DEEPE, 2000; HORWATH; FECHER; DEEPE, 2015).

Em pacientes com alterações na resposta imune celular, a infecção por *H. capsulatum* geralmente não é controlada e há uma tendência à disseminação progressiva. A infecção se estende a vários órgãos, incluindo principalmente a medula óssea, o fígado, o baço e as glândulas suprarrenais (FERNÁNDEZ ANDREU *et al.*, 2011; CDC, 2020). Estudos *in vitro*, que mimetizam a situação humana de pacientes com aids, apoiam o papel protetor das células LT CD4+ em humanos para prevenção da infecção fúngica. As células LT CD4+ e LT CD8+ influenciam a reativação, e sua eliminação eleva a carga fúngica (HORWATH; FECHER; DEEPE, 2015).

Embora muitos mecanismos relacionados à patogênese deste fungo ainda sejam desconhecidos, os principais fatores já descritos, e seus efeitos potenciais, são demonstrados no quadro 1.

QUADRO 1 - Principais determinantes da patogenicidade do *H. capsulatum* e seus efeitos potenciais

Referência	Determinante de patogenicidade	Efeitos potenciais
12	gene *YPS3*	Codifica proteína ligante de quitina na parede celular
32	Sobrevivência no interior do fagócito	Parasitismo intracelular de macrófagos
43	Secreção de glucanases	Minimiza a detecção das leveduras pelo hospedeiro
61	Dimorfismo térmico	Transformação da forma filamentosa infectante em forma leveduriforme patogênica
63	Produção de catalase e oxidase	Proteção contra o oxigênio reativo e os intermediários de nitrogênio
99	Produção de proteínas fixadoras do cálcio	Aquisição de cálcio em ambiente com limitação de nutrientes
114	Modulação do pH no microambiente	Proteção contra as enzimas lisossomais
114	Produção de sideróforos e capacidade de reduzir os íons férricos (Fe 3+)	Aquisição de Ferro em ambiente com limitação de nutrientes
114	gene *URA5*	Biossíntese de pirimidina e ácidos nucleicos em ambiente com limitação de nutrientes
114	Fenótipo rugoso (α-1-3-glucano na parede)	Incremento da virulência em algumas cepas

Fonte: compilação da autora.

2.6 Epidemiologia

A histoplasmose apresenta ampla distribuição geográfica, com casos autóctones descritos em todos os continentes, compreendendo mais de 60 países (WHEAT, 2009). No

entanto, encontra-se com um nítido predomínio nas Américas, leste da Ásia, Oceania e na África Subsaariana (KAUFFMAN, 2007; ADENIS; AZNAR; COUPIE, 2014). É uma das micoses de maior importância no continente americano devido a sua endemicidade, sendo a região central dos Estados Unidos da América (EUA) uma das áreas com maior concentração de indivíduos infectados no país (FERREIRA e BORGES, 2009).

Estudo multicêntrico nos EUA definiu a HD como a terceira micose sistêmica mais prevalente em pacientes com aids no país, com 9,1% de incidência nesta população (MARUKUTIRA *et al*., 2014). A incidência de HD, em pacientes coinfectados com HIV, na América Latina encontra-se entre 7,6% no Panamá (GUTIERREZ *et al*., 2005), 21,5% na Venezuela (REDONDO, 1995), 38,4% na Guatemala (SAMAYOA *et al*., 2017) e 38,8% na Guiana Francesa (VANTILCKE *et al*., 2014). No Brasil a incidência reportada varia entre 10,3% a 52,6% (MORA; DOS SANTOS; SILVA-VERGARA, 2008; HOFFMANN *et al*., 2016; RAMOS *et al*., 2018; FALCI *et al*., 2019). Na tabela 1 estão apresentadas os estudos referentes a incidência da coinfecção HD e aids, nas Américas.

TABELA 1- Incidência da coinfecção histoplasmose disseminada e aids, em diferentes estudos, de acordo com o país do continente americano

Referência	País	Período	n	Incidência (%)
35	Brasil	2016-2018	570	21,6
48	Panamá	1997-2003	2379	7,6
51	Brasil	2014-2015	78	10,3
65	EUA	2004-2008	303	9,1
68	Brasil	1992-2005	57	52,6
84	Brasil	2006-2010	117	41
86	Venezuela	1984-1990	200	21,5
89	Guatemala	2005-2009	263	38,4
102	Guiana Francesa	2008-2010	67	38,8

Fonte: compilação da autora.

De acordo com o Fundo de Ação Global para Infecções Fúngicas a forma disseminada da histoplasmose é de alta incidência no mundo, em pacientes com aids (15,4 casos /1.000 pessoas/ano) (NACHER *et al*., 2011), com uma letalidade total de até 30% (CÁCERES *et al*., 2012; NACHER *et al*., 2019). Adenis e colaboradores (2018) demonstraram que em alguns

países da América Latina a incidência e a mortalidade associados à histoplasmose são equivalentes ou até superiores a tuberculose nos coinfectados com HIV. Este estudo reforça que a histoplasmose não pode mais ser vista como uma doença coadjuvante em comparação à tuberculose em pacientes com aids da América Latina.

No Brasil, a HD é a terceira infecção fúngica invasiva mais frequente em pacientes com HIV/aids, resultando em alta mortalidade (PRADO *et al*., 2009). No Sul do Brasil, na capital do estado do Rio Grande do Sul (RS), Porto Alegre, a incidência de HD em pacientes coinfectados com HIV foi de 10,3% quando utilizado métodos convencionais para o diagnóstico (cultivo/histopatológico) e 17,9% com a pesquisa de antígeno urinário (HOFFMANN *et al*., 2016). Em Rio Grande/RS, no extremo sul do país, foi encontrada uma incidência de HD de 9,3% entre os pacientes que apresentavam febre e outro sintoma, no ano de 2017 em estudo multicêntrico realizado por Falci e colaboradores (2019).

Este mesmo estudo multicêntrico abrangeu diferentes estados brasileiros, demonstrando que a incidência de histoplasmose foi superior à de tuberculose nos pacientes com LTCD4+ inferior a 50 células/mm^3 (FALCI *et al.*, 2019). Pasqualotto e Telles (2018) reforçam que a maioria dos médicos aventa a possibilidade de tuberculose, mas que os diagnósticos alternativos não são frequentemente considerados. Devido a não obrigatoriedade da notificação e a dificuldade de acesso a testes não invasivos e com boa sensibilidade, a influência da histoplasmose na morbimortalidade nos pacientes coinfectados com HIV ainda é uma incógnita.

Em relação a aids, no Brasil, foram notificados no sistema de informação de agravos de notificação (SINAN) 1.011.617 casos até junho de 2020. O país tem registrado, anualmente, uma média de 39 mil novos casos de aids nos últimos cinco anos, com uma taxa de detecção de 17,8 casos a cada 100 mil habitantes. O estado do RS apresenta a terceira maior taxa de detecção (28,3 casos/100 mil habitantes) e o município do Rio Grande, local do estudo, apresenta a segunda maior taxa de detecção do país (56,6 casos/100 mil habitantes) (BRASIL, 2020).

2.7 Apresentações clínicas

A maioria das infecções por *H. capsulatum* são assintomáticas (90-95%), detectadas pela resposta intradérmica ao teste de histoplasmina (ZEMBRZUSKI *et al*., 1996) e, em alguns casos, pela presença de focos pulmonares de calcificação em exames de imagem (AIDÉ, 2009). No entanto, entre cinco e 10% dos infectados apresentam formas clínicas muito variáveis, dependentes do inóculo inalado e do estado imunológico do indivíduo. As apresentações

clínicas mais frequentes são histoplasmose pulmonar aguda, forma pulmonar crônica e HD (WHEAT *et al*., 2016).

2.7.1 Histoplasmose pulmonar aguda

A histoplasmose pulmonar aguda é a forma autolimitada da infecção por *H. capsulatum*, ocorrendo principalmente em infecções primárias e em crianças, devido à imaturidade do sistema imune, ou em indivíduos hígidos expostos a um grande inóculo (UNIS; ROESCH; SEVERO, 2005). Em sua forma benigna, é indistinguível de um resfriado comum. De início súbito, apresenta sintomatologia inespecífica: febre, mal-estar, cefaleia, mialgia, anorexia, tosse não produtiva e dor torácica. A radiografia de tórax mostra a presença de infiltrado local com linfadenopatia hilar ou mediastinal que posteriormente pode calcificar (HAGE *et al*., 2015).

A maioria dos casos tem resolução espontânea em três semanas, sem tratamento, porém em casos graves é necessária a terapia antifúngica adequada. No diagnóstico diferencial deve-se incluir blastomicose pulmonar e pneumonias comunitárias por agentes etiológicos atípicos (*Mycoplasma* sp., *Chlamydia* sp., *Legionella* sp.) (KAUFFMAN, 2007; AIDÉ, 2009). Uma anamnese cuidadosa pode auxiliar bastante no diagnóstico, com ênfase em atividades laborais e/ou recreativas que envolvam áreas de risco ou viagens para áreas endêmicas (UNIS; ROESCH; SEVERO, 2005; KAUFFMAN, 2007).

2.7.2 Histoplasmose pulmonar crônica

A histoplasmose pulmonar crônica acomete especialmente indivíduos tabagistas, com mais de 50 anos de idade e portadores de doença pulmonar obstrutiva crônica. As manifestações incluem febrícula, perda de peso, sudorese noturna, dor torácica, tosse produtiva, possível hemoptise e dispneia. As lesões são frequentes nos lobos superiores, muitas vezes confundidas com a tuberculose pulmonar (SEVERO *et al*., 1997; UNIS; SEVERO, 2005).

As manifestações clínicas são semelhantes às de outras doenças crônicas que acometem o aparelho respiratório, por isso no diagnóstico diferencial pode-se incluir tuberculose, infecções por micobactérias não tuberculosas, outras micoses endêmicas como paracoccidioidomicose e coccidioidomicose, e a sarcoidose (SEVERO *et al*., 1997; KAUFFMAN, 2007; FERREIRA; BORGES, 2009). Quando não tratada adequadamente esta forma pode evoluir para insuficiência respiratória aguda grave e consequente óbito, estimado em até 80% dos casos. Não há tendência de disseminação hematogênica nesta apresentação clínica (FERREIRA; BORGES, 2009).

2.7.3 Histoplasmose disseminada

A histoplasmose é considerada disseminada quando o fungo é encontrado em sítios extrapulmonar e/ou extralinfonodais (CDC, 1987). A forma disseminada ocorre principalmente em indivíduos com algum mecanismo de imunossupressão, como pacientes com neoplasias hematológicas (leucemias e linfomas), transplantados, em uso prolongado de corticosteroides e indivíduos com aids (WHEAT, 1995). É considerada uma doença definidora de aids (CDC, 1987) e apresenta mortalidade elevada, de até 30% (CÁCERES *et al*., 2012; NACHER *et al*., 2019).

A disseminação da doença envolve todos os sistemas do hospedeiro, porém, as manifestações clínicas podem ocorrer em alguns órgãos localizados ou em um órgão somente. Estas manifestações são variáveis e inespecíficas, e o paciente pode ter sinais de sepse grave, com falência de múltiplos órgãos (KAUFFMAN, 2007).

Em pacientes coinfectados pelo HIV, as manifestações clínicas comuns de HD incluem febre, fadiga, astenia, adinamia, perda de peso, sudorese e hepatoesplenomegalia (AIDÉ, 2009). Tosse, dor torácica e dispneia ocorrem em torno de 50% dos pacientes (WHEAT *et al.*, 1990). Aproximadamente 10% dos pacientes evoluem para choque e falência de múltiplos órgãos (CDC, 2020).

A histoplasmose deve ser considerada no diagnóstico diferencial em pacientes portadores de doença subaguda ou crônica do sistema nervoso central (SNC), visto que este comprometimento pode vir associado a doença disseminada em 40% dos casos, ocorrendo sob a forma de meningite isolada e lesões locais em 25% e encefalite em 10% dos casos (AIDÉ, 2009). Ocorrem manifestações gastrointestinais em uma porcentagem menor, embora em estudos realizados no Peru e no Panamá, a diarreia foi descrita em 40 e 50% dos pacientes, respectivamente (PÉREZ-LAZO *et al*., 2017).

As lesões cutâneas são incomuns nos pacientes diagnosticados nos EUA, ocorrendo em menos de 10% dos casos (KAUFFMAN, 2007). Já na América Latina essas injurias são relatadas em 38% a 85% dos casos de pacientes coinfectados com aids, sugerindo um tropismo cutâneo das cepas infectantes (GOLDANI *et al*., 2009). As lesões cutâneas são polimórficas e de distribuição variada, com predomínio das apresentações papulares, nódulo-eritematosas e úlceras. Raramente apresentando-se de forma vesiculopustulosas, herpetiformes ou tipo foliculite (ROCHA; SEVERO, 1994; FOCACCIA, 2021).

Exames laboratoriais podem mostrar pancitopenia, altos níveis de lactato desidrogenase (DHL), aumento de proteína C reativa, elevação da ferritina e das transaminases.

As alterações radiográficas aparecem em 50% a 70% dos pacientes que apresentam a forma disseminada. A ultrassonografia e a tomografia computadorizada do abdômen podem mostrar a presença de adenomegalias retroperitoneais e mesentéricas e hepatoesplenomegalia, por vezes com lesões focais, em ambos os órgãos, em particular, no baço (WHEAT *et al*., 1990). Os achados radiológicos torácicos mais comuns são infiltrados intersticiais difusos, reticulonodular e padrão miliar; entretanto, em muitas vezes, o primeiro exame pode não apresentar alterações (ADENIS; AZNAR; COUPPIÉ, 2014; HAGE *et al*., 2015).

A tabela 2 apresenta a frequência das manifestações clínicas e parâmetros de exames complementares em pacientes coinfectados com aids e HD.

TABELA 2 - Manifestações clínicas e exames complementares na coinfecção aids e histoplasmose disseminada

Referência	País (período)	n	Febre (%)	Perda de peso (%)	Sintomas respiratórios (%)	RX tórax infiltrado (%)	Adenomegalia (%)	Pancitopenia (%)	Lesões cutâneas (%)
13	Brasil (2011-2016)	23	87	65	60	-	-	-	52
35	Brasil (2016-2017)	123	100	91	-	56	06	52	39
42	Argentina (2000-2011)	80	84	-	72	77	-	42	56
48	Panamá (1997-2003)	104	92	62	63	75*	-	34	17
54	EUA (1983-1987)	48	81	52	21	52	19	27	-
62	Argentina (2009-2014)	171	09	13	24	30	04	-	64
80	Brasil (1987-2002)	32	85	86	78	75**	65	-	3
82	Peru (1996-2014)	27	66	29	29	-	33	70	11
84	Brasil (2006-2010)	48	92	-	70	-	41	-	31
86	Venezuela (1984-1990)	200	88	95	77	81	77	53	2
96	México (2015-2017)	85	95	94	-	74	48	...	32
97	Brasil (1977-2002)	111	97	-	61	86	35	24	44
100	Colômbia (1998-2004)	44	95	-	61	-	34	-	19
102	Guiana Francesa (2008-2010)	27	51	89	22	-	35	71	-

Fonte: compilação da autora. Legenda: *(n=54) **(n=16)

A coinfecção aids e HD pode ocorrer de forma concomitante com outras infecções oportunistas como a tuberculose, pneumocistose, toxoplasmose, citomegalovirose, micobacteriose não tuberculosa, criptococose, candidíase esofágica, criptosporidiose (GREENE *et al*., 2000; UNIS; OLIVEIRA; SEVERO, 2004; NUNES *et al*., 2016; PÉREZ-LAZO *et al*., 2017; SAMAYOA *et al*., 2017; BOIGES *et al*., 2018; CÁCERES; VALDES, 2019; FALCI *et al*., 2019; BASSO *et al*., 2020) e mais recentemente com a covid-19 (BERTOLINI *et al*., 2020; MESSINA *et al*., 2020; BARROSO *et al*., 2021; BASSO *et al*., 2021). Devido à semelhança clínica da HD com as outras infecções oportunistas, é fundamental a investigação precoce e simultânea dessas infecções, visando instituir tratamento específico para cada agente etiológico (CÁCERES *et al*., 2018a).

2.8 Diagnóstico da histoplasmose

Para o diagnóstico da histoplasmose são levados em consideração o histórico clínico do paciente, aspectos dos exames de imagem, resultado de exame micológico direto, achados histopatológicos, cultivo, exames sorológicos convencionais, detecção de biomarcadores, e/ou métodos moleculares (WHEAT, 1989; AIDÉ, 2009; WHEAT *et al*., 2016).

2.8.1 Microscopia direta

Diversas amostras biológicas podem ser submetidas ao exame microscópico: escarro e lavado brônquico, raspado de lesão cutânea, aspirado de medula óssea ou aspirado ganglionar, e outros líquidos e tecidos (FOCACCIA, 2021).

As colorações com maior sensibilidade e especificidade são a metenamina prata de Gomori ou Grocott, e Giemsa, respectivamente. Outra opção é o uso do Calcofluor *white*, um reagente fluorescente que se liga à quitina na parede celular de todos os fungos (AZAR; HAGE, 2017). O resultado positivo na microscopia é dado pela presença de pequenas células leveduriformes (2 a 5 µm de diâmetro) redondas ou ovais, circundadas por halo fino incolor, correspondente à parede celular fúngica. A parede que não é tingida pelos corantes sugere de modo incorreto, a presença de cápsula, e justifica o nome da espécie. O exame citológico pode ser pouco sensível e um resultado negativo não deve descartar a hipótese de histoplasmose. O exame direto deve ser sempre complementado com o cultivo ou a análise histopatológica da amostra biológica (GUIMARÃES; NOSANCHUK; ZANCOPÉ-OLIVEIRA, 2006).

A microscopia apesar de ser uma técnica de baixo custo, é pouco sensível e de interpretação difícil, em razão do tamanho reduzido dos blastoconídios do *H. capsulatum* e de sua semelhança com outros fungos patogênicos (AZAR; HAGE, 2017). Estima-se que cerca de 50% das amostras de aspirados de medula óssea de pacientes com aids são positivas (WHEAT, 2009).

2.8.2 Cultivo

É o método padrão-ouro e fornece o diagnóstico definitivo de histoplasmose. O crescimento fúngico é lento, de uma a seis semanas, o que dificulta o diagnóstico nos casos graves, retardando o início do tratamento (AZAR; HAGE, 2017). A especificidade é de 100% e a sensibilidade depende da apresentação clínica (pulmonar versus disseminada), do estado de imunidade do hospedeiro, e da gravidade da doença. Pacientes com histoplasmose disseminada apresentam maior taxa de culturas positivas (74%) do que pacientes com a forma pulmonar aguda (42%) (ADENIS; AZNAR; COUPIE, 2014). Para a forma disseminada, o aspirado de medula óssea é o material com maior sensibilidade neste exame (50-70%) (WHEAT, 1989; ADENIS; AZNAR; COUPIE, 2014; AZAR; HAGE, 2017).

Todo processamento da amostra biológica deve ser realizado em cabine de biossegurança classe III (GUIMARÃES; NOSANCHUK; ZANCOPÉ-OLIVEIRA, 2006; FOCACCIA, 2021). O cultivo é obtido com a semeadura das amostras biológicas na superfície de meios sólidos. Os meios devem estar em tubos de ensaio, pois a manipulação da cultura em placas de Petri oferece alto risco de infecção ao laboratorista. Amostras clínicas como sangue e aspirado de medula óssea devem ser inoculados, diretamente, em tubos contendo meio de cultura líquida para aumentar a sensibilidade do exame. Qualquer meio disponível no comércio para hemoculturas de bactérias ou fungos, tais como ágar infusão cérebro-coração (BHI – *brain heart infusion*) ou ágar Sabouraud, são suficientes para o cultivo das amostras (FOCACCIA, 2021).

H. capsulatum quando isolado em meio de cultura e incubado à temperatura abaixo de 35ºC inicia a formação de filamentos, denominadas hifas, que agrupadas formam o micélio, observado macroscopicamente como colônias brancas filamentosas. O micélio contém microconídios (menores que 5 µm), assim como macronídios tuberculados, cobertos por projeções espiculadas (FERREIRA; BORGES, 2009; FOCACCIA, 2021.

A conversão da forma filamentosa para a forma leveduriforme é essencial para a confirmação do diagnóstico, pois o *H. capsulatum* apresenta características morfológicas que podem ser confundidas com estruturas de outros fungos contaminantes dos gêneros *Renispora* spp., *Chrysosporium* spp. e *Sepedonium* spp. (FOCACCIA, 2021). A transformação para a fase leveduriforme pode ocorrer quando a cultura é incubada a 35-37°C, formando colônias de coloração branca a marrom de superfície lisa e textura cremosa A 37ºC, as leveduras são pequenas (3 a 5µ de diâmetro), ovaladas e frequentemente apresentam gemulação única (WANKE; LAZÉRA, 2004).

2.8.3 Histopatologia

Para os métodos histopatológicos em biópsias podem ser utilizadas as colorações de hematoxilina-eosina (HE), ácido periódico de Schiff (PAS) e a metenamina prata de Gomori-Grocott, com a visualização do fungo fagocitado por polimorfonucleares e macrófagos (WHEAT, 2016; FOCACCIA, 2021).

