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Fungal biomarkers in HIV-associated disseminated histoplasmosis: a multicenter diagnostic accuracy study on the Guiana shield

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ABSTRACT

Objectives: Diagnosis of HIV-associated histoplasmosis remains challenging. Our objective was to compare the performances of (1→3)-β-D-Glucan (BDG) and aspergillus galactomannan (GM) antigen for the diagnosis of HIV-associated histoplasmosis.

Methods: We performed a diagnostic accuracy study using frozen primary serum specimens issued from consecutive hospitalized people living with HIV (PLWH) and blindly tested for BDG and GM using Fungitell® and Platelia™ *Aspergillus*, respectively.

Results: We included 121 sera with 92 HIV-associated histoplasmosis cases and 29 negative controls. At thresholds of 150 pg/ml and 0.5 for BDG and GM, the sensitivity and specificity were 95% (85-100) vs 90% (77-100) and 52% (34-70) vs 83% (69-97), respectively. The receiver operating characteristics (ROC) curves showed area under the curves of 0.82 (0.68-0.91) vs 0.92 (0.80-0.98) for BDG and GM, respectively. Post-test probabilities showed best performances at lowest thresholds for a negative testing of BDG and GM and at the 0.7 threshold for a positive GM test.

Conclusions: If BDG alone may rule out histoplasmosis when negative, GM alone, either positive or negative, showed the best performances for the diagnosis of histoplasmosis. Given the poorer performances of BDG and GM than *Histoplasma* antigen detection assays commercially available, they should be considered as an alternative in settings where *Histoplasma* antigen detection assays remain unavailable. However, this study essentially provides insights in the performances of fungal biomarkers in disseminated histoplasmosis and does not represent recommendations for best practices.

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Introduction

Invasive fungal infections are neglected but common diseases mainly affecting immunocompromised hosts [1,2]. These diseases affect more than 150 million people worldwide and have high mortality rates, often above 20-30% [3]. Their overall burden is, therefore, significant and may become even more concerning due to (i) a growing number of immunocompromised individuals, (ii) persistent diagnostic and therapeutic difficulties in many countries, and (iii) the relative dearth of research on invasive fungal infections [4].

People living with HIV (PLWH) are particularly at risk for fungal infections [5]. Oropharyngeal candidiasis is diagnosed in 30% of newly diagnosed patients. *Pneumocystis jirovecii* pneumonia is the first AIDS-defining condition in western countries [6,7]. Disseminated histoplasmosis, an endemic mycosis caused by *Histoplama capsulatum,* has been an AIDS-defining condition since 1987. Latin American countries are hotspots for this disease, with an estimated burden of HIV-associated histoplasmosis at least equivalent to that of tuberculosis. In fact, both diseases are frequently misdiagnosed, leading to numerous avoidable AIDS-related deaths [8]. It is still the first AIDS-defining condition in the highly active antiretroviral therapy era in French Guiana [9], and yet lack of awareness, together with diagnostic and antifungal access difficulties, make it a neglected disease in much of Latin America and beyond [9,10]. The gold standard for diagnosis relies on fungal culture, a long (1-8 weeks) and complex process (biosafety level 3 facilities), dependent on the quality of the source sample and the experience of the laboratory. Other diagnostic methods include specific antibody detection, which is not sensitive in immunocompromised patients, or molecular methods, with good performances but not commercially available. Specific antigenic testing is now the most promising method and commercially available for testing in urine. This test has shown excellent sensitivity and specificity, although cross-reactions with other endemic mycoses may occur [11]. Still, it is not well-distributed or marketed outside the United States and, therefore, unavailable for routine use in low- and middle-income endemic areas.

Fungal biomarkers (1→3)-β-D-Glucan (BDG) and galactomannan (GM), on the contrary, are available commercially in numerous countries worldwide. These tests are based on components of the fungal wall: GM is a wall component of several *Aspergillus* species, while BDG is a pan-fungal marker (except for mucorales, *Cryptococcus*, or *Blastomyces* species). They are mainly used in hematology for the diagnosis of *Pneumocystis jiroveci* pneumonia and of fungal infections in neutropenic or bone marrow transplanted patients [12,13]. Preliminary studies reported good analytic performances for the diagnosis of HIV-associated histoplasmosis [14,15], making them relevant alternative diagnostic tools in regions where *Histoplasma* antigen detection assays are not available.

Thus, we aimed to assess and compare diagnostic performances of BDG and GM assays for the diagnosis of HIV-associated histoplasmosis. Secondary objectives included (i) determination of optimal thresholds for BDG and GM and (ii) performance assessment of a combination of both tests.

Methods

Selection of specimens and case definition

We performed a diagnostic accuracy study using frozen primary serum specimens stored in a certified biorepository (CRB Amazonie-DC-2021-4649). Samples consisted of consecutive hospitalized adult PLWH, recruited between 2013 to 2015, for a fever and/or a suspicion of infectious disease and/or an altered general condition and unexposed to oral antifungals during the month before inclusion (ANRS 12260 EDIRAPHIS study-NCT01884779). According to the online description of the original study protocol, urine and serum samples were systematically taken from enrolled patients in Suriname and French Guiana. Participants benefited from conventional methods for the diagnosis of histoplasmosis (direct examination, fungal culture, or pathology) on any tissue or fluids, only if the senior clinician suspected the disease. Because the main objective of the original study was histoplasmosis prevalence, serum specimens were systematically screened using a polyclonal *Histoplasma* antigen detection developed by the US Centers for Disease Control and an immunodiffusion anti-*Histoplasma* serology. Urine samples were systematically screened using a polyclonal antigen detection developed by the US Centers for Disease Control and a first generation of monoclonal antigen detection developed by IMMY®.

Hence, the present study used serum samples available in the biorepository and the related data sets. For all participants, clinical and paraclinical features were available to classify cases. Information on the results of conventional methods, together with the results of specific *Histoplasma* antigenic testing, using two methods on urine and one method on serum, helped us obtain a high-quality histoplasmosis case definition. No new information or samples were taken from the patients.

To assess and compare diagnostic performances of BDG and GM in serum samples, we randomly selected histoplasmosis cases among either proven (at least one sample positive for *Histoplasma capsulatum* using conventional methods, e.g. direct examination or fungal culture or pathology) or probable (positive detection of *Histoplasma* antigen in urine or serum) cases according to the latest European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria [13]. We selected negative controls who were negative in conventional methods and specific antigenic testing for *Histoplasma* in urine and serum. Patients with a proven or suspected *Pneumocystis jirovecii* infection were excluded.

Because the diagnostic accuracy may be influenced by the natural course, clinical stage, and severity of the disease, we aimed to define histoplasmosis cases accordingly. Disseminated histoplasmosis was defined as the presence of at least one positive extrapulmonary specimen, according to the EORTC-MSG criteria [13]. A severe form of histoplasmosis was defined according to the latest World Health Organization (WHO) recommendations [16]: respiratory failure (defined as presence of an acute respiratory distress syndrome or an oxygen arterial pressure $<$70 mm Hg), renal failure (defined as a two-fold increase of creatinine level), circulatory failure (defined as presence of a septic shock), coagulopathy (according to mention in medical records), severe alteration of the general condition with a WHO performance status score $>$2, or neurologic features (defined as a coma). Pauci-symptomatic forms of histoplasmosis included fever, weight loss, and/or altered general condition (WHO performance status score $<$3), without any other clinical features apart from isolated muco-cutaneous signs. Patients without either severe or pauci-symptomatic form were classified as mild disease.

Definition of other final diagnoses

To better characterize the studied specimens, notably the negative controls and false-negative cases, we classified all hospitalizations' final diagnoses -corresponding to the clinicians' conclusions upon patients discharge- as proven or suspected.

Apart from histoplasmosis cases, other final diagnoses were considered proven if there was microbiological evidence of the infectious agent causing the disease: detection of *Mycobacterium tuberculosis* or *Mycobacterium avium* complex in case of mycobacte-

rial infections, presence of *Cryptococcus spp* in cerebrospinal fluid or blood, respectively, in case of cryptococcal meningitis or sepsis (or disseminated infection), and growth of *Candida spp* or typical mucosal plaques on endoscopic examination. Subclinical cryptococcal infection was considered as a final diagnosis if cryptococcal antigen testing in blood was positive.

Suspected diagnoses of toxoplasmosis, esophageal candidiasis, or mycobacterial infection were considered if they were mentioned by clinicians as the final diagnosis on discharge without confirmatory microbiological or imaging evidence. The same applied for bacterial, viral, or undetermined infections as the final diagnoses on discharge.

Fungal biomarkers under study

Following manufacturers' instructions, serum specimens were tested for BDG and GM using Fungitell® and Platelia™ *Aspergillus* antigen assays, respectively. For BDG, a threshold of 80 pg/ml separated positive (≥80 pg/ml) from negative (<80 pg/ml) results. A result between 80 and 150 pg/ml was considered as weakly positive, between 150 and 300 pg/ml as moderately positive, and above 300 pg/ml as highly positive. For GM, a threshold of 0.5 classified positive (≥0.5) from negative (<0.5) results.

All tests were performed blindly to the case definition, final diagnosis on discharge, and results of other tests.

Gold-standard definition

Serum samples were categorized as proven or probable histoplasmosis according to the EORTC/MSG criteria [13]. When available, we took advantage of having the results of both conventional methods together with specific antigenic testing for histoplasmosis on the same patients to ascertain presence or absence of circulating *Histoplasma* antigen in the selected serum samples under study. Hence, we designed three scenarios of the gold-standard definition when assessing accuracy of BDG and GM testing in primary frozen serum samples:

(i) EORTC scenario: cases and controls were defined according to the EORTC-MSG criteria for proven endemic mycoses [13], relying on conventional methods results (direct examination, fungal culture, or pathology).
(ii) Strict scenario: cases were restricted to those positive for both conventional methods and all three specific antigenic testing for histoplasmosis, and controls were conversely negative for all methods.
(iii) Large scenario: cases were defined as either proven or probable histoplasmosis cases according to the EORTC-MSG criteria [13], whereas controls were negative for all methods.

Statistical analysis

We expressed descriptive statistics as frequency and median (interquartile range) for qualitative and quantitative variables, respectively. We performed univariate analyses using Student's or Mann–Whitney tests for quantitative variables and Pearson chi square or Fisher exact tests for qualitative variables, as appropriate. For each gold-standard scenario, we calculated diagnostic performance measures (sensitivity, specificity, positive and negative likelihood ratios, Youden index, and accuracy) and corresponding 95% confidence intervals (CIs) for BDG (three thresholds: 80, 150, and 300 pg/ml), GM (three thresholds: 0.5, 0.7, and 1), and a combination of both tests at relevant thresholds. We plotted receiver operating characteristic curves for BDG and GM testing and calculated the area under the curve (AUC) and corresponding 95% CI. We calculated post-test probability curves according to hypothetical pre-test point levels of HIV-associated histoplasmosis prevalence (1%, 5%, 10%, 20%, 30%, and 40%). Pre-test odds were calculated as [prevalence ÷ (1 − prevalence)]. We obtained the post-test odds by multiplying the pre-test odds by the positive or negative likelihood ratios. Finally, the post-test probability was [1 ÷ (1 + post-test odds)]. We used thresholds of 150, 300, and 500 pg/ml for BDG and 0.5, 0.7, and 1 for GM.

All statistical analyses were performed using STATA software, version 16.1, except for CIs for low counts, which were calculated using Wilson's method with RStudio software v. 1.4.1103 (package epi.tests).

Ethics

The ANRS 12260 EDIRAPHIS study was approved by ethical committees in Suriname and France. It was registered in clinicaltrials.gov, under the number NCT01884779. All patients were informed and gave written consent upon inclusion for their participation in the original study, urine and serum samples biobanking, and ancillary studies on fungal markers.

Results

Study population characteristics

We included 121 patients. Main demographic, clinical, and biological features are summarized in Table 1. The total population consisted of 34 proven HIV-associated histoplasmosis cases, 58 probable cases, and 29 negative controls. All proven cases had disseminated histoplasmosis.