A coloração HE é utilizada na triagem e permite a observação de células leveduriformes em brotamento único, roxo-azuladas, circundadas por halo claro, via de regra no interior dos macrófagos e polimorfonucleares, em infiltrado inflamatório de intensidade variada (FOCACCIA, 2021). Esses elementos leveduriformes podem ser visualizados no exame microscópico realizado diretamente a partir da amostra clínica. Na coloração de Giemsa (ou Wright), os fungos mostram uma massa cromática polar, azul e na forma de meia lua. Corados pelo PAS, apresentam-se na cor vermelha e pela prata metenamina de Gomori-Grocott, na cor negra ou marrom-escura (FERREIRA; BORGES, 2009). A resposta do tecido no imunodeprimido costuma ser insignificante, sendo melhor avaliada pela coloração com PAS (AIDÉ, 2009; FERNÁNDEZ ANDREU et *al*., 2011). A prata metenamina é considerada uma das melhores técnicas porque oferece um bom contraste e frequentemente cora células fúngicas que são resistentes ao PAS (WHEAT *et al*., 2016).

A observação em qualquer tipo de amostra clínica de levedura intracelular com gemulação única e sem formação de hifas é muito sugestiva de histoplasmose. No entanto, alguns agentes como o *Cryptococcus* spp. mal encapsulado, *Penicillium marneffei*, *Pneumocystis jirovecii*, *Sporothrix* spp., *Candida* não-*albicans*, *Toxoplasma gondii*, e *Leishmania* spp. podem ser encontrados intracelularmente e confundidos com *H. capsulatum*, levando a um diagnóstico incorreto, sendo o diagnóstico diferencial muitas vezes difícil até para patologistas experientes (FERNÁNDEZ ANDREU *et al*., 2011; KAUFFMAN, 2007). Outra

desvantagem é o tempo de preparação dos tecidos, que pode levar a um atraso diagnóstico (KAUFFMAN, 2007).

2.8.4 Métodos sorológicos convencionais

Os métodos de detecção de anticorpos oferecem alternativa rápida dentre as técnicas diagnósticas. Segundo Guimarães, Nosanchuk e Zancopé-Oliveira (2006), as técnicas de imunodifusão dupla (ID) e a reação de fixação do complemento (FC) são as duas principais técnicas utilizadas na rotina laboratorial devido a sua conveniência, disponibilidade e precisão.

O método de fixação do complemento (FC) é mais sensível e menos específico que a imunodifusão dupla (ID), mas requer grande perícia e habilidade do laboratorista. A especificidade varia entre 70% a 80% e as reações cruzadas podem ocorrer com blastomicose, candidíase e paracoccidioidomicose (GUIMARÃES; NOSANCHUK; ZANCOPÉ-OLIVEIRA, 2006; KAUFFMAN, 2007). Os títulos de anticorpos de 1:8 e 1:16 podem ser observados em indivíduos com infecções passadas ou que vivem em regiões endêmicas expressando resultados fracamente positivos (WHEAT, 1989; GUIMARÃES; NOSANCHUK; ZANCOPÉ-OLIVEIRA, 2006).

A imunodifusão dupla detecta a presença de anticorpos por meio da reação com precipitinas específicas H e M, fornecendo uma análise qualitativa em que se reconhece como positiva a detecção de uma ou duas linhas de precipitação, as linhas H e M. A linha H usualmente coexiste com a linha M, no entanto, frequentemente a linha M se apresenta como única (WHEAT, 2001). A presença de banda de precipitina H, relacionada com infecção ativa, é vista ocasionalmente, enquanto a banda M pode manter-se presente por anos. A sensibilidade deste método encontra-se entre 20 a 50% nos pacientes com HD (WHEAT, 1989).

Estes ensaios são pouco úteis em pacientes imunossuprimidos, considerando-se que a produção de anticorpos pode ser limitada nessa população (HAGE *et al*., 2015; WHEAT *et al*., 2016). E, em todos os casos, a interpretação dos testes sorológicos deve ser realizada em conjunto com os dados clínicos, epidemiológicos e micológicos (FERNÁNDEZ ANDREU, *et al*., 2011; AZAR; HAGE, 2017).

2.8.5 Métodos baseados na detecção de antígenos do *H. capsulatum*

Em 1989 Wheat e colaboradores desenvolveram um método rápido e promissor para a detecção do antígeno galactomanana de *H. capsulatum* em amostras de urina e sangue. Atualmente a detecção deste antígeno polissacarídeo em amostra de urina é amplamente

utilizada, e é realizada pelo método de ensaio de fluxo lateral (LFA – Lateral Flow Assay), de forma qualitativa, ou de ELISA, com resultados quantitativos. Em pacientes com aids e HD a sensibilidade do exame é de 95- 100% (ADENIS; AZNAR; COUPPIÉ, 2014; FANDIÑO-DEVIA *et al*., 2016; DANTAS *et al*., 2018.).

Um estudo conduzido por Cáceres e colaboradores (2018b) utilizando um ensaio imunoenzimático monoclonal comercial de *Histoplasma* Galactomanana (HGM) (Immuno-Mycologics [IMMY], Norman, OK, EUA), demonstrou que este teste de antigenúria comercial é de simples execução e pode ser usado para diagnóstico de HD com resultados sensíveis e específicos. Suas potenciais desvantagens são a necessidade de vários "poços" para controle de qualidade e formação da curva-padrão, o que pode reduzir seu custo-efetividade (NACHER *et al*., 2018).

Em 2019 a Organização Mundial da Saúde (OMS) incluiu a pesquisa do antígeno urinário para HD na Lista Anual de Exames Diagnósticos, como exame diagnóstico essencial (WHO, 2019). No Brasil este teste de antigenúria, através do LFA, foi liberado comercialmente pela Agência Nacional de Vigilância sanitária (ANVISA) em abril de 2021 (BRASIL, 2021). A liberação e incorporação deste teste nos laboratórios clínicos tornou-se possível, reduzindo os problemas técnicos relacionados ao desempenho de técnicas *in house*. Nos locais em que já foram implementados, foi possível diagnosticar um maior número de pacientes do que com as metodologias clássicas de diagnóstico (cultivo e histopatologia) (FALCI *et al*., 2019). Uma das vantagens do teste é a facilidade de realização da técnica sem infraestrutura específica, podendo ser realizado em laboratórios sem a estrutura de biossegurança necessária para realização de culturas ou testes moleculares (HOFFMANN *et al*., 2016; CÁCERES *et al.*, 2018b; FALCI *et al.*, 2019).

A precocidade diagnóstica decorrente da implementação destes testes para detecção de biomarcadores na rotina diagnóstica leva a redução na mortalidade associada à HD (SAMAYOA *et al.*, 2017; FALCI *et al*., 2019).

2.8.6 Métodos moleculares

O desenvolvimento de testes utilizando a reação em cadeia da polimerase (PCR) para detecção do *H. capsulatum* em tecidos e fluidos corporais tem sido o foco de muitos laboratórios para um diagnóstico mais rápido e sensível (DANTAS *et al.*, 2018). A fim de alcançar uma maior sensibilidade e especificidade, foram analisados vários métodos de PCR visando diferentes regiões do genoma de *H. capsulatum*, incluindo PCR convencional, NESTED e a

amplificação isotérmica mediada por loop (LAMP - *loop-mediated isothermal amplification*) (BUITRAGO *et al*., 2013).

Uma metanálise conduzida por Cáceres e colaboradores (2019b) encontrou cinco estudos avaliando a aplicabilidade de métodos moleculares, utilizando diferentes espécimes: respiratórios, amostra de tecido, sangue e medula óssea. Entre os estudos, não houve consenso em relação ao protocolo e genes-alvo utilizados e todos aplicaram métodos *in house*. Dessa forma, embora seja promissor o diagnóstico molecular, a falta de métodos padronizados, o pequeno número de estudos de validação e a sua indisponibilidade são limitações para o seu uso atual na rotina diagnóstica.

2.9 Tratamento da histoplasmose

Segundo a Sociedade Americana de Doenças Infecciosas (IDSA) o tratamento da histoplasmose está indicado para a forma pulmonar aguda com sintomas graves ou moderados, forma pulmonar crônica e forma disseminada (WHEAT, 2007). A escolha da terapia antifúngica e o tempo de duração do tratamento depende da gravidade e da forma clínica da doença.

Os antifúngicos de eleição utilizados para o tratamento da histoplasmose são a anfotericina B lipossomal (L- AmB), anfotericina B complexo lipídico (ABLC), anfotericina B desoxicolato (d-AmB) e o itraconazol (ITC) (WHEAT, 2007).

2.9.1 Fármacos antifúngicos para a Histoplasmose

2.9.1.1 Anfotericina B

A anfotericina B (AmB) é um agente antifúngico poliênico, produzido com base no actinomiceto *Streptomyces nodosus*. Sua atividade está relacionada a sua ligação ao ergosterol na membrana da célula fúngica, aumentando a permeabilidade da membrana, e por consequência ocasionando o extravasamento de moléculas e eletrólitos do meio intracelular para o extracelular (WANKE; LAZERA, 2004). É indicada no tratamento da maioria das infecções fúngicas sistêmicas, sendo administrada por via endovenosa, mas podendo ser administrada por via intratecal (na meningite fúngica) ou intra-articular. A absorção gastrintestinal de todas as formulações de AmB é insignificante (BENNETT, 2019). Essa droga se distribui amplamente por todo o organismo, inclusive atravessa a barreira placentária, mas com baixa penetração no líquido cefalorraquidiano, humor vítreo e líquido sinovial. É excretada

por via renal, sendo nefrotóxica por suas propriedades vasoconstritoras renais e por sua ação lesiva direta sobre o epitélio renal (WANKE; LAZERA, 2004).

Os efeitos adversos observados com o uso da AmB podem ocorrer de forma imediata durante a infusão, com manifestação de febre, calafrios, taquicardia, hipertensão arterial, náuseas, vômitos e taquipneia (MARTINEZ, 2006). A reação cessa espontaneamente em 30 a 45 minutos, podendo ser abreviada com a administração de meperidina e a diminuição da velocidade de infusão. O pré-tratamento com paracetamol oral ou o uso de hemissuccinato de hidrocortisona por via intravenosa, em uma dose de 0,7 miligrama por quilograma (mg/kg) no início da infusão, diminuem as reações. As reações febris cedem nas infusões subsequentes. Os lactentes, as crianças e os pacientes que recebem doses terapêuticas de glicocorticoides têm menor tendência a sofrer reações (BENNETT, 2019). A nefrotoxicidade e a anemia também podem ser observadas, necessitando nestes casos da correção de doses e dos intervalos da administração ou até mesmo da suspensão da terapia (MARTINEZ, 2006). Em virtude da ocorrência de acidose tubular e perda renal de potássio e magnésio durante a terapia, deve-se monitorar esses eletrólitos e, se necessário, fazer suplementação (BRASIL, 2018).

A anfotericina B é insolúvel em água, mas foi formulada para infusão intravenosa mediante a formação de um complexo do fármaco com o sal biliar, desoxicolato (BENNETT, 2019). Na década de 1990, quarenta anos após a introdução da anfotericina B desoxicolato, três formulações à base de lipídios foram desenvolvidas com o objetivo de reduzir a nefrotoxicidade sem comprometer a eficácia da medicação: L-AmB, ABCL e anfotericina B em dispersão coloidal (ABDC) (OSTROSKY-ZEICHNER *et al*., 2003).

A anfotericina lipossomal (L-AmB) apresenta o agente antifúngico incorporado em lipossomos, microesferas lipídicas com 55 a 75 nm, preparadas com lecitina de soja, colesterol e diestearoilfosfatidilglicerol, apresentando baixa toxicidade quando em comparação com a desoxicolato. Apresenta a menor toxicidade dentre todas as formulações, tanto imediata como crônica, possibilitando infusão rápida e uso seguro de doses de até 5 mg/kg/dia. Além disso apresenta melhor penetração no SNC quando comparada com as outras formulações (WHEAT; MUSIAL; JENNY-AVITAL, 2005; MARTINEZ, 2006).

A ABCL é um agregado lipídico estabilizado da anfotericina B, formando uma estrutura semelhante a um anel. Após administração endovenosa, a ABCL é rapidamente metabolizada, e altas concentrações são sequestradas pelo tecido reticuloendotelial no fígado, baço e pulmão.

Na ABDC, o antifúngico está contido, juntamente com sulfato de colesterol, em microdiscos, acarretando menos nefrotoxicidade, mas com reações imediatas que podem ser reduzidas com a infusão lenta da substância (MARTINEZ, 2006).

2.9.1.2 Derivados azólicos

Os azólicos são antifúngicos que possuem anel pentagonal na estrutura molecular, classificados em imidazólicos quando contêm três átomos de carbono e dois de nitrogênio (clotrimazol, miconazol, cetoconazol, econazol, butoconazol, oxiconazol, sertoconazol e sulconazol), ou triazólicos quando apresentam dois de carbono e três de nitrogênio (ITC, fluconazol, voriconazol, posaconazol e ravuconazol). Atuam inibindo enzimas do citocromo P450 dos fungos, o que acarreta bloqueio na demetilação do lanosterol e na síntese de ergosterol, alterando a permeabilidade da membrana e a viabilidade fúngica. Agem, também, modificando a síntese de lipídios e inativando enzimas do processo oxidativo dos fungos (MARTINEZ, 2006). Os triazóis sistêmicos são metabolizados mais lentamente e exercem menos efeito sobre a síntese de esteróis humanos do que os imidazólicos (BENNETT, 2019).

O ITC exibe atividade fungistática e está disponível em forma de cápsulas, sendo em geral bem absorvido, principalmente após refeições completas. O pico de concentração plasmática é atingido duas a cinco horas após administração oral. Essa droga passa por metabolismo hepático extenso e origina diversos metabólitos, sendo que o principal deles é o hidroxi-itraconazol, cuja concentração plasmática é aproximadamente o dobro do fármaco inalterado. A meia-vida terminal do ITC é cerca de 17 horas após dose única, aumenta para 34 a 42 horas com doses repetidas. O ITC é excretado como metabólito inativo na urina e nas fezes. A maior parte do ITC disponível no plasma está ligada à albumina (99,6%), mas também há afinidade considerável por lipídios. Este fármaco não é carcinogênico, mas, como é teratogênico em ratos, está contraindicado durante a gravidez ou para mulheres que pretendem engravidar (BENNETT, 2019). Apresenta interação medicamentosa com muitos fármacos, tendo sua absorção reduzida com uso de antiácidos, e tanto a fenitoína como a rifampicina podem reduzir seus níveis plasmáticos. Os inibidores da protease podem aumentar o nível sérico do ITC e este pode diminuir o nível sérico do efavirenz (BENNETT, 2019).

2.9.2. Abordagem terapêutica da histoplasmose disseminada de acordo com a classificação de gravidade

Em 2020, a Organização Pan-Americana para a Saúde (OPAS), em conjunto com a OMS, publicou a primeira diretriz para o diagnóstico e tratamento da HD entre pacientes vivendo com HIV/aids (PAHO/WHO, 2020). Neste documento a HD é classificada em:

• Histoplasmose grave ou moderadamente grave, definida como a presença de pelo menos um sinal ou sintoma envolvendo órgãos vitais: insuficiência respiratória ou circulatória,

sinais neurológicos, insuficiência renal, anomalias de coagulação e alteração geral do status de desempenho da OMS maior que 2, definido como confinação da pessoa a uma cama ou cadeira por mais da metade das horas de vigília e com limitações no autocuidado.

• Histoplasmose leve a moderada, definida pelo aparecimento de sinais e sintomas não incluídos entre as características supracitadas.

No tocante ao tratamento, é recomendado o uso de anfotericina lipossomal para terapia de indução em doença grave ou moderadamente grave, seguida de terapia de manutenção com ITC por 12 meses.

2.9.2.1 Indução

A primeira opção da terapia de indução para a forma grave ou moderadamente grave entre pessoas que vivem com HIV é L-AmB, 3,0 mg/kg/dia. Na indisponibilidade desta, a alternativa é a d-AmB, 0,7-1,0 mg/kg/dia. A terapia de indução deve ser administrada por duas semanas. Uma vez que a d-AmB pode estar associada à toxicidade renal, a terapia pode requerer duração mais curta de acordo com a avaliação clínica e resposta ao tratamento (CDC, 2020; PAHO/WHO, 2020). O envolvimento do sistema nervoso central pode exigir extensão da terapia de indução ou aumento da dosagem (PAHO/WHO, 2020).

Já na apresentação leve a moderada a opção terapêutica é o ITC 200 mg de 8 em 8 horas, por três dias e, em seguida, 200 mg de 12/12 horas por 12 meses (PAHO/WHO, 2020).

2.9.2.2 Consolidação

A recomendação da terapia de consolidação é ITC 200 mg de 12/12 horas por 12 meses. Um período menor que 12 meses pode ser considerado para o paciente clinicamente estável, no uso de terapia antirretroviral (TARV), que apresente carga viral indetectável e evidente melhora do status imunológico (CDC, 2020; PAHO/WHO, 2020). Duas semanas após o início da terapia, é recomendado a avaliação do nível sérico do ITC. A absorção que pode ser irregular e as potenciais interações medicamentosas com fármacos que usualmente fazem parte do arsenal terapêutico na infecção pelo HIV (antirretrovirais como efavirenz e inibidores da protease), além de outros medicamentos aleatórios, justificam esta indicação. Um nível sérico de 1 a 2 μg/mL é o indicado, e o número e a gravidade dos eventos adversos aumentam quando os níveis são ≥4 μg/mL (CDC, 2020).

2.9.2.3 Momento de início da terapia antirretroviral

A TARV no paciente com HD deve ser iniciada a partir do 14° dia do início do tratamento antifúngico, assim como em casos agudos de outras infecções oportunistas (pneumocistose e neurotoxoplasmose). Esta recomendação não se aplica se o envolvimento do sistema nervoso central na histoplasmose é suspeito ou comprovado, assim como na meningite criptocócica e na meningite tuberculosa. Esta postergação é justificada devido ao aumento da mortalidade relacionada com a síndrome da reconstituição imune (BRASIL, 2018; PAHO/WHO, 2020).

2.9.2.4 Coinfecção HIV, tuberculose e histoplasmose disseminada

A terapia da tuberculose deve ser realizada seguindo o preconizado nas diretrizes de tratamento da OMS (PAHO/WHO, 2020). Deve ser avaliado o risco de resistência ao *Mycobacterium tuberculosis* e a interação medicamentosa do regime terapêutico simultâneo de rifampicina-itraconazol (levando a níveis sanguíneos subterapêuticos do ITC e consequentemente falha no tratamento da histoplasmose). Devido a interação da rifampicina com o ITC pode ser indicada a extensão da duração da terapia de indução com AmB ou aumento da dosagem diária do ITC, bem como é indicado o monitoramento dos níveis sanguíneos do ITC, se disponível, ou a substituição da rifampicina pela rifabutina (CDC, 2020; PAHO/WHO, 2020;).

2.10 Prevenção da exposição ao *H. capsulatum*

Pacientes com HIV que apresentem LTCD4+ <150 células/mm^3 devem evitar atividades predisponentes para a aerossolização e inalação dos microconídios do *H. capsulatum*, tais como aquelas que levantem poeira do solo, limpeza de galinheiros ou construções antigas, permanência em áreas contaminadas com fezes de pássaros ou morcegos, e exploração de cavernas (CDC, 2020).

Dados de um estudo prospectivo, randomizado e controlado indicam que o ITC pode reduzir a frequência da histoplasmose, embora não a mortalidade, em pacientes com HIV e que vivem em áreas onde a histoplasmose é altamente endêmica (MCKINSEY *et al.*, 1999). Em 2020, o CDC recomendou a profilaxia primária com ITC (200 mg por dia) para pacientes com contagens de LTCD4+ <150 células/mm^3 que estão em alto risco devido à exposição ocupacional ou que vivem em uma comunidade com uma taxa hiperendêmica de histoplasmose (>10 casos/100 pacientes-ano). Esta profilaxia pode ser descontinuada em pacientes em uso de TARV, com carga viral indetectável e LTCD4+ de ≥150 células/mm^3 durante 6 meses ou mais.

A profilaxia deve ser reiniciada se a contagem de LTCD4+ cair para <150 células/mm^3 (CDC, 2020). Não há indicação de profilaxia primária utilizando antifúngicos no Brasil (BRASIL, 2018).

A profilaxia secundária está indicada para prevenção de quadros de recorrência com doença disseminada ou quando há acometimento do SNC. Deve ser instituída após a fase de consolidação, com ITC 200mg ao dia, que deverá ser mantido por 12 meses (WHEAT *et al*., 2007). O CDC, seguindo as orientações do AIDS Clinical Trial Group (ACTG), definiu a segurança da interrupção do ITC para os pacientes que tenham uma boa resposta imunológica à TARV. Os critérios para a suspensão da profilaxia secundária são: estar há mais de um ano no uso da terapia com ITC; hemoculturas negativas para fungos; antígeno urinário indetectável, e carga viral para o HIV indetectável há pelo menos seis meses. A terapia supressora deve ser retomada se a contagem de LTCD4+ diminuir para <150 células/ mm^3 (CDC, 2020).

No Brasil o tratamento (indução e consolidação) segue as orientações da IDSA. Em relação à suspensão da profilaxia secundária (ou manutenção), o Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos (BRASIL, 2018) orienta a manutenção por tempo indeterminado, justificando que não há evidência suficiente para a recomendação da interrupção do uso do ITC. Mas considera a suspensão após período mínimo de um ano de tratamento de manutenção e ausência de sintomas e LTCD4+ >150 células/mm^3 por mais de seis meses, preconizando a reintrodução se LTCD4+ <150 células/mm^3.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar aspectos clínicos, epidemiológicos e exames complementares laboratoriais e de imagem em pacientes com infecção pelo HIV e HD atendidos no Hospital Universitário Dr. Miguel Riet Corrêa Júnior, hospital terciário no extremo sul do Brasil.