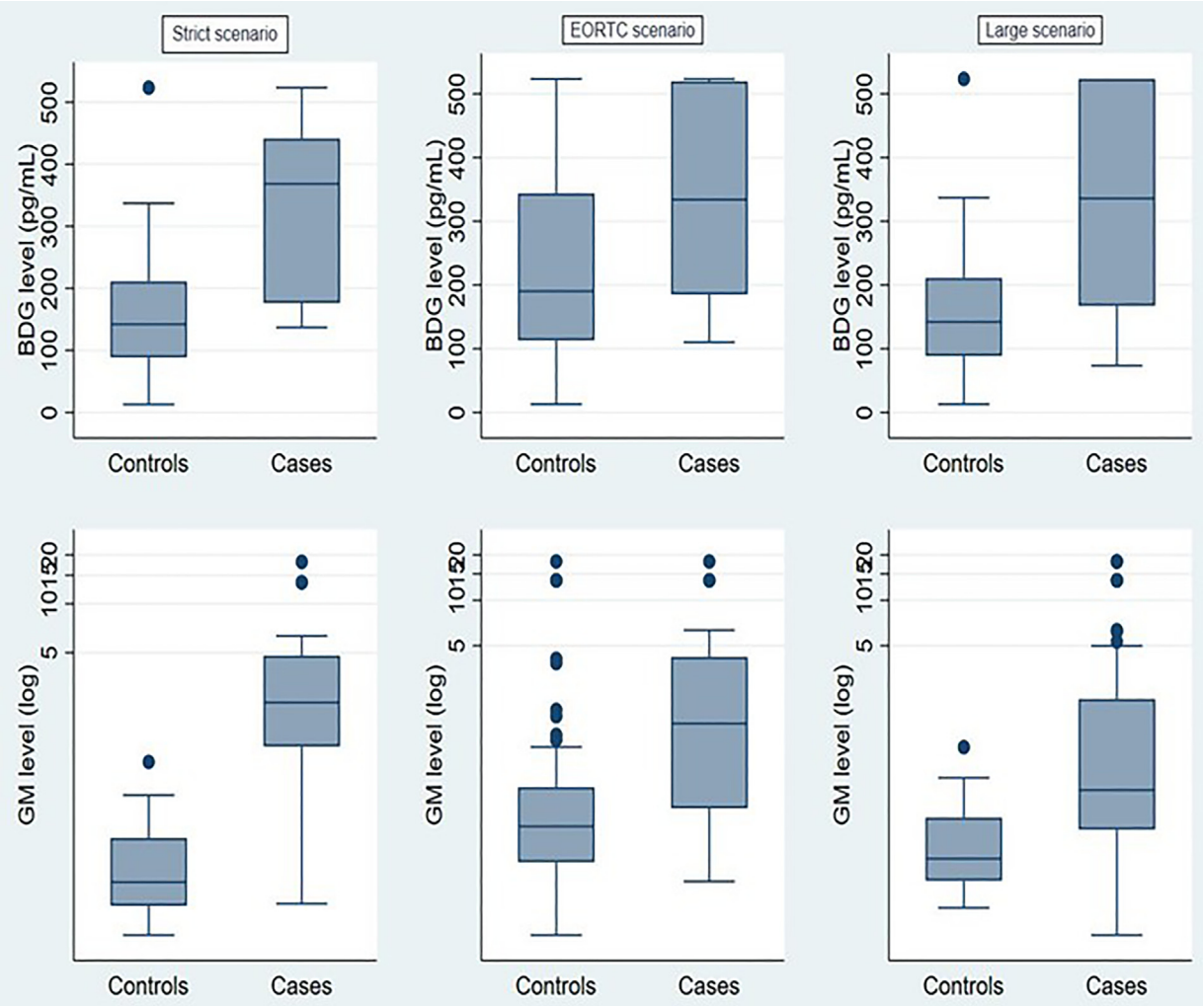
Patients with histoplasmosis were significantly younger, had lower clusters of differentiation (CD4) counts and more advanced HIV infection. Half of the HIV-associated histoplasmosis cases were defined as severe forms. Lymph node biopsy, followed by bone marrow sampling and gastro-intestinal tract biopsy, were the most frequently positive samples among histoplasmosis cases.

Overall, 26 (21%) patients had co-infections: eight of 34 in the proven cases group, 16 of 58 in the probable group, and two of 29 in the negative control group. These co-infections included histoplasmosis (n = 23) and either esophageal candidiasis (n = 9), mycobacterial infection (n = 7, with six tuberculosis and one atypical mycobacterial infection), toxoplasmosis (n = 1), suspected cryptococcosis (n = 3), or a combination of more than two co-infections (n = 3).

Diagnostic performances of fungal biomarkers

HIV-associated histoplasmosis cases had significantly higher levels of BDG and GM than negative controls in all three scenarios (Figure 1 & Supplemental Table). In the strict scenario, median BDG level was 368 (176-441) pg/ml among cases compared with 142 (89-211) among controls (P <0.001). The median GM level reached 2.5 (1.3-4.8) among cases compared with 0.19 (0.14-0.36) among controls (P <0.001). The median BDG or GM levels were not different between severe and non-severe HIV-associated histoplasmosis cases.

Across the three gold-standard scenarios, BDG had an overall high sensitivity but poor specificity, except for thresholds greater than 300 pg/ml. GM had high sensitivity and specificity in the strict scenario and high specificity but poorer sensitivity in the large scenario. Antibody detection had low sensitivity but high specificity in the three scenarios. Globally, the sequential combination of BDG, followed by GM, improved diagnostic performances of BDG alone, especially in the strict scenario (Table 2).



		All			Cases only		
		Cases	Controls	p-value	Severe	Non severe	p-value
Strict scenario	BDG	368 [176-441]	142 [89-211]	<0.001	407 [263-523]	272 [167-420]	0.29
	GM	2.5 [1.3-4.8]	0.19 [0.14-0.36]	<0.001	2.5 [1.5-5.3]	2.6 [0.9-4.8]	0.75
EORTC scenario	BDG	334 [185-520]	190 [113-344]	0.004	377 [224-523]	307 [167-422]	0.35
	GM	1.5 [0.4-4.2]	0.32 [0.18-0.57]	<0.001	1.8 [0.5-4]	1.4 [0.19-4.2]	0.35
Large scenario	BDG	336 [167-523]	142 [89-211]	<0.001	340 [138-523]	331 [175-506]	0.89
	GM	0.55 [0.3-2.2]	0.19 [0.14-0.36]	<0.001	0.63 [0.37-2.8]	0.48 [0.24-1.7]	0.86

Figure 1. BDG and GM antigen concentrations in the serum of adult patients presenting HIV-associated histoplasmosis and negative controls, according to three gold-standard scenarios[a] for the diagnosis of HIV-associated histoplasmosis, French Guiana and Suriname

BDG (1→3)-β-D-Glucan, GM, galactomannan.

Data are expressed as median (interquartile range).

Severe cases were defined according to OMS recommendations.

[a]Gold-standard scenarios were defined as:

EORTC scenario: cases and controls were defined according to the EORTC-MSG criteria [13] for proven endemic mycoses, relying on conventional methods results (direct examination, fungal culture, or pathology).

Strict scenario: cases were restricted to those positive for both conventional methods and all three specific antigenic testing for histoplasmosis, and controls were conversely negative for all methods.

Large scenario: cases were defined as either proven or probable histoplasmosis cases according to EORTC-MSG criteria [13], whereas controls were negative for all methods.

Table 1
Baseline study population data used to estimate (1→3)-β-D-Glucan and galactomannan antigen detection assays performances in the serum of adult people living with HIV for the diagnosis of HIV-associated histoplasmosis in French Guiana and Suriname.

	Global (N = 121)	Patients with histoplasmosis (N = 92)		*P*-value[a]	Patients without histoplasmosis (N = 29)	*P*-value[b]
		Proven (n = 34)	Probable (n = 58)			
Demographics						
Age	40 (33-50)	38 (32-48)	39 (33-46)	0.36	49 [=(42-54)]	**0.003**
Male	60 (50)	21 (62)	24 (41)	0.06	15 (52)	0.8
CDC HIV stage C	82 (68)	31 (91)	36 (62)	**0.002**	15 (52)	**0.03**
CD4 count level (/mm^3)	47 (18-129)	29 (14-71)	40 (17-98)	0.11	116 (62-245)	**0.03**
Viral load (log)	5.1 (4.5-5.7)	5.4 (5-6.2)	5.1 (4.7-5.7)	0.07	4.6 (2.3-5.4)	**0.02**
Sulfamethoxazole intake upon admission	32 (26)	9 (26)	19 (33)	0.53	4 (14)	0.08
Antimicrobial drugs intake upon admission[c]	6 (5)	1 (3)	4 (7)	0.65	1 (3)	1
Clinical presentation						
Gastro-intestinal signs	63 (52)	22 (65)	26 (45)	0.09	15 (52)	0.18
Fever	56 (46)	21 (62)	26 (45)	0.12	9 (31)	0.09
Skin or mucosal lesions	49 (41)	16 (47)	24 (41)	0.67	9 (31)	0.45
Weight loss	47 (39)	19 (56)	20 (34)	**0.05**	8 (28)	0.19
Respiratory signs	44 (36)	15 (44)	16 (28)	0.12	13 (45)	0.16
Neuropsychiatric signs	42 (35)	9 (26)	19 (33)	0.64	14 (48)	0.18
Lymph node enlargement	39 (32)	13 (38)	17 (29)	0.5	9 (31)	0.68
Severity						
Severe	44/92 (48)	16 (47)	28 (48)	0.91	-	-
Mild	39/92 (42)	16 (47)	23 (40)	0.5	-	-
Pauci-symptomatic	9/92 (10)	2 (6)	7 (12)	0.34	-	-
Microbiology (positive direct examination, culture, or pathology)						
Bone marrow	21/46 (46)	21/26 (81)	0/6	-	0/14	-
Lymph node biopsy	5/11 (45)	5/6 (83)	0/3	-	0/2	-
Gastro-intestinal tract biopsy	6/15 (40)	6/9 (67)	0/2	-	0/4	-
Respiratory samples	4/12 (33)	4/7 (57)	0/1	-	0/4	-
Muco-cutaneous biopsy	3/10 (30)	3/5 (60)	0/1	-	0/4	-
Blood	9/49 (18)	9/18 (50)	0/14	-	0/17	-
Cerebrospinal fluid	1/25 (4)	1/5 (20)	0/9	-	0/11	-
Urine sample	1/51 (2)	1/16 (6)	0/16	-	0/19	-
Liver biopsy	0/3 (0)	0/1 (0)	0/1	-	0/1	-
Final diagnosis at hospital discharge						
Isolated histoplasmosis[d]	32 (26)	26 (76)	6 (10)	**<0.001**	0 (0)	**<0.001**
Isolated cryptococcus infection[e]	5 (4)	0 (0)	4 (7)	0.29	1 (3)	1
Isolated esophagal candidiasis[d]	1 (1)	0 (0)	0 (0)	-	1 (3)	0.24
Isolated parasitic infection[f]	6 (5)	0 (0)	2 (3)	0.53	4 (14)	**0.03**
Isolated mycobacterial infection[g]	4 (3)	0 (0)	2 (3)	0.53	2 (7)	0.24
Co-infections[h]	26 (21)	8 (24)	16 (28)	0.8	2 (7)	**0.04**
Histoplasmosis + invasive fungal infection	15/26 (58)	7/8 (88)	8/16 (50)	0.18	0/2 (0)	0.17
Histoplasmosis + non-fungal opportunistic infections	8/26 (31)	1/8 (12)	6/16 (38)	0.35	1/2 (50)	0.53
Invasive fungal infection without histoplasmosis	3/26 (12)	0/8 (0)	2/16 (13)	0.54	½ (50)	0.22
Other infection[i]	13 (11)	0 (0)	6 (10)	**0.08**	7 (24)	**0.006**
Non-infectious diagnosis	21 (17)	0 (0)	11 (19)	**0.006**	10 (34)	**<0.001**
No final diagnosis	13 (11)	0 (0)	11 (19)	**0.006**	2 (7)	**0.008**

Data are presented as n (%) and median [interquartile range].
Footnotes:
[a] Comparison of certain and probable cases
[b] comparison of cases and controls
[c] antimicrobial treatments known to elevate BDG [17]
[d] either proven or suspected
[e] *Cryptococcus* infection, either meningitis, sepsis, or subclinical form
[f] suspected or proven parasitic infection, including toxoplasmosis
[g] mycobacterial infection, either proven or suspected. Includes *M. tuberculosis* or *M. avium* complex infections
[h] Co-infections include all patients with at least two either proven or suspected diagnoses among histoplasmosis, *Pneumocystis jiroveci* pneumonia, toxoplasmosis, esophageal candidiasis, or mycobacterial infection
[i] Other infections include bacterial, viral, or undetermined (no microbiological proof) infection. –

The AUC of GM was greater than that of BDG in all three scenarios. In the strict scenario, three different thresholds of BDG maximized the number of correctly classified patients (77.6%): 288, 331, or 405 pg/ml. These thresholds resulted in sensitivity and specificity of 60% and 90%, 55% and 93%, or 50% and 97%, respectively. For GM, the optimal threshold was 1.29, correctly classifying 92% of the patients with 80% sensitivity and 100% specificity (Figure 2).

We performed a sensitivity analysis by running the same analyses on a subgroup of patients with a clinical presentation suggestive of histoplasmosis, i.e. having less than 200 CD4/mm^3 and at least one of the following three signs: bicytopenia, polyadenopathy >2.5 cm or splenomegaly, and/or respiratory signs, yielding similar results (data not shown).

Discordant results were investigated. Patients with false-positive BDG or GM results had numerous alternative diagnoses. For example, for a BDG threshold of 80 pg/ml, the final diagnoses consisted of two mycobacterial infections, one esophageal candidiasis, three toxoplasmosis, one isosporiasis, five undetermined infections, one co-infection (suspected histoplasmosis and tuberculosis), eight non-infectious diagnoses, and two patients without a final diagnosis on discharge. For a GM at a threshold of 1.0, the only

Table 2
Diagnostic performance estimates, according to three gold-standard scenarios, for the diagnosis of HIV-associated histoplasmosis using BDG and GM antigen detection assays and anti-histoplasma immunodiffusion assay in the serum of adult people living with HIV, French Guiana and Suriname.