3.2 Objetivos Específicos

3.2.1 Avaliar a incidência da HD nos pacientes com infecção pelo HIV hospitalizados, e comparar essa taxa entre períodos pré e pós incremento da antigenúria no diagnóstico da doença;

3.2.2 Descrever dados sociodemográficos da população do estudo, os sinais clínicos associadas a HD e seus fatores associados, como: uso de antirretrovirais, níveis de LTCD4+ e carga viral para HIV;

3.2.3 Avaliar o tempo para a realização do diagnóstico da histoplasmose desde o início da sintomatologia, coinfecções, e o desfecho em 365 dias.

3.2.4 Relatar casos atípicos de coinfecções histoplasmose/micobactérias não tuberculosas e histoplasmose/covid-19.

4 JUSTIFICATIVA

O Serviço de Infectologia do Hospital Universitário Dr. Miguel Riet Corrêa Júnior (HU-FURG) é referência para o tratamento do paciente com infecção pelo HIV para 21 municípios do estado do Rio Grande do Sul, desde a década de 1990. Até dezembro de 2019, 4.700 pacientes com infecção pelo HIV estavam cadastrados no serviço. A taxa de detecção de aids no município, no ano de 2020 foi de 56,6 casos a cada 100.000 habitantes. Desde 2017 o Ministério da Saúde elaborou um ranking (seguindo parâmetros como taxa de detecção, mortalidade média e a primeira contagem de LT CD4+) dentre os municípios com mais de 100 mil habitantes, e o município do Rio Grande encontra-se em primeiro lugar no país, pelo terceiro ano consecutivo (BRASIL, 2020).

Uma vez que se conheça melhor a epidemiologia da HD, ações de vigilância locais poderão ser adotadas e desenvolvidas, reduzindo a morbimortalidade associada à doença. Também é fundamental alertar os profissionais da saúde sobre a importância desta micose. Assim, este trabalho visa documentar a incidência da HD, as coinfecções e a mortalidade associadas à esta micose sistêmica, em pacientes infectados pelo HIV atendidos no HU-FURG/EBSERH.

5 CONTRIBUIÇÕES CIENTÍFICAS

5.1 Artigo 1

Manuscrito nas normas e submetido à revista *Emerging Infectious Diseases* (Fator de impacto - FI: 6.883)

Disseminated histoplasmosis in a reference center for HIV-aids patients in Southern Brazil

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ABSTRACT

Disseminated histoplasmosis (DH) is an aids-defining disease with important morbidity and mortality in people living with HIV/aids (PLHIV) in Latin American. We aim to evaluate the clinical and epidemiological profile of patients with DH and aids coinfection in a reference service for PLHIV over a period of ten years in Southern Brazil. A retrospective study including all DH cases diagnosed in HIV/aids patients at University Hospital (UH-FURG/Ebserh) was performed. Clinical and epidemiological data of patients were analyzed, as well as diagnosis, treatment and outcome data. DH incidence rate was 12 cases per 1,000 hospitalizations of PLHIV at UH-FURG/Ebserh over the period 2010-2019, with a mortality rate of 35%. Co-infection with tuberculosis (TB) occurred in 29% of the patients. The increase of 300% in the incidence rate of this mycosis associated to a better investigation of the disease in PLHIV and to the implementation of a biomarker detection as new diagnosis method, emphasizes the necessity to investigate DH in parallel with TB in these patients in our hospital.

KEYWORDS: *Histoplasma capsulatum*, people living with HIV/aids, fungal infection, pulmonary disease.

INTRODUCTION

Histoplasmosis is an endemic mycosis caused by the dimorphic fungus *Histoplasma capsulatum* (Cáceres et al., 2018). The disease is mostly diagnosed in American continents, representing a health problem with equal or even highest impact than tuberculosis (TB) (Pasqualotto & Quieroz-Telles, 2018). Disseminated histoplasmosis (DH) is an aids-defining disease and one of the major causes of mortality in people living with HIV/aids (PLHIV) (13 to 48%) (Scheel et al., 2009; Baddley et al., 2008; Gómez, 2011; Cáceres et al., 2012; Nacher et al., 2013; Cáceres et al., 2016; Centre d'Investigation Clinique Antilles Guyane & Centre Hospitalier de Cayenne, Université de Guyane, 2016; Medina et al., 2017; Samayoa et al., 2017).

DH has a status of neglected disease, mainly due to its nonspecific symptoms, frequently misdiagnosed as tuberculosis, associated with a limitation of access to diagnosis methods with high sensitivity rates. Thus, an early diagnosis are not common to occur, and consequently the correct treatment are prescribed lately (Centre d'Investigation Clinique Antilles Guyane & Centre Hospitalier de Cayenne, Université de Guyane, 2016; Adenis et al., 2018). Worsening this scenario, in Brazil, an epidemic of aids is undergoing, with more than 800,000 new cases diagnosed in the last decades (Traebert et al., 2018). Efforts are necessary to understand the epidemiology of DH/aids co-infection in different endemic areas in this country.

More specifically, Rio Grande city (RG) at the southern state of Brazil (Rio Grande do Sul, RS) has the highest rate of HIV/aids patients among all Brazilian cities with more than 100,000 inhabitants (Brasil, 2020). Therefore we aimed to evaluate the clinical and epidemiological profile of patients with DH and aids coinfection in a reference service for PLHIV over a period of ten years in Southern Brazil, and to compare the incidence rate between periods before and after an internal improvement on DH investigation, even using urinary antigen test as a diagnostic method.

METHODS

Study area and design

A retrospective study was performed including all DH cases diagnosed in HIV/aids patients at a regional reference service in University Hospital *Dr. Miguel Riet Corrêa Jr.*,

Universidade Federal do Rio Grande/ *Empresa Brasileira de Serviços Hospitalares* (UH-FURG/Ebserh). The hospital is localized in RG, a city which covers a total of 2,817 km^2 of area and comprising an estimated population of 211,965 inhabitants in Southern Brazil (IBGE, 2020). The UH-FURG/Ebserh is a tertiary hospital with 207 beds, serving as a regional reference center to 27 cities, with an annual mean of 257 hospitalizations of HIV-aids patients/year, and a mortality rate of 19% in these patients by the last epidemiological bulletin (2015-2019) (Brasil, 2020). The study was approved by our university ethics committee (CEP/FURG; Nº 234/2018).

Case definition

DH cases were defined by 1) Classical methods: Growth of *H. capsulatum* in mycological culture of biological samples and/or presence of small oval blastoconidia suggestive of *H. capsulatum* in microscopy through Gomori-Grocott stain technique (direct mycological examination - DME or histopathology); 2) Serological method: positive serum sample by immunodiffusion test (IMMY®, Immuno-Mycologics, Oklahoma, United States); 3) Urinary antigen: positive urine sample in immunoenzymatic assay (IMMY®, Immuno-Mycologics, Oklahoma, United States). Patients with clinical suspicion and, at least one of these diagnosis criteria, were included in the study.

Clinical-epidemiological data and statistical analyses

Databases from the hospital were analyzed to collect data regarding gender, age, diagnosis test, symptoms, blood parameters, and chest computed tomography results, rates of CD4+ lymphocytes and HIV viral load, use of antiretroviral therapy at the DH diagnosis, coinfections (TB and non-TB), antifungal therapy, time elapsed between the appearance of symptoms until the diagnostic, number of TB or DH investigations previous to the diagnosis confirmation, and outcome (discharge or death) after 12 months. In cases with a simultaneous diagnosis of HIV/aids, DH was considered as the AIDS-defining illness.

Overall incidence rate of DH was calculated per one-thousand hospitalizations of patients with aids at UH-FURG/Ebserh. This rate was compared between the period pre (2010-2016) and post (2017-2019) improvement of the DH investigation in these patients, including the urinary antigen detection as another diagnosis method, by the formula: (number of DH hospitalization/number of patients with aids hospitalization – total and per year) x 1000 (Horta et al., 2015). Descriptive and frequencies analyses were performed by the statistical program SPSS 25.0® (IBM, USA).

RESULTS

A total of 31 cases of DH were included in the study, representing an overall incidence rate of 12 new cases per 1,000 PLHIV hospitalized at UH-FURG/Ebserh. In the period pre implementation of the urinary antigen test (2010-2016), this rate was 8/1,000 hospitalizations, and post implementation (2017-2019) it increased to 24/1,000 hospitalizations, representing an increase of 300% in the DH diagnosis.

The majority of the patients were men (74%; *n*=23), with a mean age of 41 years old (ranging from 21 to 61). Besides respiratory disorder, fever, and weight loss were reported by all patients. Digestive symptoms also occurred in 81% (*n*=25), cutaneous lesions in 52% (*n*=16), and generalized lymph node enlargement in 35% (*n*=11). Splenomegaly and hepatomegaly were also showed by 81% (*n*=25) and 55% (*n*=17) of the patients, respectively. All patients showed blood parameters alterations such as anemia and/or thrombocytopenia, and neurological impairment occurred in 52% (*n*=16) (Table 1).

Except for three patients, all of the others had co-infections diagnosed concomitantly, such as candidiasis (61%; *n*=19), confirmed (positive microscopy and cultive) TB (29%; *n*=9), empiric (without laboratorial confirmation) neurotoxoplasmosis (29%; *n*=9), and empiric (also without laboratorial confirmation) pneumocystosis (23%; *n*=7). Herpetic encephalitis, herpes zoster, syphilis, medullary cytomegalovirus infection, *Mycobacterium avium* infection, herpes simplex, cryptococcosis and hepatitis C were also reported in one patient each. In addition, 26% (*n*=8) and of the DH patients were empirically treated to TB (rifampicin – 150 mg, isoniazid – 75 mg, pyrazinamide – 400 mg, and ethambutol – 275 mg for two months, and rifampicin – 450 mg, and isoniazid – 225 mg for four months).

The use of antiretroviral therapy at the DH diagnosis was irregular or inexistent in 90% (*n*=28) of the patients. Only four patients had > 200 cells/mm^3 of CD4+ lymphocytes, and 48% (*n*=15) had ≤ 50 cells/mm^3 (mean: 109 cells/mm^3 - ranging from 7 to 752) (Table 1). Three patients had DH associated with a systemic inflammatory response syndrome (SIRS).

In six patients (19%), DH was the AIDS-defining illness. In the remaining known PLHIV (*n*=25) the diagnosis of histoplasmosis occurred after a mean of 136 days (ranging from 14 to 400) from the beginning of the respiratory symptoms, being only three patients investigated and diagnosed during the first month of the clinical manifestation. On the other hand, up to 12 previous clinical samples from these patients were submitted to TB investigations (mean: 5 exams) before the suspicion of DH.

Classical mycological exams were the diagnostic method of DH in 14 patients (45%), such as culture (*n*=9) and/or DME/histopathology (*n*=14). Serological tests (immunodiffusion) confirmed the diagnosis in 29% (*n*=9) of patients, and urinary antigen assay diagnosed 13% (*n*=4) of patients. Four patients (13%) had at least two positive exams confirming DH (Figure 1).

The treatment of choice for 81% (*n*=25) of the patients was amphotericin B deoxycholate (0.7 to 1 mg/kg/day, until the maximum dose of 50 mg/day) for 14 days, following by itraconazole (200 mg, every 8 hours) for 3 days, and 200 mg (every 12 hours) for 12 months. Five patients (16%) were treated with itraconazole (four who had an early diagnosis of DH and one who had renal disfunction). After twelve months from the DH diagnosis, 65% (*n*=20) of the patients remained alive (Table 1). One patient died before the laboratorial confirmation of the disease, and four died in a mean period of 25 days post-diagnosis of DH (0-62 days). Three patients died after 5 to 6 months and three patients had a recurrence after itraconazole interruption, evolving to death in 6, 7 and 12 months of treatment.

Table 1. Clinic-epidemiological data of disseminated histoplasmosis cases (*n*=31) diagnosed in people living with HIV/aids (PLHIV) at the University Hospital Dr. Miguel Riet Corrêa Jr. (UH-*Universidade Federal do Rio Grande* - FURG/*Empresa Brasileira de Serviços Hospitalares* - Ebserh) from 2010 to 2019.

Variable	**Frequency %** (n/total of patients included in the variable)
Male	74% (21/31)
Female	26% (8/31)
Signs and symptoms	
Weight loss	100% (31/31)
Fever (> 37.8°C)	100% (31/31)
Respiratory (cough and dyspnea)	100% (31/31)
Cutaneous (papular and ulcerated)	52% (16/31)
Neurological (disorientation, focal deficit, paresthesia, confusion, headache and hemiplegia)	52% (16/31)
Digestive (abdominal distension and pain, diarrhea and nausea)	81% (25/31)
Hepatomegaly	55% (17/31)
Splenomegaly	81% (25/31)
Generalized lymph node enlargement	35% (11/31)

Image exams	
Interstitial lung pattern	55% (17/31)
Reticulonodular lung pattern	32% (10/31)
Pulmonary nodules	6% (2/31)
Mediastinal lymphadenopathy	26% (8/31)
Blood Parameters	
Anemia	100% (31/31)
Inflammatory marker*	100% (31/31)
Liver damage marker**	84% (26/31)
Tissue injury marker***	87% (27/31)
Thrombocytopenia	74% (23/31)
HIV parameters	
CD4+ lymphocytes $\leq 100/mm^3$	71% (22/31)
CD4+ lymphocytes $\leq 50/mm^3$	48% (15/31)
HIV Viral load ≥ 50 copies	90% (26/29)
Antifungal treatment	
None	3% (1/31)
Amphotericin B deoxycholate	81% (25/31)
Itraconazole	16% (5/31)
Outcome after 12 months	
Alive	65% (20/31)
Dead	35% (11/31)

*C-Reactive Protein increased **alkaline phosphatase increased *** lactate dehydrogenase increased

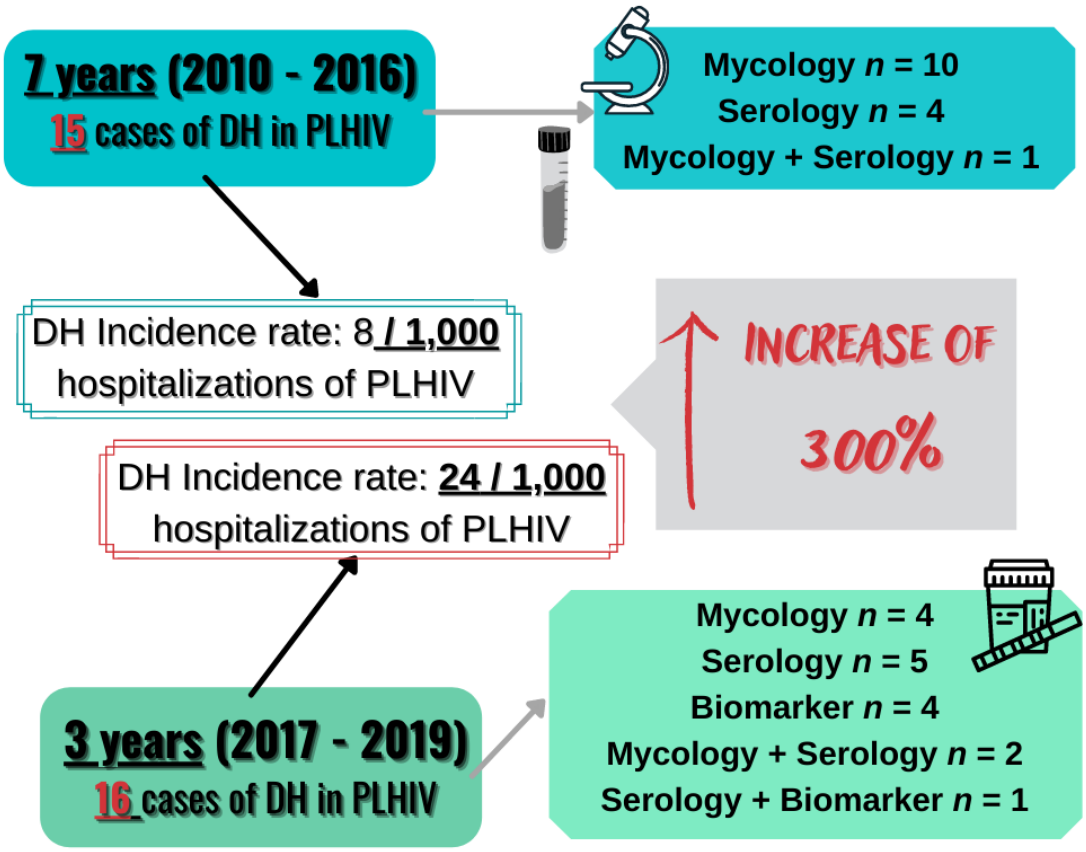

Figure 1. Approach used for the diagnostic of 31 cases of disseminated histoplasmosis (DH) in people living with HIV/aids (PLHIV) from a tertiary hospital in southern Brazil, between 2010 and 2019. And, the incidence rate of DH between periods before (2010-2016) and after (2017-2019) the implementation of the urinary antigen test, showing an increase of 300%.

DISCUSSION

Our study shows data regarding the DH casuistic in a tertiary hospital from southern Brazil, a neglected disease that can cause severe clinical manifestations in PLHIV leading to death (Almeida et al., 2019). Improve knowledge of local epidemiology of DH in references services to PLHIV are essential to reduces the underdiagnosis and contribute to the survival of these patients (PAHO, 2020), especially in a harbor city with the highest rate of HIV/aids patients among all Brazilian cities with more than 100,000 inhabitants, which was the population of our study (Brasil, 2020).

DH was the AIDS-defining illness in 21% of our patients, being the disseminated symptoms of histoplasmosis the immunosuppression indicator. Co-infections diagnosed in our patients, such as candidiasis, neurotoxoplasmosis and pneumocystosis are well-reported in the

literature (Falci et al., 2019; Nacher et al., 2020). Respiratory signs, splenomegaly, and cutaneous lesions were signs more commonly described in our patients than in other studies, possibly due to a more severe extension of the disease associated with late-diagnosed (Couppié et al., 2019; Falci et al., 2019; Nacher et al., 2020; Nacher et al., 2021). The high rate of neurological impairment described in our patients can also be attributed to their co-infections, since 69.2% (9/13) of the patients with signs such as disorientation, confusion and focal deficit were also diagnosed with neurotoxoplasmosis or herpetic encephalitis. On the other, neurological signs, also detected in five patients without other neuropathogenic condition, merits additional investigations.

A high rate of our patients were exhaustively investigated to TB diagnosis (up to 12 respiratory samples examinations), and more than 20% were empirically treated to TB, and had a mean period of more than four months showing respiratory dysfunction, before clinicians include DH as a diagnosis hypothesis, delaying the confirmation of the DH diagnosis. Although TB represents an important opportunistic disease in PLHIV hospitalized at HU/FURG-Ebserh (Boffo et al., 2004), 29% of our DH patients had concomitant TB. Thus, the investigation of both diseases must occur simultaneously (Falci et al., 2019), and DH must be investigated in all PLHIV with CD4+ lymphocytes < 200 cells/mm^3 (PAHO, 2020).

Diagnostic methods with high rates of sensitive and specificity are vital in endemic areas to histoplasmosis, aiming an early diagnostic and a positive outcome to patients (Cáceres et al., 2018; Falci et al., 2019). An improvement in the investigation of DH in PLHIV with respiratory symptoms by classical methods associated with serology and/or urinary antigen assay began to occur in the UH-FURG/Ebserh in 2017. Since an early investigation associated with the detection of urinary *Histoplasma* spp. antigens is the gold-standard to DH diagnosis in immunosuppressed patients (PAHO, 2020), we showed an increase of 300% in the incidence rate of DH in our reference service to PLHIV after the improvement of these conditions.

Our mortality rate (35%) was similar to the national rate (33%) referred in a systematic review from Brazil histoplasmosis cases (Almeida et al., 2019). Therefore, the underdiagnosis of histoplasmosis, especially of the DH in PLHIV, is a national problem, which must be urgently changed. In our hospital, DH was responsible for high morbidity of PLHIV patients, up to 24/1,000 hospitalizations, and high mortality (35%). In addition, we highlighted that 29% of patients were co-infected with TB, a disease with overlap symptoms with histoplasmosis, thus, the simultaneous investigation for the two infectious diseases in all PLHIV patients living in endemic areas of this mycosis must be mandatory.

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5.2 Artigo 2

"*Histoplasma capsulatum* and *Mycobacterium avium* co-infection in an immunocompromised patient: Case report and literature review."

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Histoplasma capsulatum and *Mycobacterium avium* co-infection in an immunocompromised patient: Case report and literature review

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ABSTRACT

We report a case of fungal and mycobacterial co-infection in an immunosuppressed patient from Southern Brazil. Histoplasmosis was diagnosed in an AIDS patient admitted to the hospital with nonspecific respiratory signs. However, 4 months post hospital discharge, the patient worsened and a co-infection with *Mycobacterium avium* was detected. Physicians must consider and investigate a broad spectrum of diseases which can occur as co-infections and which share the same clinical symptoms and signs in immunosuppressed patients.

1. Introduction

Patients immunocompromised by human immunodeficiency virus (HIV) infection are at increased risk of opportunistic diseases, even with the advance of antiretroviral therapy, which

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represent a better prognosis for these patients [1]. Among the main opportunistic agents are *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, *Cryptococcus neoformans*, *Toxoplasma gondii*, cytomegalovirus, *Histoplasma capsulatum* and *Mycobacterium avium* [2].

Advanced and more accurate diagnosis techniques directed at the large diversity of pathogens that can infect HIV-AIDS patients, have indicated that co-infections can occur more frequently than previously expected. Thus, a full diagnostic investigation is necessary to find the correct treatment and improve the prognosis [1]. *Histoplasma capsulatum* and *M. tuberculosis* co-infection in HIV patients has been described, however, co-infection reports of *H. capsulatum* and non-tuberculous mycobacteria, such as *M. avium*, in these patients are scarce in the literature [3]. Therefore, we report a case of histoplasmosis and mycobacteriosis by *M. avium*, in a HIV/AIDS patient from southern Brazil and perform a bibliographic review to report all cases of this co-infection described thus far.

2. Case

A 52 year-old man, living on a farm, with HIV infection diagnosed in 2007, was admitted to the University Hospital Dr. Miguel Riet Corrêa Jr., Rio Grande, Rio Grande do Sul, Brazil (UH-FURG) in January 2017, reporting loss of appetite, nausea, vomiting and weight loss of 10 kg in the previous four months. The patient had a history of poor adherence to and abandonment of antiretroviral therapy several times. He was a smoker since age 13 and an alcoholic for 25 years.

Given his medical history, his symptoms of asthenia, adynamia, night sweats, fever and productive cough, and the high tuberculosis endemicity of the region, an investigation for *M. tuberculosis* infection was performed. Negative microscopy and liquid culture (BD BACTEC MGIT™ - BD Medical, United States of America) of three sputa made a diagnosis of tuberculosis unlikely. Unfortunately, the investigation had to be interrupted since the patient left the hospital without medical consent.

Four months later (May 2017, Day 0), he was again hospitalized with the same symptomatology previously reported. Laboratory tests showed immunosuppression (LTCD4+ cells=20 cells/mm^3 and HIV viral load of 178,304 copies), and several laboratory abnormalities, such as anemia (hemoglobin of 6.9 g/dL and hematocrit of 21.3%), leukopenia (leukocytes of 2,760/ mm^3), increase in serum ferritin (1,389 ng/mL) and in inflammatory markers (erythrocyte sedimentation rate of 120 mm and C-reactive protein of 82.2mg/L). A bilateral ground-glass pattern and a consolidation area in the left upper lobe with two cavities (the largest 4 cm) and bronchiectasis areas were detected in the computed tomography of the chest, as well as hepatosplenomegaly (Figure 1).

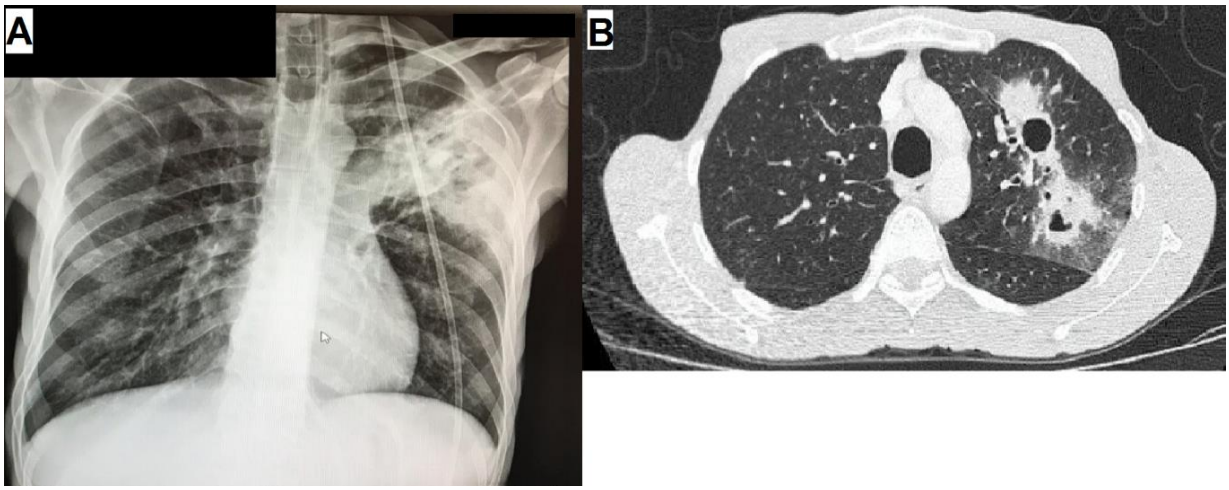

Figura 1. A. Chest radiograph (May 2017, Day 0) showing bilateral ground-glass pattern and left upper lobe bronchiectasis. B. Chest computed tomography shows consolidation area in the left upper lobe with two cavities, the largest 4 cm, and bronchiectatic areas, ground glass attenuation areas and septal thickening.

On this occasion, three more sputum smear samples were negative for *M. tuberculosis* infection, as well as for fungal and bacterial. *Cryptococcus* antigen tested by latex agglutination was also negative. On the other hand, histoplasmosis was diagnosed via a serum positive immunodiffusion test and urinary antigen test (both kits from IMMY®, Immuno-Mycologics, Oklahoma, United States). The patient was given amphotericin B deoxycholate (1mg/kg/d) intravenously, showing clinical improvement after 14 days of antifungal therapy (weight gain, decreased cough, resolution of fever). Then he was discharged with prescriptions for itraconazole (400mg/d) and antiretroviral therapy (tenofovir, lamivudine, efavirenz) and sulfamethoxazole-trimethoprim, and azithromycin prophylaxis. The patient did not return for follow-up appointments at Infectology Service (UH) for three months.

In September 2017 (Day 120), and he was hospitalized again with productive cough with mucopurulent sputum, hemoptysis, daytime fever, weight loss (6 kg). Diffuse wheezing was noted on respiratory auscultation. He was in the day 96 of itraconazole and no cutaneous lesions or other physical examination abnormalities were detected. His blood lymphocyte CD4+ count was 74 cells/mm^3 and HIV viral load was 1815 copies/mL. The chest radiograph showed consolidation areas in the left upper lobe with lower density than the previous image on May 2017 (Day 0) (Figure 2).

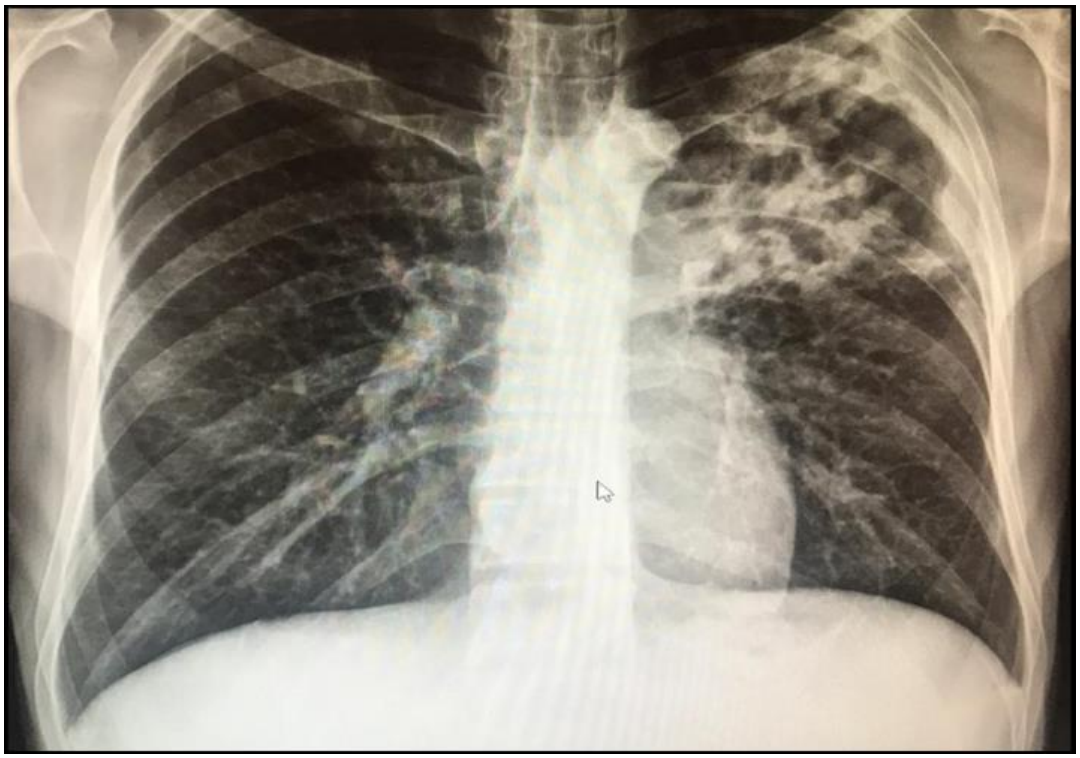

Figura 2. Chest X-ray (September 2017, Day 120) showing consolidation area in the left upper lobe, paraseptal thickening and bronchial thickening in the peripheral region.

His sputum was cutured for mycobacteria, and grew in two consecutive samples non-tuberculosis mycobacteria, identified as *M. avium* by sequencing of the hsp65 gene. Therapy with clarithromycin, ethambutol, streptomycin, and levofloxacin, as per the Health State Department of Brazil [4] recommendations, was introduced. He was maintained on itraconazole therapy for histoplasmosis treatment, and antiretrovirals for HIV therapy. In January 2018 (Day 240), the patient returned to UH-FURG asymptomatic, and had been adherent to treatment. His blood parameters were improved (hematocrit of 30.1%, hemoglobin of 10.0 g/dL, leukocytes of 6,310/ mm^3, erythrocyte sedimentation rate of 21 mm and C-reactive protein of 5.42mg/L), including an undetectable HIV viral load.

3. Discussion

A bibliographic search was performed in the PubMed database, using the descriptors "AIDS and *Histoplasma capsulatum* and *Mycobacterium avium*," to collect articles referring to *H. capsulatum* and *M. avium* coinfection. Table 1 show the seven studies found in this search, which described 15 cases of *H. capsulatum* and *M. avium* coinfection in HIV patients.

Table 1. Cases of *H. capsulatum* and *M. avium* coinfection in HIV patients reported in scientific literature

Case	Year	Country	Age (years), sex	Clinical Presentation	Histoplasmosis Diagnosis method	Treatment	Outcome	Reference
1	1980	USA	30, Male	Disseminated	Culture	Ketoconazole, isoniazid, ethionamide, ethambutol	Died	[5]
2	1981	USA	26, Male	Disseminated	Serological and Culture	Amphotericin B, rifampin, ethambutol, cycloserine, streptomycin	Died	[6]
3	1981	USA	30, Male	Disseminated	Serological and Culture	Amphotericin B, isoniazid, rifampin, ethambutol, cycloserine, ethionamide	Died	[6]
4, 5, 6, 7, 8	1983 -1987	USA	NDA	Disseminated	Histopathology or culture	Ketoconazole and amphotericin B	NDA	[7]
9	1996	USA	30, Male	Disseminated	NDA	Clarithromycin, ethambutol, and itraconazole, rimethoprim-sulfamethoxazole, zidovudine-lamivudine, nelfinavir	Recovered	[8]
10	2015	USA	32, Male	Disseminated	Culture	Amphotericin B, itraconazole, clarithromycin, ethambutol	Recovered	[9]
11, 12, 13, 14	2015 -2017	Mexico	NDA	Disseminated	Histoplasma urinary antigen and culture	NDA	NDA	[10]
15	2018	Brazil	52, Male	Pulmonary	Culture post mortem	Not treated	Died	[11]

*NDA: No data available

Opportunistic co-infection diseases in HIV patients are widely described in the literature, however, *H. capsulatum* and *M. avium* coinfection is hardly mentioned, with only seven reports of this co-infection in a period of 38 years [5, 6, 7, 8, 9, 10, 11]. There are many diagnostic challenges, owing to nonspecific and overlapping symptoms for both diseases [3]. Our report is the first with a premortem diagnosis of this co-infection, with survival, in a Brazilian patient.

Intense investigation is necessary to uncover the etiologies in co-infections [12, 13, 14]. In this case, one of the most sensitive tests (95%) [15] for disseminated histoplasmosis (galactomannan antigen detection) and semi-automatized liquid culture for mycobacteria with molecular identification, yielded the diagnoses. Then correct treatment favored the patient outcome, adding antibacterial therapy to the antifungal therapy already started. The molecular identification enabled a treatment specific for nontuberculous mycobacteria, whereas the therapeutic scheme used for *M. tuberculosis* would not have been efficacious [3, 16].

Once established, the progression of a systemic infection, such as histoplasmosis or mycobacteriosis, will each lead to depression of, or exhaustion of, cell-mediated immunity; thus one infection may make establishment of a second infection more likely. Co-infections in HIV patients often do not have a favorable outcome, owing to the lack of clinical suspicion and consequently a late diagnosis. This is illustrated in our literature review, emphasized by the high mortality rate [3, 5, 6, 7, 8, 9, 10, 11]. Considering the emergent importance of histoplasmosis in HIV patients on Latin America, particularly Brazil [13, 14, 19], it is important that clinicians are knowledgeable about the availability of rapid diagnosis tests, such as *Histoplasma* antigen detection.

Our patient was likely exposed to infection with both pathogens (*H. capsulatum* and *M. avium*) during his farm occupation, which provides contact with soil contaminated by chicken and pig excrement [17, 18]. Due to the fact that a diversity of co-infection can be caused by environmental pathogens, immunocompromised patients should be advised about the risks of exposure to such infection sources, and to take preventive measures. Pertinent to this is the "one-health concept," in which environment, animals and humans can share potential pathogens [20].

Acknowledgements

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Conflict of Interest

All authors declare that they have no conflicts of interest pertaining to this work.

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5.3 Artigo 3

"COVID-19-Associated Histoplasmosis in an AIDS Patient".

Este artigo foi o primeiro relato da coinfecção histoplasmose disseminada no paciente com aids e COVID-19 no Brasil. Foi publicado em novembro de 2020 no periódico *Mycopathologia* (Fator de impacto - FI: 2.574).

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SHORT COMMUNICATION

COVID-19-Associated Histoplasmosis in an AIDS Patient

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COVID-19 associated histoplasmosis in an AIDS patient

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ABSTRACT

Most reports associating fungal infections with Covid-19 have been cases of invasive aspergillosis. Here we report a case of severe histoplasmosis and Covid-19 infections in an HIV patient in Rio Grande, Southern Brazil. Histoplasmosis must be included as a diagnosis hypothesis of opportunist fungal co-infections in Covid-19 patients with AIDS, mainly in endemic areas.

KEYWORDS: AIDS, Covid-19, *Histoplasma capsulatum*, mycoses, SARS-CoV-2.

INTRODUCTION

The Covid-19 pandemia has so far affected more than 39 million people all over the world, causing more than one million deaths [1]. Several co-infections are known to affect patients with severe Covid-19, which may be challenging particularly in terms of diagnosis and treatment, potentially affecting patient outcomes [2, 3]. The frequency of Covid-19 in AIDS is not higher than the frequency of Covid-19 in the general population, even though the true role of the SARS-CoV-2 virus in HIV patients remains to be fully elucidated [4, 5].

Even more challenging is the diagnosis of fungal infections in patients with Covid-19, with most of the infections being associated with *Candida* and *Aspergillus* species [2, 3]. Indeed, coronavirus-associated pulmonary aspergillosis (CAPA) is a concern in Covid-19 patients, occurring in 20-35% of patients with severe acute respiratory syndrome (SARS), mainly in those requiring admission to the intensive care unit (ICU) and mechanical ventilation [2, 6, 7]. Similarly, candidemia occurs in 2.5% to 6.9% of Covid-19 patients in the ICU, mainly catheter-related infections and often with unfavorable outcomes [3, 8]. In addition, individual reports of *Pneumocystis* spp. co-infection in Covid-19 patients are being described [9, 10].

On the other hand, histoplasmosis, an emergent systemic fungal infection in HIV patients in Brazil and other Latin American countries [11, 12], has not yet been described in the context of Covid-19. Here we report about a case of Covid-19 in a patient who was subsequently diagnosed with histoplasmosis.

CASE REPORT

A 43year-old homeless woman from Rio Grande (South of Brazil) with a 21-year history of HIV infection and drug abuse (crack cocaine) was poorly adherent to active antiretroviral treatment (ART). She had two hospitalizations (2012 and 2017) due to neurotoxoplasmosis.

Her most recent CD4 count was low, at 113 cells/mm^3, and her HIV viral load was 38,503 RNA copies/ml (4.58 log).

On July 9, 2020, she was admitted to the hospital due to disorientation, cough, dyspnea, and low grade fever. Chest computed tomography (CT) showed multiple centrilobular nodules, with ground-glass attenuation in both lungs, diffuse thickening of bronchial walls, and lymph node enlargement in the mediastinal and cervical chains and the retroperitoneal upper abdomen (measuring up to 2.7 cm on its smallest axis). Abdominal CT showed hepatosplenomegaly. Sputum microscopy stained by Gomori-Grocott showed abundant small oval blastoconidia suggestive of *Histoplasma capsulatum* (Figure 1), but sputum culture was negative for both *Mycobacterium tuberculosis* and fungi. *Histoplasma* and *Aspergillus* precipitins tests were negative, as well as PCR test for SARS-CoV-2 virus (kit *Bio-Manguinhos*/FIOCRUZ, Lot 66/2020). She was put on empirical therapy for neurotoxoplasmosis and discharged from the hospital with antiretroviral therapy (tenofovir/lamivudine and atazanavir/ritonavir).

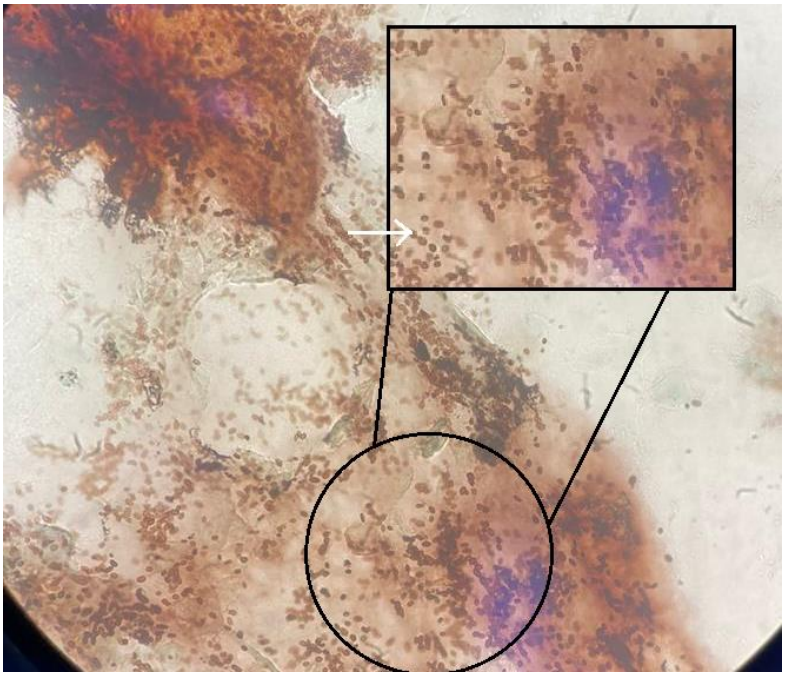

Fig 1. Sputum microscopy showing abundant small oval blastoconidia (arrow) without pseudo-hyphae (Gomori-Grocott stain)

On July 17, 2020, the patient returned with worsening dyspnea and oxygen saturation of 83%. Dexamethasone (6 mg, intravenous), ceftriaxone, azithromycin, and oxygen supplementation by nasal catheter were prescribed. At this time, she was diagnosed with Covid-19 by a positive PCR test (kit *Bio-Manguinhos*/FIOCRUZ, Lot 66/2020). Blood testing revealed anemia (hemoglobin of 9.6 g/dl), mild leukocytosis (10,190/mm^3), thromocytopenia

(130,000/mm^3), and elevated C-reactive protein (140 mg/l) and d-dimer (2 µg/ml). Chest CT showed several sparse micro nodules in the lungs, with a nodule in the posterior segment of the upper lobe of the right lung (measuring 4 mm) and intraparenchymal nodule (7 mm), lymph node enlargement in the mediastinal and cervical region (up to 2.5 cm). Abdominal CT showed confluent lymph node enlargements in the retroperitoneal region (biggest measuring 3 x 1.7 cm) and hepatosplenomegaly (Figure 2). She was discharged from the hospital but since the urine sample result became positive for *H. capsulatum* antigen (galactomannan) in immunoenzymatic assay (catalog number HGM201; IMMY®, Immuno-Mycologics, Oklahoma, United States; index of >2) she was asked to return to the hospital again. She refused hospitalization and itraconazole PO was commenced at 200 mg twice daily.

Patient was last seen on Sept 14, 2020. Her HIV viral load was 400 copies/ml (2.6 log) and CD4 count was 593 cells/mm^3. Follow-up abdominal CT showed persistence of hepatosplenomegaly and abdominal lymph node enlargement, while chest CT revealed an increase in the number of lymph nodes affected compared to the previous exam from July (Figure 2). Anemia persisted and an increase in inflammatory markers was detected, suggesting active disease. Her medical team is doing their best in an attempt to readmit the patient to receive IV antifungal therapy.