	C (n)	NC (n)	TP (n)	FP (n)	TN (n)	FN (n)	Sensitivity (%)	Specificity (%)	LR+	LR-	Accuracy	Youden index	Area under the curve
Strict criteria	20	29											
BDG 80			20	23	6	0	100	21 (6-36)	1.3 (1.1-1.5)	-	0.53	0.21	0.82
BDG 150			19	15	14	1	95 [85-100]	52 (34-70)	2 (1.4-3]	0.1 (0.01-0.7)	0.69	0.47	(0.68-0.91)
BDG 300			11	3	26	9	55 [33-77]	90 (79-100)	5.5 (1.8-17)	0.5 (0.3-0.8)	0.76	0.45	
BDG 500			4	1	28	16	20 [2-38]	97 (91-100)	6.7 (1-56)	0.8 (0.63-1)	0.65	0.17	
GM 0.5			18	5	24	2	90 [77-100]	83 (69-97)	5.3 (2.4-12)	0.12 (0.03-0.45)	0.86	0.73	0.92
GM 0.7			16	1	28	4	80 [62-98]	97 (91-100)	27 (3.9-188)	0.21 (0.09-0.51)	0.90	0.77	(0.80-0.98)
GM 1			16	1	28	4	80 [62-98]	97 (91-100)	27 (3.9-188)	0.21 (0.09-0.51)	0.90	0.77	
Serology ID*			4	0	29	16	20 [2-38]	100	-	0.8 (0.64-1]	0.67	0.20	-
Combination GM 0.7+BDG 150			15	1	28	5	75 [56-94]	97 (91-100)	25 (3.6-174)	0.26 (0.12-0.56)	0.88	0.72	-
Combination GM 1.3+BDG 288			9	0	29	11	45 [23-67]	100	-	0.55 (0.37-0.82)	0.78	0.45	-
EORTC criteria	34	69											
BDG 80			34	63	6	0	100	9 (2-16)	1.1 (1.02-1.2)	-	0.39	0.09	0.67
BDG 150			30	40	29	4	88 [77-99]	42 (30-54)	1.5 (1.2-1.9)	0.3 (0.11-0.78)	0.57	0.3	(0.57-0.76)
BDG 300			20	21	48	14	59 [42-76]	70 (59-81)	2 (1.3-3.2)	0.6 (0.39-0.92)	0.66	0.29	
GM 0.5			25	22	47	9	74 [59-89]	68 (57-79)	2.3 (1.5-3.4)	0.38 (0.21-0.68)	0.7	0.42	0.76
GM 0.7			21	13	56	13	62 [46-78]	81 (72-90)	3.3 (1.9-5.8)	0.47 (0.3-0.73)	0.75	0.43	(0.66-0.84)
GM 1			21	10	59	13	62 [46-78]	86 (78-94)	4.4 (2.3-8.3)	0.44 (0.28-0.68)	0.78	0.48	
Serology ID*			5	3	66	29	15 [3-27]	96 (91-100)	3.75 (1-15)	0.89 (0.77-1)	0.69	0.11	-
Combination GM 0.7+BDG 150			18	10	59	18	53 [36-70]	86 (78-94)	3.8 (2-7.3)	0.55 (0.39-0.77)	0.75	0.39	-
Large criteria	92	29											
BDG 80			91	23	6	1	99 [97-100]	21 (6-36)	1.25 (1.04-1.5)	0.05 (0.006-0.4)	0.80	0.20	0.78
BDG 150			73	14	15	19	79 [71-87]	52 (34-70)	1.6 (1.1-2.4)	0.4 (0.23-0.68)	0.72	0.31	(0.70-0.85)
BDG 300			50	3	26	42	54 [44-64]	90 (79-100)	5.4 (1.8-16)	0.51 (0.4-0.66)	0.63	0.44	
GM 0.5			53	5	24	39	58 [48-68]	83 (69-97)	3.4 (1.5-7.7)	0.51 (0.38-0.68)	0.64	0.41	0.78
GM 0.7			43	1	28	49	47 [37-57]	97 (91-100)	16 (2-113)	0.55 (0.45-0.67)	0.59	0.44	(0.70-0.85)
GM 1			37	1	28	55	40 [30-50]	97 (91-100)	13.3 (1.9-93)	0.62 (0.52-0.74)	0.54	0.37	
Serology ID[a]			10	0	29	82	11 [5-17]	100	-	0.89 [0.83-0.96]	0.32	0.11	-
Combination GM 0.7+BDG 150			37	1	28	55	40 [30-50]	97 (91-100)	13.3 (1.9-93)	0.62 [0.52-0.74]	0.54	0.37	-

Data are expressed as n with a 95% confidence interval when available.
BDG, (1→3)-β-D-Glucan; C, HIV-associated histoplasmosis cases; GM, galactomannan; FN, false negative; FP, false positive; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NC, negative controls; Se, sensitivity; Sp, specificity; TN, true negative; TP, true positive.
[a]The type of histoplasmosis antibody detection used was an immunodiffusion technique.

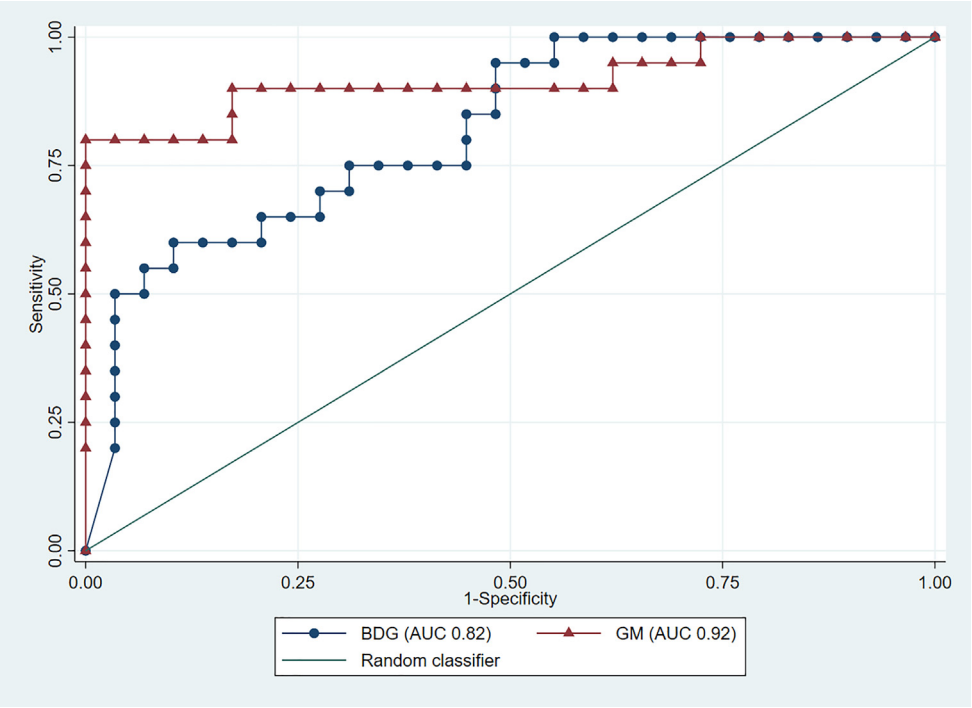


Figure 2. Comparative receiver operating characteristic curves estimates of BDG and GM antigen detection assays in the serum of adult people living with HIV according to a strict gold-standard scenario for the diagnosis of HIV-associated histoplasmosis, French Guiana and Suriname.
AUC, area under the curve; BDG, (1→3)-β-D-Glucan; GM, galactomannan.

false-positive patient had a non-infectious final diagnosis. False-negative patients had heterogenous clinical and biological presentations of histoplasmosis. Previous use of antimicrobial drugs or sulfamethoxazole-trimethoprim were not different or less frequent in the false-positive than in the true-positive patients for both tests (data not shown).

At commercial thresholds, the positive agreement between BDG (80 pg/ml) and GM (0.5) assays was 48% and the negative agreement was 6%. At optimal thresholds of 288 pg/ml for BDG and 1.29 for GM, positive agreement reached 16% and negative agreement 38%.

Pre and post-test probabilities

Post-test probabilities of having histoplasmosis, after a positive or a negative test result, exhibited better performances for GM than BDG in the three scenarios, even at low prevalence levels (Figure 3). When fungal biomarkers were combined with a sequential testing strategy using BDG, followed by GM, previous BDG and GM post-test probabilities were found improved, especially at low prevalence levels (Supplemental Table).

Discussion

In our study, GM and BDG were found to have good diagnostic performances and high AUCs for the diagnosis of HIV-associated histoplasmosis. To the best of our knowledge, this is the first study to compare diagnostic performances of BDG and GM for the diagnosis of HIV-associated histoplasmosis.

The study population was young, had advanced HIV infection, and had multiple concurrent opportunistic infections, making the evaluation of performances challenging. Half of HIV-associated histoplasmosis cases had a severe disseminated form of the disease, which may have led to an over-estimation of the sensitivity level reported.

BDG and GM diagnostic performances are reported across three gold-standard scenarios. Conventional methods performed on any tissue or fluid, mostly taken from the patients through invasive procedures, are considered the gold standard for histoplasmosis diagnosis according to the EORTC-MSG criteria for the diagnosis of proven endemic mycoses [13]. Still, conventional methods results were not available for all selected patients from the original study data set (n = 17 of 121 [14%] patients without any results using conventional methods for the diagnosis of histoplasmosis) because their realization were clinically guided and not mandatory in the original study. On the other hand, it is known that conventional methods for the diagnosis of invasive fungal infections are not perfectly reliable, and, therefore, imperfect for the definition of the gold standard, e.g. sensitivity of fungal culture being low and varying according to sample type and laboratory expertise [18]. In the present study, conventional methods results were issued from diverse sample types, with <40% of patients tested on bone marrow, a sample known to yield the highest number of positive histoplasmosis diagnosis. Therefore, we chose to express a more stringent scenario, the so called strict scenario, taking advantage of adding the results of the multiple *Histoplasma* antigen detections that were available for the whole population. In this way, we intended to avoid measurement-classification bias to increase internal validity of the study and our confidence in selecting "true" histoplasmosis cases and "true" histoplasmosis-free controls. Finally, considering that one is considering specific antigenic testing as the gold standard for histoplasmosis diagnosis and to increase the number of cases and, therefore, the statistical power of our study, we studied a larger scenario gathering proven and probable cases within the case definition of histoplasmosis. The present discussion will focus on the results of the strict scenario, which seemed to be the most stringent, reliable, and robust scenario.

BDG was highly sensitive for the diagnosis of HIV-associated histoplasmosis. This is consistent with two recent studies report-

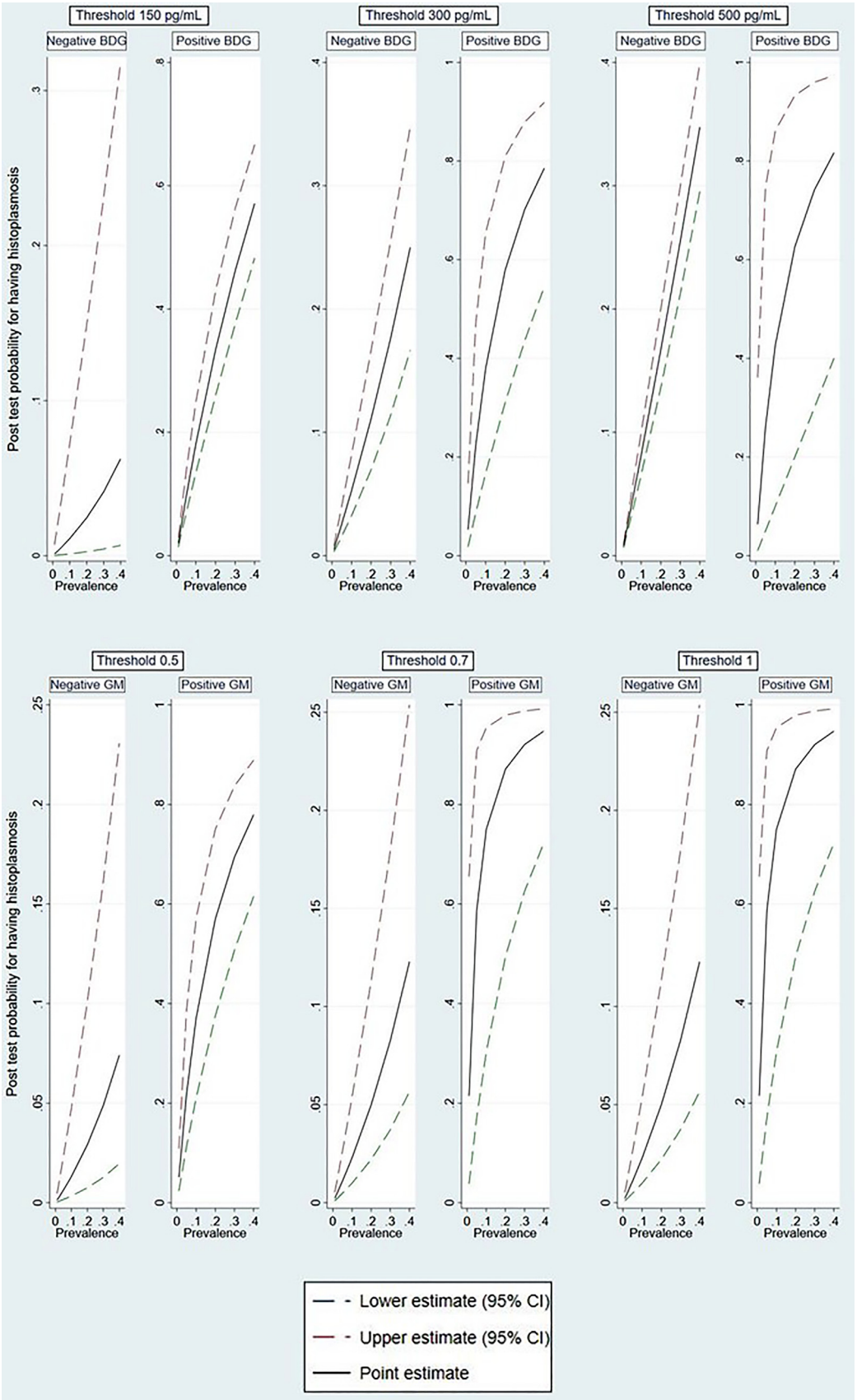


Figure 3. Post-test curves according to different serum concentration thresholds of BDG and GM antigen detection assays in a strict gold-standard scenario for the diagnosis of HIV-associated histoplasmosis in adult people living with HIV, French Guiana and Suriname.
CI, confidence interval; BDG, (1→3)-β-D-Glucan; GM. galactomannan.