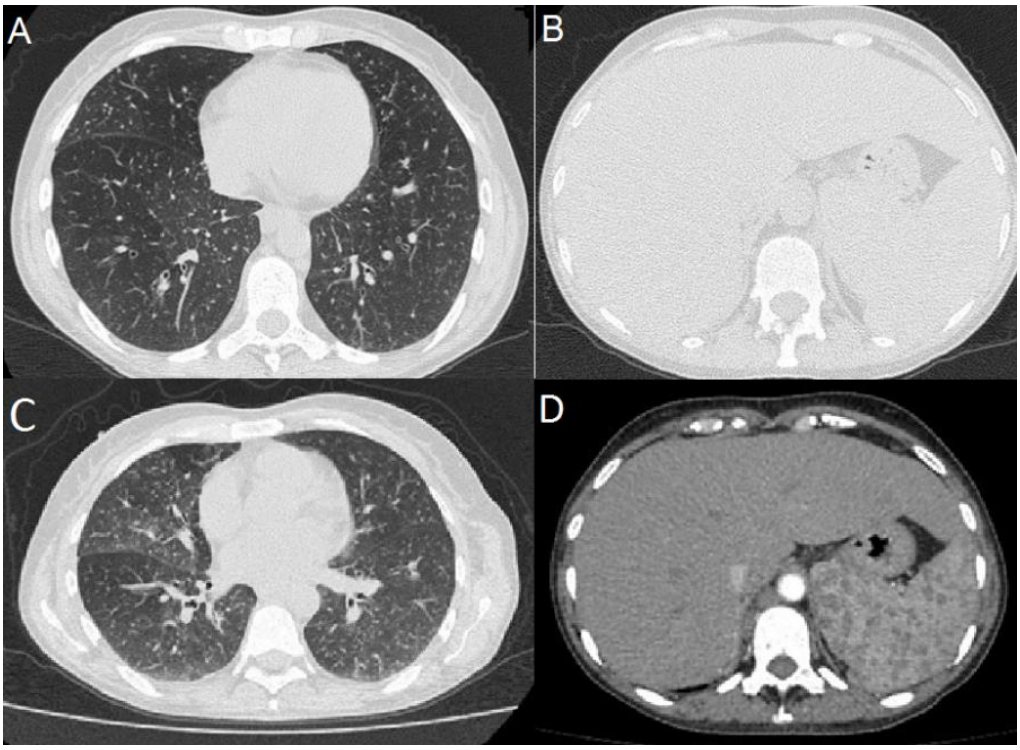

Fig 2 Computed tomography of the chest (A) and abdomen (B) of July 17, 2020. (C) and (D) images show follow up computed tomography performed on September 14, 2020

DISCUSSION

Here we present the first Brazilian report of disseminated histoplasmosis associated with Covid-19 in an HIV-infected patient. Despite the co-infections with aggressive pathogens, our patient did not develop severe lung deterioration, nor was intensive care and mechanical ventilation necessary. The favorable outcome of Covid-19 in this patient might be related to her low CD4 cell count, which might have prevented the occurrence of a cytokine storm, as described in patients with severe Covid-19, as well as in those initiating antiretroviral therapy with immune reconstitution syndrome [5].

Disseminated histoplasmosis (DH) is one of the most common opportunistic diseases in HIV/AIDS patients and presents many diagnostic challenges. The symptoms are nonspecific and overlap with other opportunistic diseases, and there is variation in the sensitivity of diagnostic methods (30 to 95%) [13, 14]. Our patient was investigated for DH with the use of the three diagnostic methods available locally, i.e., microscopy, culture, and agar gel immunodiffusion test. Despite being highly suggestive of histoplasmosis, findings at sputum microscopy were not considered definitive and the diagnosis was only made by *H. capsulatum* antigen testing, performed in other institution. Even though antigen testing has a high sensitivity in diagnosing DH (95%) [14], the method is at the moment available for research purposes only in Brazil. This led to a delay in the diagnosis of DH and consequently in the initiation of treatment, which can affect a patient's outcome. Efforts to increase the availability of the test for urinary antigen detection in DH diagnosis are urgent, especially in regions with a high HIV prevalence [11, 15]. The patient in this report comes from Rio Grande, the municipality with the highest prevalence of HIV/AIDS among Brazilian cities with more than 100,000 inhabitants [16].

Co-infections in Covid-19 and especially in the SARS-CoV-2/HIV patients should be widely investigated since correct treatment would contribute to a favorable outcome [4, 5]. Moreover, fungal infections play an important role as opportunistic infections in HIV or Covid-19 patients, and possible co-infections in both populations should be considered, and appropriate screening tests performed [2, 3, 9].

DECLARATIONS

Conflict of Interest All authors declare that they have no conflicts of interest pertaining to this work.

Ethical Approval This project was approved by the Health Research Ethics Committee of the *Universidade Federal do Rio Grande* (FURG), under number 234/2018 and patient consent was obtained and all procedures performed in studies were in accordance with the ethical standards of the institution.

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Authors' contributions

All authors contributed to accomplishment of this manuscript. RPB: Care and monitoring of the patient; searched the clinical information; first draft of the manuscript. VRP: Mycological analyses; searched the clinical information; first draft of the manuscript. JLB: Mycological analyses. DAS: Critical correction. HEZ: Care and monitoring of the patient. ICSV: Mycological analyses. ACP: Mycological analyses and critical correction. MOX: Mycological analyses and critical correction

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6 CONCLUSÕES

Foi encontrada uma taxa elevada de HD dentre os pacientes internados com infecção pelo HIV (12 casos por 1.000 hospitalizações), no período de 2010-2019. A implementação da pesquisa de antígeno urinário, em 2017, associada ao reforço da suspeição da doença, levou a um aumento de 300% na taxa de incidência desta micose.

A maioria dos pacientes foi do sexo masculino (74%) e a HD foi a doença definidora de aids em seis pacientes. A HD foi observada em pacientes severamente imunodeprimidos e em falha virológica, atribuída ao uso irregular ou abandono da TARV.

A suspeição e investigação da HD foi tardia e as coinfecções ocorreram na maioria dos pacientes (n=28). Todos os pacientes foram investigados para tuberculose, que foi diagnosticada em 29% dos pacientes, reforçando a necessidade da investigação simultânea. A taxa de mortalidade foi de 35%.

Dois dos artigos da tese relataram e ressaltaram a importância da investigação concomitante de doenças infecciosas. No relato da coinfecção HD e micobacteriose por *Mycobacterium avium* foi observado um desfecho positivo, porém ao revisar a literatura evidenciou-se a alta mortalidade relacionada a esta coinfecção. De fato, este foi o primeiro caso de um paciente vivo com esta coinfecção, relatado no Brasil. O artigo da coinfecção covid-19, aids e HD foi o primeiro relato destas coinfecções publicado no Brasil e o segundo no mundo.

7 CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

O estudo proposto demonstrou a taxa de incidência da HD, bem como os dados clínicos e laboratoriais dos pacientes coinfectados com HIV, atendidos no HU-FURG. Este hospital é referência para 21 municípios do extremo sul do país no atendimento destes pacientes e para o município do Rio Grande, o qual apresenta um dos maiores coeficientes de mortalidade pelo HIV no país.

Considerando que estudos similares na região sul são escassos na literatura científica, estes dados poderão agregar informações importantes sobre a histoplasmose nos pacientes com aids, podendo servir de alerta para aumentar a suspeição diagnóstica, contribuindo para um diagnóstico precoce e uma consequente diminuição da letalidade desta infecção.

As coinfecções na aids são frequentes e a investigação simultâneas de várias doenças deve ser preconizada, mesmo após se diagnosticar uma etiologia. A inespecificidade das manifestações clínicas da HD reforça que ao aplicar-se a "navalha de Ockham", eleva-se o subdiagnóstico de outras doenças oportunistas e a mortalidade nesta população.

A suspeição clínica e a implementação da pesquisa do antígeno urinário *H. capsulatum*, um método muito sensível e não invasivo, incrementou o diagnóstico da HD, oportunizando uma terapêutica adequada e desfechos positivos.

Como perspectivas futuras, encontra-se em discussão a construção a rede de diagnóstico micológico para as unidades básicas de saúde, em conjunto com a Secretaria Municipal da Saúde, o SAE Infectologia do HU-FURG e o Laboratório de Micologia da Famed-FURG.

Planejam-se:

- Capacitações periódicas sobre infecções fúngicas para os profissionais da saúde;
- Fluxograma para direcionamento do diagnóstico com a introdução da pesquisa do antígeno urinário no rastreamento dos pacientes com aids e LTCD4+ inferior a 200 ou concomitante a investigação de tuberculose;
- O matriciamento dos casos e se necessário o encaminhamento para investigação no Serviço de Atendimento Especializado em Infectologia do HU-FURG;
- A notificação municipal das micoses sistêmicas e
- A padronização e introdução da anfotericina lipossomal no HU-FURG.

Aliar a pesquisa com o retorno social foi fundamental para a escolha do tema deste doutorado. Conciliar a rotina médica, a vida familiar, a maternidade e uma pandemia foi exaustivo, mas recompensador.

Pensar nos fungos tornou-se uma paixão.

"Think fungus, save lives"

Centers for Disease Control and Prevention (CDC – USA).

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APÊNDICE A

"Histoplasmosis, An Underdiagnosed Disease Affecting People Living With HIV/AIDS in Brazil: Results of a Multicenter Prospective Cohort Study Using Both Classical Mycology Tests and Histoplasma Urine Antigen Detection."

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Open Forum Infectious Diseases
MAJOR ARTICLE

Histoplasmosis, An Underdiagnosed Disease Affecting People Living With HIV/AIDS in Brazil: Results of a Multicenter Prospective Cohort Study Using Both Classical Mycology Tests and *Histoplasma* Urine Antigen Detection

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ABSTRACT

Background. Histoplasmosis is highly endemic in the American continent. This condition is associated with a high mortality, particularly in people living with HIV/AIDS (PLWHA). Diagnosis of histoplasmosis is usually late in South America, as Histoplasma antigen detection is rarely available. Here we determined the prevalence, risk factors, and outcome of histoplasmosis in PLWHA in Brazilian hospitals. **Methods.** This was a prospective cohort study (2016–2018) involving 14 tertiary medical centers in Brazil. We included hospitalized

PLWHA presenting with fever and additional clinical findings. Patients were investigated at each participant center with classical mycology methods. Also, Histoplasma antigen detection was performed in urine samples (IMMY). Probable/proven histoplasmosis was defined according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria. **Results.** From 616 eligible patients, 570 were included. Histoplasmosis was identified in 21.6% (123/570) of patients. Urine antigen testing increased the diagnostic yield in 53.8%, in comparison with standard mycology methods. Variables independently associated with histoplasmosis were CD4+ count <50 cells/mm3, use of an antiretroviral (protective effect), and sample collection in the Northeast region of Brazil. Dyspnea at presentation was independently associated with death. Histoplasmosis was more frequent than tuberculosis in patients with low CD4+ counts. Overall 30-day mortality was 22.1%, decreasing to 14.3% in patients with antigen-based diagnosis. **Conclusions.** Histoplasmosis is a very frequent condition affecting PLWHA in Brazil, particularly when CD4+ counts are lower than 50 cells/mm3. Antigen detection may detect earlier disease, with a probable impact on outcomes. Access to this diagnostic tool is needed to improve clinical management of PLWHA in endemic countries.

Keywords: diagnosis, epidemiology, histoplasmosis, HIV

Histoplasma capsulatum is the etiologic agent of histoplasmosis, a systemic mycosis that is highly endemic in the Americas and is also being identified in other parts of the globe [1, 2]. Histoplasmosis may disseminate in immunosuppressed patients, particularly those with HIV infection. Accordingly, disseminated histoplasmosis has been an AIDS-defining disease since 1987 [3]. Histoplasmosis is often described as the most common endemic mycosis in the United States, with a definite endemic area around the Mississippi valley [4]. More recently, it has become appreciated as a highly prevalent disease in AIDS and a major cause of morbidity and mortality in the Americas [3, 5, 6]. Similar to syphilis and tuberculosis (TB) in the history of medicine, the protean manifestations of histoplasmosis require it to be included in the differential diagnosis of diverse clinical syndromes associated with AIDS in the Americas [2].

Brazil is the largest country in Latin America, with a population of >200 million. It is currently struggling with an HIV epidemic, with almost 1 000 000 cases [7]. Nevertheless, the frequency of disseminated histoplasmosis has not been well characterized in Brazil. There are no nationwide studies showing prevalence rates; available data are retrospective and limited to specific regions of the country in single-center studies. Most studies are not population based

or representative of the epidemiology as a whole, as data have often been obtained through convenience samples rather than through properly performed epidemiologic surveys [8–10]. Moreover, histoplasmosis is usually diagnosed late in Latin America, mainly due to a lack of non-culture-based diagnostic methods. In this setting, skin lesions are frequently observed; this could reflect a genetic variation of the pathogen, but, more likely, it confirms another marker of late diagnosis [11].

Histoplasma antigen detection by antigen-capture enzyme-linked immunosorbent assay (ELISA) has a high sensitivity using urine samples (95%) [12]. However, its availability is very limited in South and Central America, as its reagents are not commercially available (Miravista Diagnostics, Indianapolis, IN). To overcome these difficulties, other tests have been developed: an ELISA kit (Alpha Histoplasma antigen enzyme immunoassay [EIA]; Immuno-Mycologics, Norman, OK) that uses polyclonal antibodies with variable sensitivity (62%–81%); an in-house double-polyclonal-antibody sandwich ELISA from the US Centers for Disease Control and Prevention (CDC), which has high sensitivity (>80%) but is not currently available; and more recently, a commercial Histoplasma antigen (Histoplasma galactomannan [HGM]) single-monoclonal-antibody sandwich ELISA (Immuno-Mycologics, Norman, OK) [13–16]. The last performed very well in a recent study, conducted in Latin America, with a very high sensitivity and specificity (both >95%) [17].

Here, our goal was to determine the prevalence, risk factors, and outcomes of histoplasmosis in a large cohort of people living with HIV/AIDS (PLWHA). We used for diagnosis both classical mycology tests and a previously validated monoclonal Histoplasma galactomannan (HGM) enzyme-linked immunosorbent assay (Immuno-Mycologics [IMMY], Norman, OK) for Histoplasma antigen detection.

METHODS

Setting and Study Design

This was a multicenter prospective cohort study with PLWHA from 14 tertiary Brazilian hospitals. Institutions were located in all 5 regions of the country: South—Porto Alegre (Hospital de Clinicas de Porto Alegre, Santa Casa de Misericordia de Porto Alegre, and Hospital Nossa Senhora da Conceicao), Rio Grande (Hospital Universitario de Rio Grande), Santa Maria (Hospital Universitario de Santa Maria), and Curitiba (Hospital de Clinicas da Universidade Federal do Parana); Southeast—Sao Paulo (Hospital Sao Paulo, and Instituto de Infectologia Emilio Ribas); Midwest—Goiania (Hospital de Doenças Tropicais); Northeast—Salvador (Hospital Couto Maia), Natal (Hospital Giselda Trigueiro), and Fortaleza (Hospital São Jose de

Doencas Infecciosas); and North—Manaus (Hospital Universitario Francisca Mendes) and Macapa (Laboratorio Central de Saude Pública do Amapa [LACEN]). The study was carried out between October 2016 and February 2018.

We included adult (≥18 years old) patients with documented HIV infection who were admitted to 1 of the participant hospitals. Inclusion criteria required fever plus 1 of the following: weight loss (>10% of usual body weight), diarrhea, miliary pattern on thorax imaging, pancytopenia, lymphadenopathy, splenomegaly, or hepatomegaly. Patients were excluded if they refused to consent to study participation. Outpatients were not studied. Patients were also excluded for inability to perform urine Histoplasma antigen tests or for insufficient clinical information. The attending physician was entirely in charge of patients' diagnostic and therapeutic decisions.

Data on patient demographics, clinical manifestations, HIV disease (CD4+ cell counts, HIV viral load, use of antiretroviral drugs), exposure to known risk factors for fungal disease, radiological abnormalities, laboratories, diagnosis by classical mycology methods, use of antifungals, presence of other opportunistic infections, and clinical outcome were collected by the institutions and recorded into a clinical research form.

Laboratory Methods

Urine samples were collected from each patient for Histoplasma antigen detection. Urine was collected at each research center, and samples were immediately frozen. Samples were shipped to a reference laboratory (Molecular Biology Laboratory at Santa Casa de Misericordia de Porto Alegre) and run in batches to optimize the use of kits. Antigen detection was performed using the Histoplasma galactomannan (HGM) single-monoclonal-antibody sandwich ELISA (Immuno-Mycologics, Norman, OK), according to the manufacturer's instructions. According to test validation performed by Cáceres et al., a cutoff of 0.5 ng/mL was used to determine positivity [17]. Physicians were not informed of Histoplasma antigen test results.

Clinical Data

Information on demographics and clinical manifestations of disease was compared between patients with histoplasmosis or other diagnoses. Probable/proven histoplasmosis was defined according to modified European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria [18]. The only modification made in the EORTC/MSG criteria was the addition of positive serology (immunodiffusion test

for antibody detection) for Histoplasma capsulatum to the "probable histoplasmosis" criterion. Any positivity in H or M bands was considered a positive result. Centers were allowed to use their usual diagnostic tests for histoplasmosis. We evaluated the performance of available classic methods (microscopy, fungal culture, antibody detection, and histopathology) in comparison with Histoplasma antigen detection. For the determination of outcomes, covariates were compared in survivors vs nonsurvivors at 30 days. Independent risk factors for death were determined using multivariate analysis.

Ethical Approval

Participants signed an informed consent granting permission for using their biological samples in the study, as well as clinical data. The protocol was approved by the ethical committees of the general coordination center (Santa Casa de Misericordia de Porto Alegre) and each participant institution, according to Brazilian research regulation guidelines.

Statistical Analysis

Sample size was calculated in 138 PLWHA, considering an estimated prevalence of 10% of histoplasmosis in PLWHA presenting symptoms or signs of possible disease (margin of error, 5%; confidence level, 95%) [5, 19]. Statistical analyses were conducted using JMP for Mac, version 9 (SAS Institute, Cary, NC). We used descriptive statistics to analyze and present data. The Kolmogorov-Smirnov test was performed to assess for normal distribution. Statistical differences between groups were analyzed using the chi-square or Fisher exact test for categorical data. For continuous data, the Student t test or Wilcoxon rank-sum test was employed. All tests were 2-tailed, and a P value of $\leq .05$ was considered significant. A logistic regression model was constructed using a forward stepwise approach. Variables with $P < .20$ were included in the model and remained in the final model if $P < .05$ and/or because of biological significance. Collinearity was assessed when applicable.

RESULTS

From 616 eligible patients, 46 were excluded due to loss of clinical information, accident during sample transportation, absence of urine sample for testing, and/or lack of inclusion criteria after critical revision. Therefore, 570 patients were included in the cohort. From the original 14 medical centers participating in the study, 3 were excluded as no patient was included (Manaus, Macapa, and Curitiba), resulting in 11 centers enrolling patients.

Table 1 presents the general characteristics of patients included in the study. A total of 123 (21.6%) were diagnosed with probable/proven histoplasmosis, including 88 (15.4%) patients with a positive Histoplasma urine antigen test. Among 123 patients with histoplasmosis, 109 (88.6%) were properly investigated by classical mycology methods, and 78 of these (71.5%) had confirmed infection at the local institutions. These figures translate into 78 patients in the "proven" category of modified EORTC/MSG criteria and 45 patients in the "probable" category. In the "no histoplasmosis" group, 157/447 (35.1%) were tested with classical methods and found to be negative, and 290/447 (64.9%) were not cultured/biopsied as the attending physician perceived a low pretest probability of disease. A positive test for H. capsulatum antigen in the urine was the only criterion for probable/proven disease in 42 (34.1%) patients. In 2 cases (1.6%), positivity was found both on antigen testing and antibody detection; in 1 patient (0.8%), a positive antibody detection test was the only criterion for infection (Figure 1). Among the 78 patients who had been diagnosed at a local hospital, 26/33 (78.8%) had identification of H. capsulatum on microscopy, 32/61 (55.7%) patients were culture positive for H. capsulatum, 10/16 (62.5%) patients had confirmed infection by histopathology, and 11/48 (22.9%) had positive blood culture. Only 8/41 (19.6%) patients with a local-institution diagnosis had detected antibodies against *H. capsulatum.*

Table 1.

Patients' Baseline Characteristics in PLWHA With or Without Disseminated Histoplasmosis (Probable/Proven Histoplasmosis)

Variables	No Histoplasmosis (n = 447)	Probable/Proven Histoplasmosis (n = 123)	*P* Value
Age, median [IQR], y	41 [33–48]	39 [33–45]	.162
Female gender, No. (%)	159 (35.6)	33 (26.8)	.069
CD4+ cell, median [IQR], count/mm^3	86 [31–240] (n = 428)	39 [14–91] (n = 115)	<.001
CD4+ cell category, No. (%)	(n = 428)	(n = 115)	<.001
<50 cells/mm^3	146 (34.1)	66 (57.3)	
50–99 cells/mm^3	87 (20.3)	24 (20.8)	
100–149 cells/mm^3	45 (10.5)	13 (11.3)	
150–199 cells/mm^3	28 (6.5)	3 (2.6)	
≥200 cells/mm^3	122 (28.5)	9 (7.8)	
No CD4+ count available	19 (4.2)	8 (6.5)	.297
Receiving antiretroviral treatment, No. (%)	217 (48.5)	42 (34.1)	.005
City of enrollment,[a] No. (%)			<.001
Goiania	76 (17.0)	50 (40.6)	
Fortaleza	39 (8.7)	23 (18.7)	
Porto Alegre	138 (30.9)	13 (10.6)	
Natal	16 (3.6)	13 (10.6)	
Rio Grande	97 (21.7)	10 (8.1)	
Sao Paulo	73 (16.3)	7 (5.7)	
Previous diagnosis of histoplasmosis, No. (%)	10 (2.2)	12 (9.8)	<.001
Previous empirical use of antifungals,[b] No (%)	8 (1.8)	39 (32.0)	<.001
Fluconazole	2/8 (12.5)	6/39 (15.4)	
Itraconazole	2/8 (12.5)	9/39 (23.1)	
d-AmB	6 (75.0)	30/39 (77.0)	
L-AmB	0 (0.0)	2/39 (5.1)	
ABLC	0 (0.0)	1/39 (2.5)	
Reported exposure to environmental risk factors, No. (%)	(n = 269)	(n = 91)	
Rural activity	69 (25.7)	26 (28.5)	.585
Poultry farms	83 (30.9)	30 (33.0)	.708
Caves	6 (2.2)	3 (3.3)	.573
Places with bats	32 (11.9)	17 (18.7)	.103
Tunnels	2 (0.7)	0 (0.0)	>.999
Construction	76 (28.3)	23 (25.3)	.582
Bird exposure	64 (23.8)	14 (15.4)	.092

Abbreviations: ABLC, amphotericin B lipid complex; d-AmB, amphotericin B deoxycholate; IQR, interquartile range; L-AmB, liposomal amphotericin B; PLWHA, people living with HIV/AIDS.

[a]Municipalities with <10 patients in the cohort were omitted from this table.

[b]In the last 7 days before sample collection.

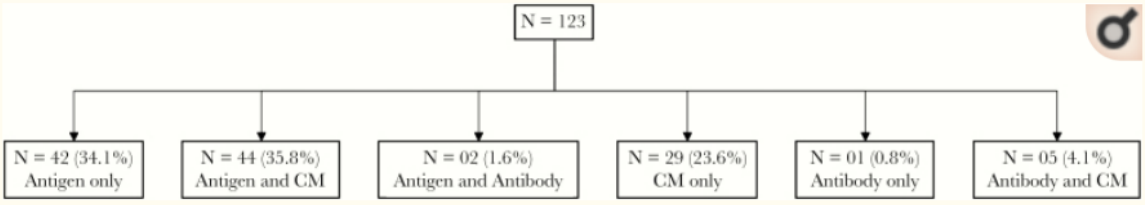

Figure 1.

Criteria for diagnosis of histoplasmosis in the 123 patients with probable/proven disease. From the original cohort of 570 patients, 447 were excluded due to negative results. Abbreviation: CM, classical methods (mycological and histopathological examination).

The detection of Histoplasma antigen in the urine increased the diagnosis of disseminated histoplasmosis by 53.8% in comparison with classical methods. When all patients with positive antigen were considered (n = 88), 74 (84.1%) were properly investigated with classical methods, and the diagnosis was confirmed locally in 44 of these patients (59.5%). Conversely, urine testing detected only 44 (56.4%) of the 78 patients with a diagnosis made by classical methods. However, it is to be noted that 39.7% of patients with proven histoplasmosis were using antifungals before sample collection (at least 7 days of use), which may have influenced results.

Twenty-two (3.9%) patients had a previous diagnosis of histoplasmosis. In 20 of these (90.9%), data were available on the time between previous diagnosis and current disease, with a median (range) of 12 (1–143) months. Regarding therapy, 9 of these patients (40.9%) were using antifungals before study entry: itraconazole (n = 7, 31.8%), amphotericin B deoxycholate (n = 6, 27.2%), and amphotericin B lipid complex (n = 1, 4.5%).

In the multivariate analysis, CD4+ cell count <50 cells/mm3 (odds ratio [OR], 2.45; 95% confidence interval [CI], 1.60–3.78), use of antiretroviral at study entry (OR, 0.54; 95% CI, 0.34–0.83), and being enrolled in a city in the Northeast region of Brazil (OR, 2.61; 95% CI, 1.53–4.40) were independently associated with probable/proven histoplasmosis.

Clinical presentation of patients was very exuberant, especially in patients with histoplasmosis. Most patients were investigated with imaging techniques and laboratory tests. Table 2 summarizes the main clinical, imaging, and laboratorial findings of patients with histoplasmosis, in comparison with patients with other diseases. With the exception of C-reactive protein and serum hemoglobin, all other tests were significantly more abnormal in probable/proven histoplasmosis patients, compared with patients without histoplasmosis. A

multivariate model for clinical variables predicting the occurrence of histoplasmosis is described in Table 3.

Table 2.

Findings on Clinical Examination, Diagnostic Imaging, and Laboratory Tests in PLWHA With or Without Probable/Proven Histoplasmosis at Study Inclusion

Clinical Manifestations	No Histoplasmosis (n = 447)	Probable/Proven Histoplasmosis (n = 123)	*P* Value
Weakness, No. (%)	374 (83.7)	111 (90.2)	.070
Anorexia, No. (%)	254 (56.8)	99 (80.5)	<.001
Night sweats, No. (%)	183 (40.9)	69 (56.1)	.003
Weight loss, No. (%)	336 (75.2)	112 (91.1)	<.001
Amount of weight loss, median [IQR], kg	10 [6–15]	10 [7–15]	.412
Time losing weight, median [IQR], d	60 [30–90]	60 [30–90]	.486
Dyspnea, No (%)	206 (46.1)	53 (43.1)	.555
Generalized lymph node enlargement, No. (%)	69 (15.4)	7 (5.7)	.005
Oral lesions, No. (%)	131 (29.3)	53 (43.1)	.004
Diarrhea, No (%)	149 (33.3)	40 (32.5)	.865
Splenomegaly, No. (%)	55 (12.3)	29 (23.6)	.002
Hepatomegaly, No. (%)	78 (17.5)	43 (35.0)	<.001
Skin lesions, No. (%)	114 (25.5)	48 (39.0)	.003
Papular rash, No. (%)	11 (2.5)	11 (8.9)	.001
Thorax imaging	(n = 348)	(n = 101)	
Normal exam	13 (3.7)	3 (3.0)	.715
Miliary pattern	42 (12.1)	28 (27.7)	<.001

Pleural effusion	51 (14.7)	22 (21.8)	.088
Cavities	27 (7.8)	6 (5.9)	.538
Abdomen imaging	(n = 136)	(n = 56)	
Adrenal enlargement	9 (6.6)	1 (1.8)	.286
Hepatomegaly	85 (62.5)	46 (82.1)	.008
Splenomegaly	79 (58.1)	30 (53.6)	.566
Intra-abdominal lymph node enlargement	47 (34.6)	16 (28.6)	.422
CNS imaging	(n = 48)	(n = 12)	
Cerebral abscess	7 (14.6)	0 (0.0)	.326
Single mass lesion	10 (20.8)	7 (58.3)	.010
Multiple mass lesions	16 (33.3)	3 (25.0)	.735
Laboratory tests			
Pancytopenia	110 (24.6) (n = 447)	65 (52.9) (n = 123)	<.001
C-reactive protein, median [IQR], mg/L	59 [13–106] (n = 378)	59 [28–100] (n = 91)	.312
Lactate dehydrogenase, median [IQR], IU/L	332 [201–602] (n = 355)	901 [428–1880] (n = 107)	<.001
Alkaline phosphatase, median [IQR], IU/L	104 [75–200] (n = 355)	171 [85–488] (n = 105)	<.001
Ferritin, median [IQR], ng/mL	462 [171–1152] (n = 164)	1389 [488–5446] (n = 39)	<.001
Ferritin >1000 ng/mL, No. (%)	48 (29.3) (n = 164)	21 (53.9) (n = 39)	.004
Aspartate aminotransferase, median [IQR], IU/mL	31 [21–60] (n = 421)	55 [30–116] (n = 120)	<.001
Alanine aminotransferase, median [IQR], IU/L	26 [15–46] (n = 420)	34 [21–59] (n = 119)	.004
Gamma-glutamyltransferase, median [IQR], IU/L	83 [39–179] (n = 348)	112 [70–298] (n = 103)	.001
Hemoglobin, median [IQR], g/dL	10.1 [8.7–12.0] (n = 443)	9.8 [8.4–10.9] (n = 123)	.054
Platelet count, median [IQR], cells/mm^3	191 000 [129 000–281 000] (n = 441)	138 000 [78 000–217 000] (n = 123)	<.001
Leucocyte count, median [IQR], cells/mm^3	5360 [3100–7980] (n = 444)	4400 [2730–7000] (n = 123)	.011
Lymphocyte count, median [IQR], cells/mm^3	895 [510–1450] (n = 438)	564 [345–920] (n = 121)	<.001

Table 3.

Multivariate Model for the Clinical Prediction of Probable/Proven Histoplasmosis in the PLWHA Cohort

Clinical Variable	Odds Ratio (95% Confidence Interval)
CD4+ <50 cells/mm^3	2.11 (1.17–3.82)
Pancytopenia	1.79 (1.00–3.21)
Miliary pattern on thorax imaging	2.72 (1.35–5.46)
Hepatomegaly on clinical examination	2.47 (1.28–4.76)
Generalized lymphadenopathy	0.37 (0.11–0.96)
Lactate dehydrogenase >1000 IU/L	3.60 (1.94–6.69)

Abbreviation: PLWHA, people living with HIV/AIDS.

Patients presented not infrequently with other opportunistic infections, particularly active TB (n = 143, 25.1%). There were 19 cases of histoplasmosis and TB coinfection (15.4% of histoplasmosis cases). As shown in Figure 2, histoplasmosis becomes more frequent than TB in patients with CD4+ cell counts <50 cells/mm3 (difference not statistically different; P = .085). Pneumocystosis was diagnosed in 57 (10.0%) patients. In addition, 135 (23.7%) patients had bacterial infections, and 78 (13.7%) patients had cytomegalovirus (CMV) disease. CMV disease was reported in 30.5% of patients with histoplasmosis, in comparison with 11.9% of individuals without histoplasmosis (P = .016). Cryptococcosis and invasive candidiasis were uncommon (31 cases each, 5.4%).

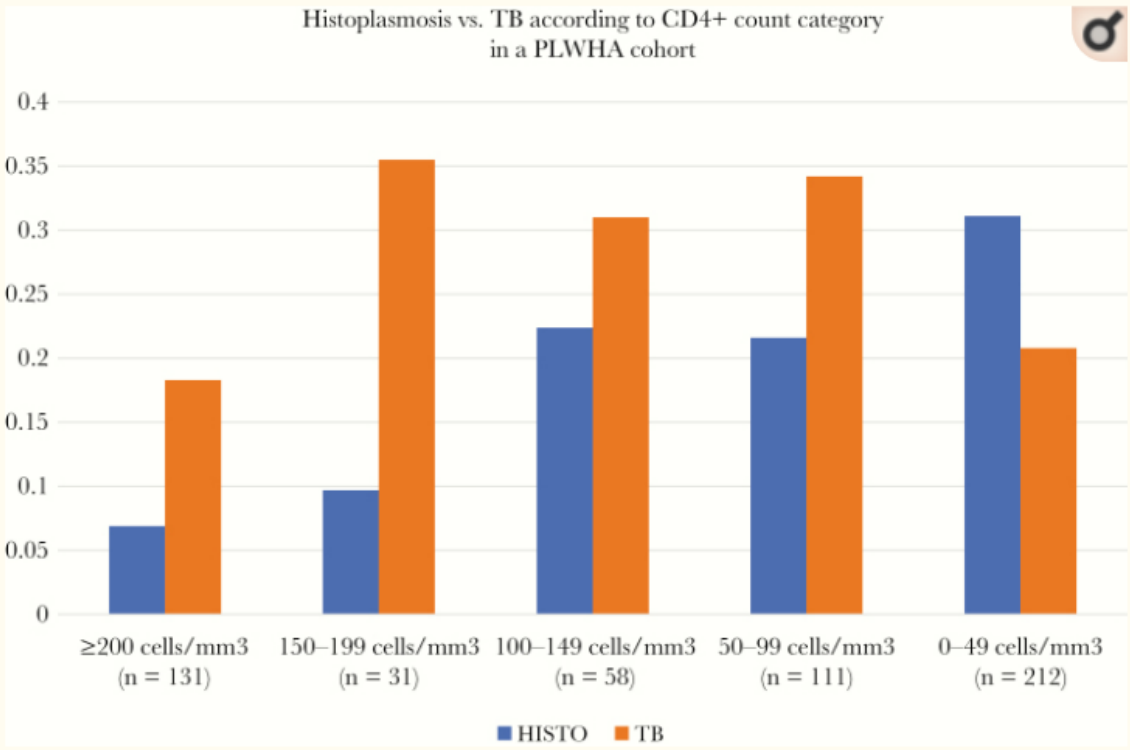

Figure 2.

Comparative frequency of histoplasmosis and tuberculosis in a cohort of PLWHA in Brazil. Abbreviations: HISTO, histoplasmosis; PLWHA, people living with HIV/AIDS; TB, tuberculosis.

Overall mortality in patients with histoplasmosis at 30 days was 22.0%. Mortality in patients diagnosed only by urinary antigen was 14.3% (6/42 patients), in comparison with patients diagnosed by classical methods (26.9%, 21/78; P = .114). Clinical features and their association with death in 30 days are presented in Table 4. In a multivariate analysis, only the presence of dyspnea at clinical presentation (OR, 2.83; 95% CI, 1.19–7.06) was independently associated with death.

Table 4.

Clinical Predictors of 30-Day Mortality in 123 PLWHA With Probable/Proven Histoplasmosis (Bivariate and Multivariate Analysis)

Variable	Bivariate Analysis			Multivariate Analysis
	30-d Survivors (n = 96)	Death in 30 d (n = 27)	*P* Value	OR (95% CI)
Demographics				
Age, median [IQR], y	40 [34–46]	36 [30–43]	.078	
Female gender, No. (%)	25 (26.0)	8 (29.6)	.710	
HIV disease status				
Receiving antiretroviral treatment at study entry, No. (%)	34 (35.4)	8 (29.6)	.575	
CD4+ count, median [IQR], cells/mm^3	38 [18–84]	49 [8–133]	.948	
CD4+ <50 cells/mm^3, No. (%)	55 (58.5)	11 (52.4)	.608	
Previous history				
Previous diagnosis of histoplasmosis, No. (%)	9 (9.4)	3 (11.1)	.724	
Symptoms and clinical examination				
Weakness, No. (%)	86 (89.6)	25 (92.6)	.642	
Anorexia, No. (%)	73 (76.0)	26 (96.3)	.025	
Night sweats, No (%)	52 (54.2)	17 (63.0)	.416	
Weight loss, No. (%)	87 (90.6)	25 (92.6)	>.999	
Dyspnea, No. (%)	36 (37.5)	17 (63.0)	.018	2.83 (1.19–7.06)
Oral lesions, No. (%)	41 (42.7)	12 (44.4)	.872	
Diarrhea, No. (%)	29 (30.2)	11 (40.7)	.302	

Table 4.

Clinical Predictors of 30-Day Mortality in 123 PLWHA With Probable/Proven Histoplasmosis (Bivariate and Multivariate Analysis)

Variable	Bivariate Analysis			Multivariate Analysis
	30-d Survivors (n = 96)	Death in 30 d (n = 27)	*P* Value	OR (95% CI)
Splenomegaly at clinical examination, No (%)	23 (24.0)	6 (22.2)	.851	
Hepatomegaly at clinical examination, No (%)	33 (34.4)	10 (37.0)	.798	
Skin lesions, No. (%)	37 (38.5)	11 (40.7)	.836	
Imaging				
Miliary pattern on thorax imaging, No. (%)	24 (30.8)	4 (17.4)	.291	
Diagnosis of histoplasmosis				
Diagnosis by classic methods, No. (%)	57 (67.9) (n = 84)	21 (84.0) (n = 25)	.116	
Positive direct microscopy, No. (%)	21 (53.9) (n = 39)	7 (77.8) (n = 9)	.270	
Positive culture for *H. capsulatum*, No. (%)	25 (38.5) (n = 65)	10 (41.7) (n = 24)	.784	
Only antigen testing positive, No. (%)	36 (37.5) (n = 96)	6 (22.2) (n = 27)	.139	
Therapy				
Empirical antifungal therapy	77 (80.2) (n = 96)	23 (85.2) (n = 27)	.558	
Histoplasmosis-directed antifungal therapy	53 (68.8) (n = 77)	18 (78.3) (n = 23)	.381	
Use of antifungals before study entry	32 (33.3) (n = 96)	7 (25.9) (n = 27)	.465	

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IQR, interquartile range; LDH, lactate dehydrogenase; OR, odds ratio; PLWHA, people living with HIV/AIDS.

Regional disparities inside Brazil were demonstrated (Figure 3). Centers located in the Northern states (Northeast and Midwest regions) had a higher prevalence of histoplasmosis than centers in the Southern states (South and Southeast regions). Even though there was a difference in mortality across centers, this difference was not present when only patients with histoplasmosis were evaluated (data not shown).

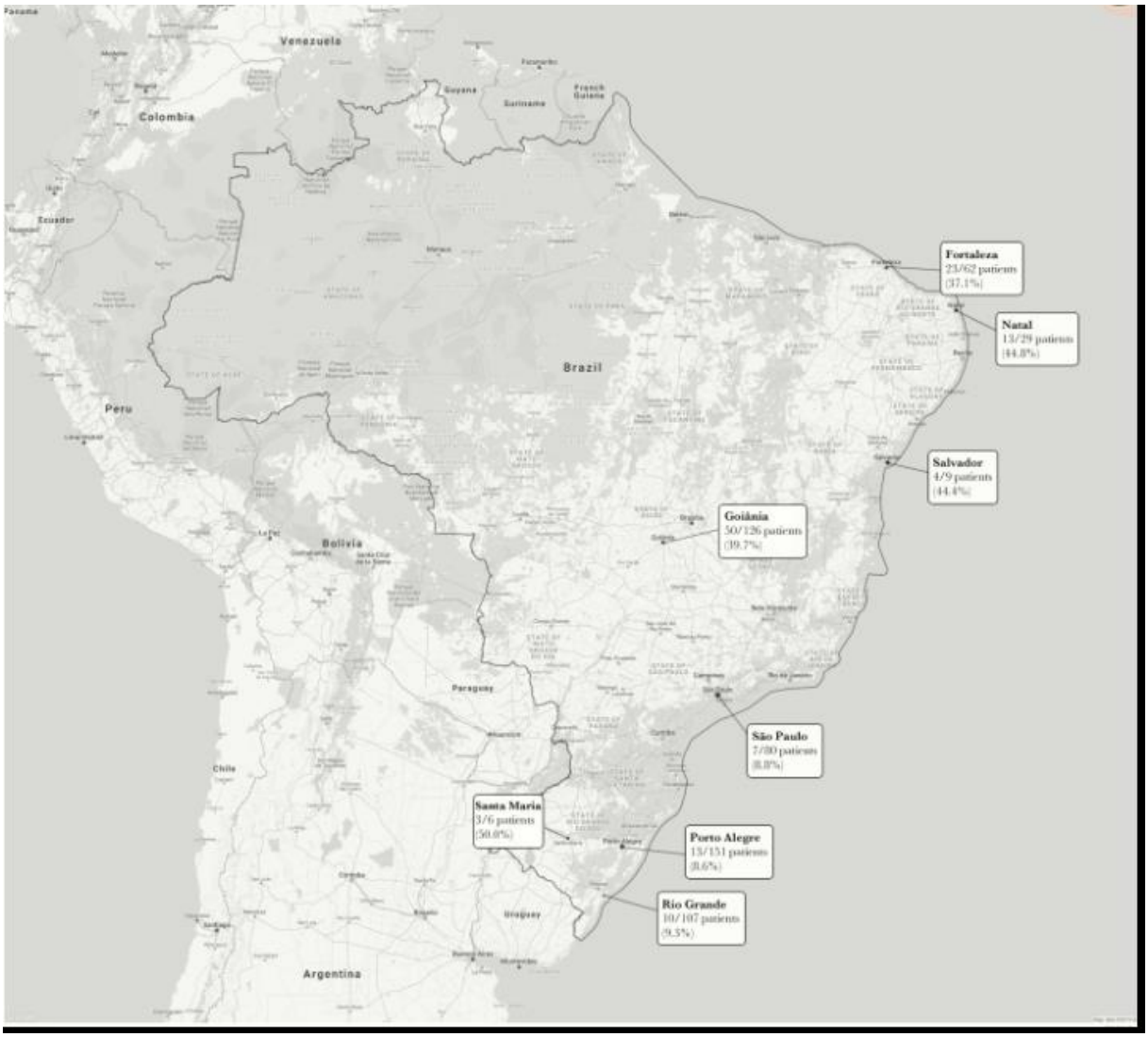

Figure 3.

Prevalence of probable/proven histoplasmosis in people living with HIV/AIDS according to city of sample collection.

Discussion

This is the largest prospective cohort study of AIDS patients with disseminated histoplasmosis ever conducted [20–23]. We found a high rate (21.6%) of probable/proven

histoplasmosis in febrile PLWHA admitted to Brazilian hospitals. These findings have tremendous impact in terms of public health and disease awareness in South America, considering that Brazil is the largest country and has the greatest population in this region.

Disseminated histoplasmosis is an opportunistic infection that usually occurs when the CD4+ cell count is <150 cells/mm3, and its prevalence rates vary widely. The mortality of AIDS-associated histoplasmosis ranges between 20% and 70% in studies carried out mainly in developing countries [21, 22, 24–26]. Clinical manifestations of disseminated histoplasmosis may often mimic other diseases, like TB. Pulmonary involvement is also common, along with systemic symptoms such as fever and weight loss. Skin lesions, hoarseness, gastrointestinal ulceration or strictures, meningitis, and adrenal masses are other clinical manifestations often encountered in this condition [2, 3]. Such unspecific clinical presentations and the high lethality observed lead to an urgent need for rapid and accurate diagnostic tools, along with the identification of clinical features that increase the probability of this diagnosis. In our cohort, weight loss, hepatomegaly, pancytopenia, miliary pattern on thorax imaging, lactate dehydrogenase (LDH) >1000 IU/L, and the absence of generalized lymphadenopathy were independently associated with diagnosis of probable/proven histoplasmosis in PLWHA. These findings are in agreement with other South American reports [21, 22, 26]. In a descriptive study of 25 years of experience in French Guiana, including 200 cases, there were many similarities regarding laboratory findings with our study. In particular, increased LDH, aminotransferases, alkaline phosphatase, ferritin, and thrombocytopenia were more frequent in patients with histoplasmosis in both these large studies. In contrast, the study from French Guiana identified a majority (55.3%) of patients presenting with enlarged lymph nodes [27]. As already postulated as the reason for increased frequency of skin lesions in a Brazilian case series, genetic differences in fungal strains may influence enlargement of lymph nodes [11, 22]. Incomplete records in clinical notes may also have accounted for such differences.