ing a sensitivity of 91.7% among 36 patients with disseminated HIV-associated histoplasmosis [14] and one of 88% among 40 patients with disseminated histoplasmosis, with or without HIV [19]. A 295 pg/ml (108-500) median level of BDG was reported and consistent with our findings of 368 pg/ml (176-441). However, the BDG assay lacked specificity, especially at low thresholds of 80 or 150 pg/ml (21% and 52%, respectively). These performances are in line with previous studies and recommendations for the use of BDG in the diagnosis of invasive fungal infections in patients with hematologic malignancies [20,21]. Given the AUC of 0.82 (0.68-0.91), BDG testing may be considered as a discriminant diagnostic tool. Still, when BDG post-test probabilities were estimated in a context of high histoplasmosis endemicity (prevalence of 30-40%), the positive predictive value was poor (post-test probability <60%), making a positive BDG result inconclusive for clinicians, whereas the negative predictive value remained high, regardless of prevalence levels. Hence, even at low prevalence levels and considering a low BDG threshold (80 pg/ml), a negative BDG result may allow clinicians to rule out HIV-associated histoplasmosis, avoid invasive sampling, and preemptive antifungal therapy for histoplasmosis.

Relevance of the GM assay for the diagnosis of HIV-associated histoplasmosis has been previously reported, within a cross-reactivity context of an antigen detection assay first marketed for the diagnosis of aspergillosis [22–24]. Consistent with our findings, a previous retrospective report on selected laboratory samples in a high endemic area using GM detection at a 0.4 threshold showed a sensitivity of 82%, a specificity of 100%, and an AUC of 0.96 for the diagnosis of HIV-associated histoplasmosis [15]. However, in a context of multiple concurrent co-infections in advanced HIV infection, we found that a higher threshold of 1.29 maximized the sensitivity (80%) and specificity (100%) of GM detection, correctly classifying 92% of our population.

When considering post-test probabilities at different point prevalence levels, if negative predictive values of GM and BDG were similar, the GM positive predictive value estimates were better than BDG, regardless of the estimated histoplasmosis point prevalence levels. Thus, given the wide use of BDG and its low specificity, clinicians may consider a two-step diagnostic strategy when facing a positive BDG result. In this context, GM could be used as a confirmatory test for the diagnosis of HIV-associated histoplasmosis and, when positive, will inform clinical decision in promptly starting antifungal therapy. On the other hand, when facing a negative BDG result, fungal infection and histoplasmosis seem very unlikely and other differential diagnoses should be investigated. Moreover, because GM alone had better performances at a cheaper price than BDG (average costs of 4.5€ vs 20€ per test in France, respectively), solely testing GM as the diagnostic strategy should arguably be preferred to a sequential testing strategy, especially in resource-limited settings.

As many authors have pointed out, the diagnosis of histoplasmosis remains challenging [11,25,26]. Fungal culture and antibody detection are not sensitive enough, *Histoplasma* antigen detection is not widely distributed or even available despite better performances and having been listed as a WHO essential diagnosis test, and no molecular detection is commercially available. In this context, the wide availability of alternative fungal markers, such as BDG and GM, may significantly improve clinicians' ability to diagnose and treat a disease where rapid treatment initiation is key to improve the patients' prognosis. Moreover, they could also lead to substantial savings if they allow to avoid an expensive and potentially toxic exposure to amphotericin B or the multiple drug-drug interactions of itraconazole.

One might be concerned about the risk of differential diagnosis with aspergillosis. However, this disease has become anecdotal in PLWH in the post-highly active antiretroviral therapy era where iatrogenic neutropenia no longer exists [5]. As an example, no cases of HIV-associated aspergillosis were documented in the French Guiana HIV cohort, and *Aspergillus* species accounted for less than one-third of clinical isolates (only one *A. fumigatus*) in a recent study on fungal diversity in French Guiana [27,28]. Other endemic mycoses, such as blastomycosis, paracoccidioidomycosis, and talaromycosis, may cross-react and result in a positive GM test—invasive fungal infections that were not reported to be endemic within the area of patients' recruitment in the present study [29]. This may limit the applicability of our results beyond the Amazon biome and call for further studies in endemic areas with other dimorphic fungi represented.

Our study presents some limitations. Performance estimates of tests under study may have been impacted by using frozen samples stored several years, with a potential decrease in the antigens concentrations levels in samples under study notably. Still, according to the high level of certification and storage conditions of the biorepository, we feel confident on our ability to test the scientific hypotheses under study. The sample size in the "strict scenario" was small, resulting in wide CIs and low precision estimates. For the same reasons, we could not study the performances of fungal biomarkers according to CD4 count strata. Our large scenario, by increasing the power, allowed us to obtain refined estimates. Nevertheless, because of imperfect performances of *Histoplasma* antigen detection tests, considered as part of the gold-standard definition in this scenario, it is possible that cases classified as probable histoplasmosis did not actually have histoplasmosis, which could distort the performance estimates obtained. Because we studied only one BDG assay among the four BDG assays commercially available, our results may not reflect performances of other BDG assays in similar contexts. When exploring potential sources of false positivity for BDG or GM assays, information on recent surgery, previous use of blood-derived treatments, or hemodialysis were lacking in the original study dataset. However, previous use of antimicrobial drugs or sulfamethoxazole-trimethoprim did not appear to influence BDG or GM detection levels.

Despite these limitations, to the best of our knowledge, this is the first study to compare BDG and GM diagnostic performances based on robust data regarding the gold-standard definitions. We reported information on how to interpret results and use a combination of the two tests. If BDG alone may rule out histoplasmosis when negative, GM alone, either positive or negative, showed the best performances for the diagnosis of HIV-associated histoplasmosis. This contribution may, therefore, address an unmet diagnostic need and improve patient management and prognosis. Further studies are needed to refine these results on larger sample sizes and other geographic settings. However, it should be remembered that effective, sensitive, and specific antigenic tests are available, specifically designed for the diagnosis of histoplasmosis. Our efforts must, therefore, be focused on guaranteeing low-cost access to these tests in all histoplasmosis-endemic settings [30,31]. Given their poorer performances, fungal biomarkers described here should only be an alternative when specific antigenic testing for histoplasmosis is not available.