Laboratory diagnosis of histoplasmosis is often difficult and overlooked. Available tests include culture, microscopy, histopathology, antibody detection, antigen detection, and molecular assays. Culture can take up to 4 weeks to reveal growth. Microscopy is more rapid but may have low sensitivity and specificity. Histopathological findings of small intracellular budding yeasts using specific stains are diagnostic, although they can be mistaken with other organisms such as Leishmania species (also endemic in Brazil). In addition, both microscopy and histopathology require highly trained professionals to make a proper diagnosis [28]. Antibody detection has limited sensitivity in immunocompromised individuals with disseminated disease. Hence, quick, noninvasive, and sensitive diagnostic methods are needed,

and so far only antigen detection and polymerase chain reaction (PCR) tests meet these requirements [2]. Unfortunately, PCR is not available in most laboratories in Latin America and the Caribbean. Also, H. capsulatum antigen detection is not available in endemic areas for histoplasmosis, including Brazil [29]. Therefore, histoplasmosis is frequently diagnosed as a late-stage disease.

We used a previously validated Histoplasma antigen test (Histoplasma galactomannan single-monoclonal-antibody sandwich ELISA; Immuno-Mycologics, Norman, OK) which is known to have a better performance than gold standard tests in the diagnosis of histoplasmosis (ie, classical methodological methods). Nevertheless, we observed a lower sensitivity of the antigen test (71.5%) in comparison with a previous study [17]. We believe that the reduced test sensitivity observed in our study could be attributable to previous use of antifungal drugs (which occurred in 39.7% of "proven" histoplasmosis patients). Some of the discordant results between antigen detection tests and classical mycology tests could be justified by the time between sampling for fungal culture and the urinary Histoplasma antigen test (average 39 days).

Our cohort study supports these arguments, demonstrating high 30-day mortality (26.9%) in patients with proven disease—that is, confirmed by classic mycological methods, which is concordant with current reports. Antigen detection using the Histoplasma galactomannan test improved diagnostic yield in 53.8%. In patients with only a positive antigen test, 30-day mortality was lower (14.3%), though it was not significantly different, than in patients diagnosed with classical methods. Indeed, patients only with a positive antigen test could be false positives, which should lead to lower mortality. However, these were all febrile inpatients individuals with HIV/AIDS, many of whom had very low CD4+ counts. The pretest probability for histoplasmosis is much higher than a false-positive result in these settings. It also should be noted that centers were not notified of antigen results on time to make clinical decisions; therefore, patients were not treated based on antigen results, something that may prove to substantially decrease mortality in future studies. These findings illustrate the potential benefit of the availability of this diagnostic tool in the management of PLWHA.

Brazil is a continental country and has many disparities in terms of climate, soil composition, and economics. Despite the HIV infection being widespread in the country, some opportunistic diseases still occur in a typically endemic behavior. We observed a huge (>40%) prevalence of probable/proven histoplasmosis among febrile PLWHA in the Central-Northeast region of Brazil, especially in the cities of Fortaleza and Natal. Despite these figures, cities from South, including Porto Alegre and Sao Paulo, had prevalence rates under 10%. These findings

should allow for an increase in disease awareness nationwide, with a special emphasis on particular regions of the country.

TB is a huge threat to public health in Brazil. Brazil is 1 of the 20 countries with the highest TB burden in the world [30]. However, as shown in our study, histoplasmosis is an HIV opportunistic infection that has a comparable frequency. The endemicity of histoplasmosis, along with the occurrence of disseminated disease in immunocompromised people, is now a well-defined phenomenon in Central and South America, and it represents a significant threat to PLWHA with low CD4+ counts [5]. We demonstrated that in these low–CD4+ cell count categories, even though histoplasmosis is a major opportunistic infection affecting PLWHA in Brazil, it tended to be more frequent than TB (with the lack of statistical difference being probably due to the limited number of patients in this category included in the trial). This should prompt revisiting of the argument for itraconazole prophylaxis in the region, particularly in the Northern states where the prevalence is very high. Itraconazole has significant drug–drug interactions that should be taken into account, considering the possibility of TB coinfection and the need of rifampin as the basis of TB therapy [31]. Another interesting but speculative approach could be an aggressive screening and preemptive therapy strategy for histoplasmosis in patients with low CD4+ cell counts from areas with high prevalence. This strategy is currently recommended for cyrptococcal infection using cryptococcal antigen detection [32, 33] and may also be reasonable for histoplasmosis. In areas with lower prevalence, this screen-and-treat strategy should be more restricted considering cost-effectiveness, just like what has been done with cryptococcosis [34, 35].

In summary, histoplasmosis is a frequent opportunistic infection in Brazil, and antigen detection could improve the diagnostic capacity of this condition, potentially improving clinical outcomes, including mortality. Knowledge on histoplasmosis epidemiology is absolutely necessary given the high mortality associated with this condition. Access to diagnostic tools and antifungal drugs, including point-of-care technologies and antiretroviral treatment, is urgently needed to decrease disease burden in South America.

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APÊNDICE B

"Histoplasmosis Outbreaks in Brazil: Lessons to Learn About Preventing Exposure"

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ORIGINAL PAPER

Histoplasmosis Outbreaks in Brazil: Lessons to Learn About Preventing Exposure

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Abstract

Histoplasmosis is considered the most common invasive opportunistic fungal disease in the Americas, with outbreaks and micro-epidemics reported for over 80 years. In Brazil, this disease has been described since 1946, reaching a remarkable incidence in the population, especially during the HIV–AIDS pandemic. In this study, published and unpublished outbreaks and micro-epidemics of histoplasmosis in Brazil were revisited by accessing different database sources and evaluating epidemiological and clinical features. We have found reports spanning 1946–2017, across 10 Brazilian states and with involvement of 370 humans and 2 dogs, and 13 disseminated cases and 3 deaths were reported. Rio de Janeiro had the largest number of outbreaks (n = 20/40; 50%) reported in this study. The majority of outbreaks and micro-epidemics was reported in caves (n = 21/40; 52.5%), followed by reports in abandoned/deactivated sites (n = 6/40; 15%), mines (n = 5/40; 12.5%), chicken coops (n = 4/40; 10%). Histoplasmosis is a serious health issue in Brazil considering the attractive and growing market of ecotourism throughout more than 7000 caves, and all levels of poultry farming activity are important to raise awareness about how dangerous this neglected disease can be and

establish ways to decrease exposure to contaminated environmental sources through adequate preventive measures.

Introduction

Histoplasmosis is a worldwide-distributed systemic mycosis caused by the dimorphic fungus Histoplasma capsulatum sensu lato. This mycosis has been reported for over a century on all continents with the exception of Antarctica. The American and African continents are the most affected, especially in underdeveloped countries [1,2,3]. Bahr et al. [2] describe histoplasmosis worldwide through evaluation of histoplasmin skin tests (reaching reactivity up to 89% in some areas), showing the highest prevalence in the Americas and increasing rate of infections in Asian and African countries.

Histoplasmosis has first been described by Samuel Darling in 1906 in a case with the disseminated form from Panama [4]. A series of important studies were followed by characterizing the pathogen antemortem [5] as well as the identification of dimorphism and the first case of this infection in a dog [6]. The notorious severity of this neglected disease came with the HIV pandemic due to the high mortality ratio involving HIV–H. capsulatum co-infection [7,8,9]. Histoplasma sp. are mainly found in its filamentous form within enclosed areas enriched with bat and/or bird droppings (rich sources of nitrogen and phosphate compounds), low luminosity, and moderate temperature (~ 25 °C). Caves, basements, old buildings, and chicken coops are natural environmental sources of this fungus [10]. The fungal ecology has been studied for a long time and has been elucidated by pioneer studies of Dr. Chester Wilson Emmons and Dr. Libero Ajello, among other researchers, which have showed the influence, especially of bats and birds in the growth and dispersion of Histoplasma spp., but also demonstrated the isolation of the pathogen from guano-enriched soils [11,12,13,14]. Many mammalians, like humans, dogs, and cats are potential hosts [11, 15].

During the saprobic phase, Histoplasma spp. produce infectious structures known as macro- and microconidia, the latter being the main infective spores due to their reduced size. After microconidia aerial dispersion and their inhalation by mammalian hosts, those spores reach lungs' alveoli, switch their morphology to the yeast phase, and develop the infection [16, 17]. In addition, conidia can be easily dispersed by wind, reservoir hosts such as bats, and through soil disturbance, generating the possibility of new infection foci in unprecedented regions [10]. The onset of the infection depends on the fungal–host interaction, which is modulated by three key factors: immunological status of the host, the virulence of a given fungal strain, and the amount of fungal spores inhaled [16, 17]. Usually, histoplasmosis is asymptomatic in immunocompetent hosts, with minor cases displaying low fever, dry cough

and fatigue, which can lead to misdiagnosis due to its similarity with other community-acquired pneumonia such as the flu or tuberculosis [3, 9, 18]. Immunocompromised patients such as people living with HIV/AIDS, individuals under chemotherapy and transplant patients are the primary concern because their immune system may not be able to withstand the infection [1]. Among these patients, there is a notorious ability for the fungus to disseminate to other organs through the bloodstream, along with high mortality rates, especially if they are not treated in the early stages of the infection [19]. The most common drugs used for treatment are liposomal amphotericin B and itraconazole [1].

With the advances of molecular systematics, new species diagnostics and identification methods have been developed, allowing to better understand the Histoplasma spp. evolution and taxonomy. The genotypic distribution of Histoplasma spp. from different countries around the globe was accessed via Multi-Locus Sequencing Typing (MLST), revealing at least eight species-level clades as follows: North America clade 1 (NAm 1), North America clade 2 (NAm 2), Latin America clade A (LAm A), Latin America clade B (LAm B), Panama, Australia, Netherlands, Eurasia, and Africa [20]. By expanding this work, Teixeira et al., 2016 provided substantial shreds of evidence that the genus Histoplasma is composed by at least 17 different monophyletic lineages; the LAm A and LAm B clades were split into LAm A1, LAm A2, LAm B1, LAm B2, RJ, and BR1-4, along with the description of the new phylogenetic species BAC1 [21]. Recently, four monophyletic lineages of Histoplasma were re-classified by using whole-genome phylogenetic concordance as follows: Histoplasma capsulatum sensu stricto Darling (Panama), Histoplasma mississippiense (NAm 1), Histoplasma ohiense (NAm 2), and Histoplasma suramericanum (LAm A) [22]. The Africa clade was not ranked as a new species due to low taxa sampling. So far, only a few genomes of Histoplasma strains from South and Central America were sequenced by next-generation sequencing, and further taxonomical refinement in Latin America is needed, where the mycosis broadly occurs.

Currently, histoplasmosis outbreaks or micro-epidemics are defined as a sudden pulmonary infection affecting more than one individual in a defined community and period [23]. Hundreds of outbreaks have been reported throughout the endemic areas of histoplasmosis around the globe, especially in the Americas, followed by Africa and Asia [24, 25]. In this study, we aimed to critically review the histoplasmosis outbreaks/micro-epidemics in Brazil by accessing the clinical features and the potential causative sources as well as by discussing how preventive actions might protect people from a high burden of Histoplasma exposure [26]. Thus, we reviewed at least 40 published and unpublished outbreak cases and micro-epidemics over 71 (1946–2017) years. We herein characterize general clinical features, geographic

location, and the most likely recurrent sites of infection of two or more individuals. Finally, a set of recommendations are suggested to avoid exposure to areas with a high likelihood of Histoplasma contamination.

Materials and Methods

The methodology was based on the concept of a descriptive study, whereas the main objective is to determine the distribution of a particular disease or health situation. To analyze this parameter, we used databank sources with secondary data, such as published papers, epidemiological bulletins, M.Sc. and PhD. reports and unpublished cases found on the internet. Searching Criteria and Data Filtering for Referential Articles

Starting on February 21, 2019, international and national databases such as NCBI Pubmed, Google, Google Scholar, Scielo, Web of Science were searched for combinations of keywords "histoplasma," "histoplasmosis," "histoplasmose and surtos," "histoplasmosis outbreaks in Brazil," "micro-epidemics/epidemics and histoplasmosis," also including Brazilian Universities, cities, states, authors, and year of publishing. All relevant references of the articles were checked to gather as much information as possible. Inclusion criteria were based on studies in any languages that necessarily reported an outbreak or a micro-epidemic of histoplasmosis where two or more people or one person and a non-human mammalian presented symptoms compatible with the disease and got infected from the same exposure event. Only reports within Brazil were considered and isolated cases were excluded from the study. Epidemiological and clinical features like sex, occupation, and others were taken into consideration. Relevant information extracted from the studies consisted of the reported date of the outbreak (month and year), location (state and city), geographic coordinates, number of people involved, amount of positive cultures, X-ray photography data, immunodiagnostic results, dissemination, and disease outcome.

Cartography

Histoplasmosis outbreaks and micro-epidemics were mapped, state by state, through a search of coordinates of locations described in the reports to illustrate disease's distribution across the country. The software used to produce the maps with the data that was found in the research was QGIS 3.6.2 'Noosa', released on april 19, 2019. This software can make a match between the specific location and the cleaned data, favoring the understanding between the outbreaks and their location. Graphpad Prism v6.0 was used for graphs through analysis of data present in Table 1.

Table 1 Summary of data collected

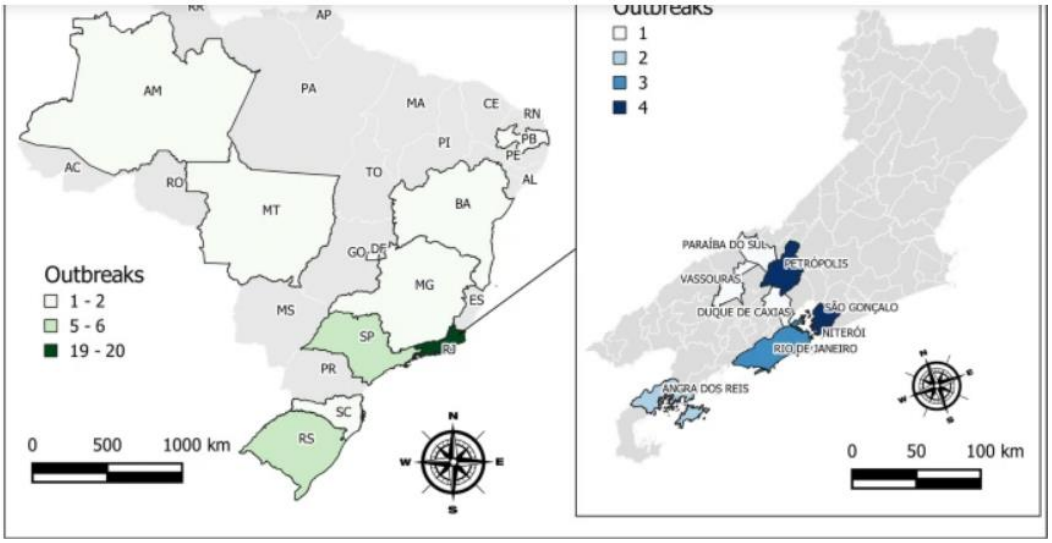
Study	Reference	Date	City	State	Number involved	Diagnostic	Pulmonary involvement	Dissemination	Deaths	Occupation
1	[49]	September, 1943	Eldorado	SP	1 human and 1 dog	Yes	NA	Yes	Yes	Was born in a straw hut
2	[50]	October, 1958	Paraíba do Sul	RJ	13 humans and 1 dog	Yes	Yes	Yes	No	Bats-inhabited cave
3	[51]	1959	Rio de Janeiro	RJ	8	NA	Yes	NA	No	Swimming in abandoned site
4	[52]	April, 1966	North Cost of SP (Between Caraguatatuba and Ubatuba)	SP	8	Yes	Yes	No	No	Bats-inhabited house
5	[53]	July, 1967	Brasília	DF	14	Yes	Yes	No	No	Bats-inhabited cave
6	[51]	1972	Vassouras	RJ	5	NA	Yes	Yes	Yes	Bats-inhabited cave
7	[54]	1975	Rio de Janeiro	RJ	5	Yes	Yes	NA	No	Bats-inhabited cave
8	[51]	1978	Near Angra dos Reis	RJ	7	NA	Yes	NA	No	Caving
9	[55]	October, 1978	Canoas	RS	2	Yes	Yes	No	No	Bats-inhabited tree
10	[56]	1980	Angra dos Reis	RJ	8	Yes	Yes	NA	NA	Caving
11	[51]	September, 1981	Niterói	RJ	10	Yes	Yes	Yes	No	Caving
12	[51]	May, 1981	São Gonçalo	RJ	14	Yes	Yes	NA	No	Caving
13	[51]	October, 1981	Petrópolis	RJ	10	Yes	Yes	NA	No	Caving
14	[57]	May, 1981	São Gonçalo	RJ	10	Yes	Yes	Yes	No	Abandoned mine
15	[57]	July, 1981	São Gonçalo	RJ	4	Yes	Yes	NA	No	Abandoned mine
16	[57]	October, 1981	Petrópolis	RJ	10	Yes	Yes	NA	No	Abandoned mine
17	[51]	February, 1982	Petrópolis	RJ	5	Yes	Yes	NA	No	Caving
18	[58]	August, 1982	São Gonçalo	RJ	6	Yes	Yes	Yes	No	Bats-inhabited cave
19	[57]	February, 1982	Petrópolis	RJ	5	Yes	Yes	NA	No	Abandoned mine
20	[57]	July, 1982	Niterói	RJ	6	Yes	Yes	Yes	No	Abandoned mine
21	[51]	July, 1984	Duque de Caxias	RJ	12	NA	Yes	NA	No	Chicken coop
22	[51]	1984	Niterói	RJ	17	Yes	Yes	NA	No	Abandoned site—bats-inhabited
23	[59]	July, 1986	Borborema	PB	6	Yes	Yes	No	No	Chimney cleaning—bat droppings
24	[60]	1990	Rio de Janeiro	RJ	8	Yes	Yes	No	No	Cleaning a chicken coop
25	[61]	March, 1993	Manaus	AM	8	Yes	Yes	No	No	Caving
26	[62]	1993	Taquari	RS	2	Yes	Yes	Yes	No	Chicken coop cleaning
27	[63]	May, 1997	Pedro Leopoldo	MG	4	Yes	Yes	No	No	Caving
28	[64]	2000	Jequié	BA	4	Yes	Yes	No	Yes	Basement cleaning—bat droppings
29	[65]	2006	Blumenau	SC	2	Yes	Yes	No	No	Dust cleaning—bats
30	[66]	September, 2007	Arapeí	SP	35	Yes	Yes	No	No	Caving
31	[67]	September, 2007	Areias	SP	35	Yes	NA	NA	NA	Bats-inhabited cave
32	[68]	August, 2007	Cáceres	MT	34	Yes	Yes	No	No	Caving
33	[69]	2013	Unaí	MG	8	Yes	Yes	No	No	Bats-inhabited cave
34	[70]	May, 2017	Brazlândia	DF	18	No	Yes	No	No	Caving
35	[71]	1971–1973	North Cost of SP (Sununga beach)	SP	10	Yes	NA	NA	NA	Caving
36	[72]	NA	São Gabriel	RS	3	Yes	Yes	Yes	No	Chicken droppings
37	[72]	NA	Porto Alegre	RS	2	Yes	Yes	Yes	No	NA
38	[72]	NA	Porto Alegre	RS	2	Yes	Yes	Yes	No	NA
39	[73]	NA	Niterói	RJ	5	Yes	Yes	Yes	No	Bats-inhabited cave
40	[74]	NA	Vale do Paraíba	SP	4	Yes	Yes	Yes	No	Bats-inhabited cave

NA non-available

Results

From 1946 to 2017, we found 40 episodes of multiple Histoplasma infections that were characterized as an outbreak or micro-epidemic affecting 370 people and 2 dogs in total (Table 1). Complete epidemiological and clinical information was not available in all reports (shown in Table 1 as non-available "NA"). From those human patients, 193 were male and 81 were female according to the reported data. Most outbreaks were observed in Rio de Janeiro (RJ) (Fig. 1b), which accounts for (n = 20/40; 50%) of all outbreaks, followed by São Paulo (SP) (n = 6/40; 15%), Rio Grande do Sul (RS) (n = 5/40; 12.5%), Minas Gerais (MG) and the Distrito Federal (DF) (n = 2/40; 5%) each, Bahia (BA), Mato Grosso (MT), Paraíba (PB), Amazonas (AM), and Santa Catarina (SC) (n = 1/40; 2.5%) each (Fig. 1a). Cave visitation/training exposures accounted for 52.5% of infections (n = 21/40) while 15% were reported in abandoned/deactivated sites (n = 6/40), 12.5% in mines (n = 5/40), 10% in chicken coops cleaning/management (n = 4/40), 5% from other sources (n = 2/40) and 5% of unknown/non-described sources (n = 2/40—Fig. 2). Diagnostics by either fungal culture, X-ray photography, histoplasmin intradermic reaction, or antibody detection were described in 87, 5% (n = 35/40) of the cases (Table 1). In all but three studies, deep pulmonary involvement was confirmed, dissemination was observed and described in 13 reports and, three deaths occurred in individuals without known immunodeficiency status (Table 1).

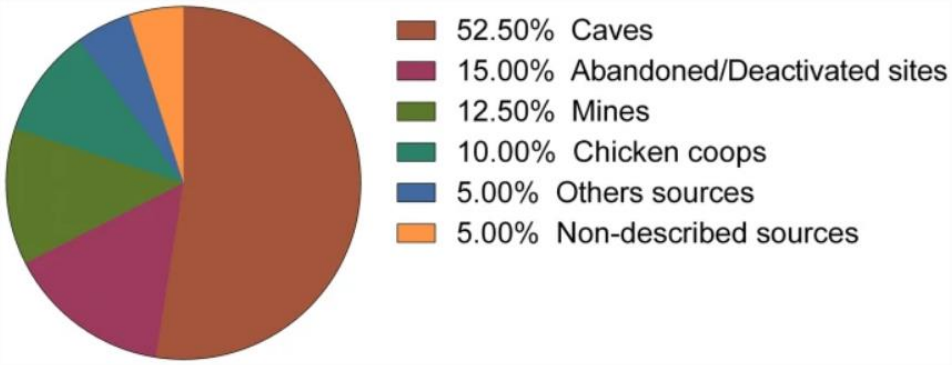
Fig. 1



Histoplasmosis outbreaks in Brazil. a Outbreaks and micro-epidemics reported in Brazil from 1946 to 2017. Outlined states in white (AM, MT, BA, MG, PB, SC, DF), gray (SP, RS), and black (RJ) had 1–2; 5–6 and 19–20 reports, respectively. b Outbreaks and micro-epidemics in the state of Rio de Janeiro (RJ). Cities were pinpointed according to their respective geographic coordinates. Outlined cities in white (Vassouras, Paraíba do Sul, Duque de Caxias),

gray (Angra dos Reis), darker gray (Rio de Janeiro) and black (Petrópolis, São Gonçalo, Niterói) had 1, 2, 3 and 4 outbreaks reported, respectively

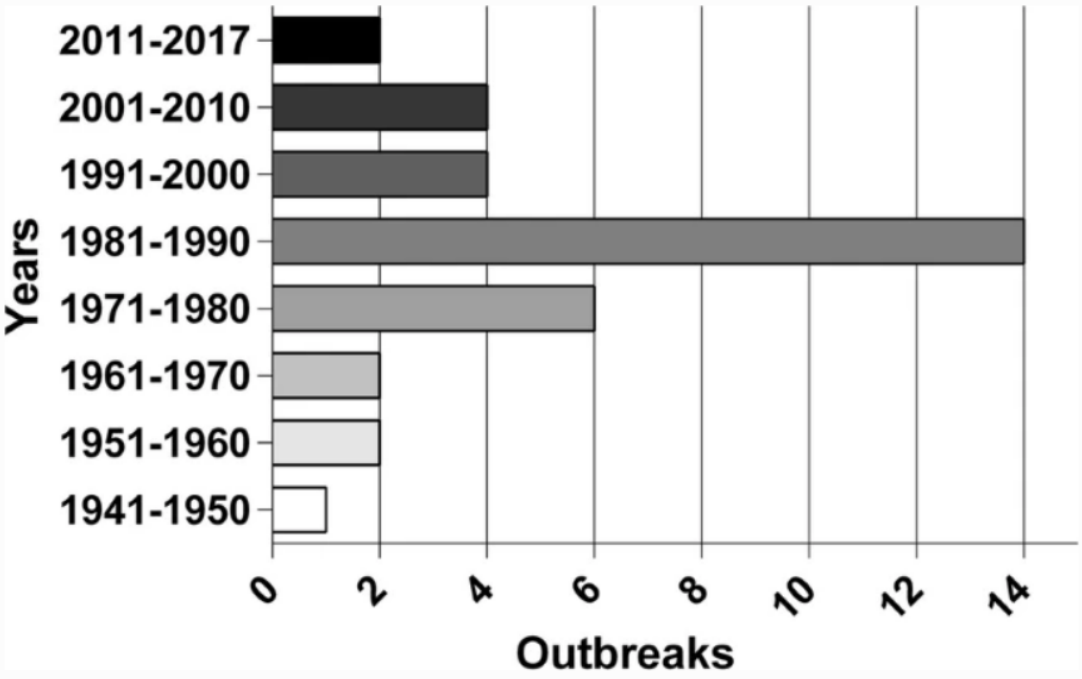
Fig. 2



Exposure sources of *Histoplasma* spp. outbreaks reported in Brazil

Most outbreaks (n = 29/40; 72.5%) were reported in the last century, with the majority being reported during 1980s decade (n = 14/40; 35%). There was an increased number of reported outbreaks in the 1970s and 1980s (n = 17/40; 42.5%) in total (Fig. 3). Most of the outbreaks were published from July to October (Table 1).

Fig. 3



Reported histoplasmosis outbreaks from 1946 to 2017 in Brazil

Discussion

Histoplasmosis is caused by a dimorphic fungus mostly found in areas with high concentrations of bat guano and/or bird droppings coupled with enclosed warm environments and low luminosity, especially in caves and chicken coops [24]. In Brazil, there are several published studies concerning HIV/AIDS patients [9, 27,28,29]; however, a considerable number of outbreaks have also been reported all over the country with alarming statistics. Brazil is a hotspot for histoplasmosis outbreaks, especially because of its growing speleology activity throughout the vast number of caves all over the country coupled with the adequate conditions for fungal growth and dispersion found in those environments. Specific diagnostic tests for histoplasmosis are not always available in all country's regions and most clinical data generated ends up filed and stored, resulting in almost no notification whatsoever and thus avoiding any ways of effective countermeasures to decrease the number of cases. In addition, many studies are not published, and M.Sc. projects and PhD. thesis are often locally archived masking the true numbers of this disease. Despite the high rates of infection in immunocompetent patients and the high mortality among people living with HIV/AIDS, unfortunately histoplasmosis is not a notifiable disease in the Brazilian territory, except for the Goiás state. The example of Goiás state must be followed by other state-level and country-level health agencies, especially in other states with intense mining and agricultural activities.

We found through our research that caves are the most common sites of infection (n = 21/40; 52.5%). According to the Sociedade Brasileira de Espeleologia (SBE), there are 7862 catalogued caves in Brazil, which brings an alert to potential exposure to Histoplasma sp. for thousands of ecotourists all over the country (http://www.cavernas.org.br/cnc/). Due to the defective knowledge about the disease and the lack of notification by Brazilian health agencies, histoplasmosis awareness is scant and people at risk are not conscious on how to avoid potential infection through the use of simple measures, mostly, personal protective equipment (PPE) such as appropriate clothing, N95 masks, or other types of protective respirators [30].

In immunocompetent people, the disease is usually asymptomatic and most of the symptomatic patients display minor, self-limited symptoms and rapidly respond to appropriate treatments [31,32,33]. Nevertheless, there were some cases of severe pulmonary manifestations, dissemination, and even deaths in apparently immunocompetent people. Therefore, we can speculate that this disease is a major concern in immunocompromised patients and even in immunocompetent individuals.

It is important to notice a possible connection between HIV pandemics that happened in the early 1980s [19, 34] with increased outbreak reports in the same period (Fig. 3). Data from the US Center for Disease Control and Prevention (CDC) show that the mortality rate in co-infected Histoplasma spp.-HIV/AIDS patients ranges from 20 to 40%, showing that histoplasmosis should be carefully approached when dealing with immunocompromised patients such as HIV/AIDS, diabetes, transplanted, under chemotherapy or corticosteroids treatment [9]. However, the lack of deaths on the outbreaks that occurred in the 1980s weakens this hypothesis. Another plausible explanation for the excess of cases in the 1980s is that during that decade a medical mycology group from RJ was focused on the active search of histoplasmosis cases, which led to the description of (n = 37/40; 92.5%) of the Brazilian outbreaks on this decade. Also, the observed increase in histoplasmosis cases in the 80s in RJ might be due the economic growth of Southeast Brazil. This finding suggests that active surveillance programs can improve early histoplasmosis diagnosis and detection and managements of outbreaks [35].

We observed a considerable distribution of the disease across the country based on outbreak investigations and this could be explained by the fact that conditions for this fungus' growth are similar in several regions, but also that Histoplasma sp. can adapt itself through necessary genetic regulations to survive in different environments and interactions with suitable hosts [21, 22, 36]. It is worth mentioning that the numbers herein reported are not representative of the country-wide real situation, they are just an estimative of cases that have been reported with symptomatic patients, not counting the asymptomatic ones. Also, we observed that despite Rio de Janeiro having a low number of caves compared to other states, it had the most number of reported cases (n = 20/40; 50%). One of the reasons for the most reports being in this state could be that specific training of doctors in medical mycology along with adequate laboratory support by health and research Institutions in this state might contribute to identify those micro-epidemics related to Histoplasma. Thus, specific diagnostics are more efficient and available to the population or this increased number of outbreaks in Rio de Janeiro might be correlated with the high population density of southeastern Brazil. Certainly, there are many micro-epidemics that are not notified and may mask the real numbers of this disease [24].

It is worth noting that regions where an outbreak had already been reported may be a potential source for future re-exposition and consequently new outbreaks. Reports show that people who returned to the same place of a previous outbreak were infected or reinfected (reports 12 and 21—Table 1). An important aspect is that infection dynamics also includes domestic animals, described in studies 1 and 2 (Table 1), which showed dogs susceptible to

infection by Histoplasma spp. probably due to the inhalation of a large number of infective spores [11, 37]. Also, dogs follow their owners in outdoors activities with increased dust exposure, different than cats for example. So far, it has been described that histoplasmosis cannot be transmitted from animals to humans and the only form of infection is through inhalation of spores from the environment [11, 37, 38].

An intriguing result is that out of 23 studies in which the month of the outbreak was reported, 14 occurred within the period of July–October, but a statistical correlation could not be applied since the majority of the reports did not provide the number of involved individuals not infected or asymptomatic. As of today, histoplasmosis is mostly associated with dry seasons in countries with similar climate as Brazil such as the Guianas [39], but other related factors remain unclear. Long drought seasons may contribute for spore production and dispersion under specific conditions in other dimorphs [40, 41]. However, this information has been studied in other mycosis such as coccidioidomycosis, paracoccidioidomycosis with similar ecological characteristics such as growth, development, and infection, and these might help understanding the role of the environment in the dispersion and growth of Histoplasma spp. The conditions of each season, for example increased dust exposure, high precipitations, and drought periods are thought to be deeply related to increased incidence of coccidioidomycosis [41]. On the other hand, absolute air humidity, soil-related jobs, mostly farming due to aerosolization of dust, and climate variation phenomena such as El niño showed positive correlations with increased incidence of paracoccidioidomycosis [42].

In comparison to a similar work performed in the USA, where histoplasmosis is also endemic in several states in the middle and eastern areas of the country, Brazil has less than a half of scientifically reported USA outbreaks (40 and 105, respectively—[25]). These discrepancies do not necessarily mean that there are more outbreaks in the USA, but could be a reflection of different investment in healthcare, including more advanced and available diagnostics, surveillance programs, and alerts to the population about the risks of histoplasmosis provided by the USA health agencies in known hotspots of the disease. Also, histoplasmosis infections are mostly related to chicken coops as well as construction and demolition in the USA while cave visitation was the main source of exposure in Brazil; however in both countries, the majority, if not all cases, occurred in locations harboring high load of guano/bird droppings which increases the likelihood of infection by this fungus [25]. These observations provide strong arguments that Histoplasma spp. can develop in any region of North and South America under appropriate growth conditions [10, 11, 14].

Healthcare-associated infections (HAI) due to Histoplasma sp. should also be considered since residues generated by these facilities (i.e., any organic matter contained in trash) are not always properly discarded. The accumulation of such materials can attract pigeons and other birds and their droppings are a great source of nutrients for Histoplasma spp. growth, endangering local environment and, especially in hospitals, immunocompromised patients [16, 43]. Moreover, this fungus is a risk-group 3 pathogen, especially when dealing with its filamentous form due to high spore (microconidia) production, and should be manipulated specifically at biosecurity level 3 laboratories. Laboratory-acquired histoplasmosis has been reported in the past [44, 45] and since the 1960s no laboratory-acquired infections have been reported due improved biosecurity protocols that must be strictly followed to avoid potential infections.

Taking altogether, some precautions must be considered to decrease overall exposure to histoplasmosis in Brazil and other countries of Latin America: immunocompromised people with underlying diseases (e.g., AIDS and diabetes) or patients under immunosuppressive treatments (e.g, transplanted, chemotherapy, corticosteroid treatment) must avoid visitation to caves, chicken coops and mines, or contact with bird/bat droppings [9, 46,47,48]; long-term visitations in caves should be avoided by all personnel; avoid generation of aerosol by disturbance of soils with high concentration of bat guano/bird droppings; follow protocols of waste disposal; use of appropriate PPE during training in caves or any speleological practices in which a high concentration of bat guano/bird droppings are present; avoid places and periods of high bat activity; make awareness of areas in which histoplasmosis has been identified using warning signs; demand better notification of health-related departments and the government itself through every mean of communication, especially social medias. For a better understanding of the histoplasmosis situation in Brazil, investment in health programs, notification, and research development is necessary and further studies are vital to build a more concrete basis around environmental factors, spore dispersion, host–pathogen interaction, and diagnostics.

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Contributions

Conceived of or designed study—R.A.P., L.S.D., M.O.X., B.W., R.M.ZO., M.M.T.; Performed research—B.T.G., F.A.S., R.P.B., J.P.R.A.B., M.A.A.; Analyzed data—B.T.G., F.A.S., R.A.P., R.P.B., J.P.R.A.B., M.A.A., M.M.T.; Contributed new methods or models—B.T.G., F.A.S., J.P.R.A.B., B.W., R.M.ZO., M.M.T.; Wrote the paper—B.T.G., F.A.S., J.P.R.A.B., M.M.T.;

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Conflict of interest These authors have no conflict of interests or any potential influence or impart bias on the submitted work.

APÊNDICE C – Formulário de dados do paciente

INFORMAÇÃO DO PACIENTE	
1) Data: ____/_____/_____	**2) Data nascimento:** ____/_____/_____
3) Nome:_____	**4) Cidade procedência:**_____
5) Data início sintomas: ____/_____/_____	**6) Sexo:** ☐Masculino ☐ Feminino
SOBRE A INFECÇÃO PELO HIV	
7) Data diagnóstico HIV: ____/____/_____	**8) Já tratava com ARV na última consulta?** ☐ Sim ☐ Não
9) Contagem CD4: _____	**10) Data CD4:** ____/_____/_____
11) Contagem CD8: _____	**12) Data CD8:** ____/_____/_____
13) Carga viral HIV: _____	**14) Data CV:** ____/_____/_____
DIAGNÓSTICO LABORATORIAL DE HISTOPLASMOSE	
15) Diagnóstico prévio de histoplasmose? ☐Sim ☐Não ☐ Não obtido	**16) Data diagnóstico prévio:** ____/_____/_____
DADOS EPIDEMIOLÓGICOS	
17) Profissão atual:_____ **18) Profissões anteriores:** _____	**19) Naturalidade:**_____ **20) Moradias anteriores:**_____

SINAIS E SINTOMAS

21) Manifestações gerais:

Febre: ☐ Sim ☐ Não ☐ Não obtido

Fraqueza ☐ Sim ☐ Não ☐ Não obtido

Anorexia ☐ Sim ☐ Não ☐ Não obtido

Sudorese noturna: ☐ Sim ☐ Não ☐ Não obtido

Perda de peso: ☐ Sim ☐ Não ☐ Não obtido

Qual a perda? _____ Em quanto tempo? _____

22) Sintomas pulmonares: ☐ Sim ☐ Não ☐ Não obtido

☐ Dispneia ☐ Tosse seca ☐ Tosse produtiva ☐ Hemoptise ☐ Sibilância ☐ Dor torácica

23) Lesão oral: ☐ Sim ☐ Não ☐ Não obtido

Localização: ☐ Tonsilas ☐ Faringe ☐ Língua ☐ Gengiva ☐ Mucosa oral ☐ Laringe

Tipo: ☐ Ulcerações ☐ Placas ☐ Verrucosas ☐ Pápulas

24) Manifestações abdominais: ☐ Sim ☐ Não ☐ Não obtido

☐ Diarreia ☐ Náusea ☐ Vômito ☐ Esplenomegalia ☐ Hepatomegalia ☐ Dor abdominal ☐ Enterorragia

☐ Disfagia ☐ Ulceração na mucosa TGI ☐ Pseudopólipos ☐ Placas ☐ Envolvimento do intestino grosso

25) Linfonodos aumentados: ☐Sim ☐ Não ☐ Não obtido

☐ Hilares ☐ Cervicais ☐ Axilares ☐ Adenomegalia generalizada ☐ Outros - Especificar: _____

26) Lesões de pele: ☐ Sim ☐ Não ☐ *Rash* ☐ Mácula ☐ Pápulas ☐ Pápulas com crostas ☐ Placa ☐ Placa com crosta ☐ Pústula ☐ Nódulo ☐ Celulite ☐ Eritemato-violáceas ☐Úlceras / Erosões

27) Sintomas neurológicos: ☐Sim ☐Não ☐ Não obtido

☐ Déficit neurológico focal ☐ Confusão mental ☐ Cefaléia ☐ Meningite crônica ☐ Meningite aguda ☐ Convulsões ☐ Hidrocefalia ☐ HIC

ACHADOS DE IMAGENS

28) Possui achados de imagens? ☐ Sim ☐ Não ☐ Não obtido

29) TÓRAX (Pulmão): ☐ Normal ☐Padrão miliar ☐ Padrão intersticial ☐ Consolidação ☐ Infiltrado reticulonodular ☐Infiltrado focal ☐ Infiltrado difuso ☐ Derrame pleural ☐ Adenopatia mediastinal ☐ Fibrose pulmonar ☐ Granuloma ☐Granuloma calcificado ☐Cavidades

30) Anormalidades Bilaterais: ☐ Sim ☐Não ☐ Não obtido ☐ LSD ☐ LID ☐ LM ☐ LSE ☐ LIE ☐ Mediastino

Outros achados pulmonares: _____

31) ABDÔMEN: ☐ Comprometimento da adrenal, a saber: ☐ Necrose ☐ Calcificações ☐ Aumento de volume ☐ Hepatomegalia ☐ Esplenomegalia ☐ Adenomegalias intra-abdominais

32) SISTEMA NERVOSO CENTRAL:

☐ Abscesso cerebral ☐ Meningite ☐ Ventriculite ☐ Aracnoidite ☐ Lesão simples ☐ Lesões múltiplas

DIAGNÓSTICO LABORATORIAL DE HISTOPLASMOSE

33)Data do diagnóstico de histoplasmose: _____/_____/_____

34) Diagnóstico de histoplasmose (no hospital local):

☐Positivo ☐Negativo

335) Testes realizados:

☐ Micológico direto – material analisado: _____

Resultado: _____

☐ Cultura – material analisado: _____

Resultado: _____

☐ Biópsia - material analisado: _____

Resultado: _____

☐ Leveduras em sangue periférico

Resultado: _____

Pancitopenia: ☐Sim ☐Não

☐ Sorologia: ☐Imunofluorescência Direta

☐ Immunoblotting

	Valor de referência / Resultado
☐ Proteína C reativa	_____ / _____
☐ LDH	_____ / _____
☐ Fosfatase alcalina	_____ / _____
☐ Ferritina	_____ / _____
☐ TGO / AST	_____ / _____
☐ TGP / ALT	_____ / _____
☐ Gama GT	_____ / _____
☐ Hemoglobina	_____ / _____
☐ Plaquetas	_____ / _____
☐ Leucócitos	_____ / _____
☐ Linfócitos	_____ / _____

MEDICAMENTOS ANTIFÚNGICOS

36) Foram prescritos medicamentos antifúngicos? ☐ Sim ☐ Não ☐ Não obtido

37) Os medicamentos prescritos foram todos para tratamento de histoplasmose? ☐ Sim ☐ Não ☐ Não obtido

38) Se sim, quais?	Data que iniciou	Data última dose	Quantidade da última dosagem (mg/d)
☐ Fluconazol	____/____/_____	____/____/_____	
☐ Itraconazol	____/____/_____	____/____/_____	

☐ Anfotericina B convencional	____/____/_____	____/____/_____	
☐ Anfotericina B lipídica	____/____/_____	____/____/_____	
☐ Sulfametoxazol	____/____/_____	____/____/_____	
☐Outra: _____	____/____/_____	____/____/_____	

COMORBIDADE E RESULTADOS

39) Diagnóstico de outras infecções:

☐TB MÉTODO_____☐ n° de investigações de TB antes da HD ☐ MNT ESPÉCIE _____

☐tratamento empírico para TB ☐PCP ☐Criptococose ☐Infecção bacteriana ☐toxoplasmose

☐CMV ☐Outras infecções virais_____ ☐ Infecção de origem desconhecida

☐Outra infecção (especificar):_____☐Outra comorbidade não infecciosa_____

RESULTADOS

40) Desfecho: ☐ Sobreviveu ☐ Morreu Data da morte: ____/____/_____ ☐ Não obtido

41) Tempo entre o diagnóstico e o óbito _____ meses

42) Outros resultados ou observações:_____