Declarations of competing interest

The authors have no competing interests to declare.

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Author contributions

AM: literature search, study design, data collection, data analysis, data interpretation, writing, figures and tables, revising. SM: study design, data collection, data interpretation, writing, figures and tables, revising. MB: study design, data collection, data interpretation, writing, figures and tables, revising. ME: study design, data collection, data interpretation, writing, figures and tables, revising. SV: study design, data collection, data interpretation, writing, figures and tables, revising. TC: study design, data collection, data interpretation, writing, figures and tables, revising. DC: study design, data collection, data interpretation, writing, figures and tables, revising. BG: study design, data collection, data interpretation, writing, figures and tables, revising. MN: study design, funding acquisition, data analysis, data interpretation, writing, figures and tables, revising. OL: literature search, study design, funding acquisition, data collection, data analysis, data interpretation, writing, figures and tables, revising. AA: literature search, study design, funding acquisition, data collection, data analysis, data interpretation, writing, figures and tables, revising. OL and AA contributed equally to this study.

Disclaimer

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the views of the US Centers for Disease Control and Prevention.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107360.

References

[1] Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. *J Fungi (Basel)* 2017;**3**:E57. doi:10.3390/jof3040057.
[2] Rodrigues ML, Nosanchuk JD. Fungal diseases as neglected pathogens: a wake-up call to public health officials. *PLoS Negl Trop Dis* 2020;**14**:e0007964. doi:10.1371/journal.pntd.0007964.
[3] Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* 2012;**4**:165rv13. doi:10.1126/scitranslmed.3004404.
[4] Denning DW. The ambitious '95–95 by 2025' roadmap for the diagnosis and management of fungal diseases. *Thorax* 2015;**70**:613–14. doi:10.1136/thoraxjnl-2015-207305.
[5] Armstrong-James D, Meintjes G, Brown GD. A neglected epidemic: fungal infections in HIV/AIDS. *Trends Microbiol* 2014;**22**:120–7. doi:10.1016/j.tim.2014.01.001.
[6] World Health Organization *HIV/AIDS surveillance in Europe*; 2009 *https://data.europa.eu/doi/10.2900/36146* [accessed 04 March 2022].
[7] Buchacz K, Lau B, Jing Y, Bosch R, Abraham AG, Gill MJ, et al. Incidence of AIDS-defining opportunistic infections in a multicohort analysis of HIV-infected persons in the United States and Canada, 2000–2010. *J Infect Dis* 2016;**214**:862–72. doi:10.1093/infdis/jiw085.
[8] Adenis AA, Valdes A, Cropet C, McCotter OZ, Derado G, Couppie P, et al. Burden of HIV-associated histoplasmosis compared with tuberculosis in Latin America: a modelling study. *Lancet Infect Dis* 2018;**18**:1150–9. doi:10.1016/S1473-3099(18)30354-2.
[9] Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, Hanf M, et al. What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. *Am J Trop Med Hyg* 2011;**84**:239–40. doi:10.4269/ajtmh.2011.10-0251.
[10] Nacher M, Adenis A, Aznar C, Blanchet D, Vantilcke V, Demar M, et al. How many have died from undiagnosed human immunodeficiency virus-associated histoplasmosis, a treatable disease? Time to act. *Am J Trop Med Hyg* 2014;**90**:193–4. doi:10.4269/ajtmh.13-0226.
[11] Toscanini MA, Nusblat AD, Cuestas ML. Diagnosis of histoplasmosis: current status and perspectives. *Appl Microbiol Biotechnol* 2021;**105**:1837–59. doi:10.1007/s00253-021-11170-9.
[12] Giacobbe DR, Del Bono V, Viscoli C, Mikulska M. Use of 1,3-β-D-glucan in invasive fungal diseases in hematology patients. *Expert Rev Anti Infect Ther* 2017;**15**:1101–12. doi:10.1080/14787210.2017.1401467.
[13] Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the mycoses study group education and research consortium. *Clin Infect Dis* 2020;**71**:1367–76. doi:10.1093/cid/ciz1008.
[14] Saccente M, Krishnan G. Comparison of blood (1->3)-β-d-glucan levels in AIDS-related Pneumocystis jirovecii pneumonia and AIDS-related progressive disseminated histoplasmosis. *Clin Infect Dis* 2021;**73**:1100–2. doi:10.1093/cid/ciab277.
[15] Iriart X, Blanchet D, Menard S, Lavergne RA, Chauvin P, Adenis A, et al. A complementary tool for management of disseminated Histoplasma capsulatum var. capsulatum infections in AIDS patients. *Int J Med Microbiol* 2014;**304**:1062–5. doi:10.1016/j.ijmm.2014.07.016.
[16] World Health Organization. Guidelines for diagnosing and managing disseminated histoplasmosis among people living with HIV, https://www.who.int/publications/i/item/9789240006430; 2021 [accessed 08 Novomber 2021].
[17] Liss B, Cornely OA, Hoffmann D, Dimitriou V, Wisplinghoff H. 1,3-β-D-Glucan contamination of common antimicrobials. *J Antimicrob Chemother* 2016;**71**:913–15. doi:10.1093/jac/dkv419.
[18] Adenis AA, Aznar C, Couppié P. Histoplasmosis in HIV-infected patients: a review of new developments and remaining gaps. *Curr Trop Med Rep* 2014;**1**:119–28. doi:10.1007/s40475-014-0017-8.
[19] Krishnan G, Power M, Bariola JR, Dare R. Comparison of indirect fungal diagnostic tests in patients with proven histoplasmosis. *Open Forum Infect Dis* 2022;**9**:ofac609. doi:10.1093/ofid/ofac609.
[20] Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S, et al. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. *Bone Marrow Transplant* 2012;**47**:846–54. doi:10.1038/bmt.2011.178.
[21] White SK, Schmidt RL, Walker BS, Hanson KE. (1→3)-β-D-glucan testing for the detection of invasive fungal infections in immunocompromised or critically ill people. *Cochrane Database Syst Rev* 2020;**7**:CD009833. doi:10.1002/14651858.CD009833.pub2.
[22] Ranque S, Pelletier R, Michel-Nguyen A, Dromer F. Platelia Aspergillus assay for diagnosis of disseminated histoplasmosis. *Eur J Clin Microbiol Infect Dis* 2007;**26**:941–3. doi:10.1007/s10096-007-0380-7.
[23] Xavier MO, Pasqualotto AC, Cardoso ICE, Severo LC. Cross-reactivity of Paracoccidioides brasiliensis, Histoplasma capsulatum, and Cryptococcus species in the commercial platelia Aspergillus enzyme immunoassay. *Clin Vaccine Immunol* 2009;**16**:132–3. doi:10.1128/CVI.00310-08.
[24] Vergidis P, Walker RC, Kaul DR, Kauffman CA, Freifeld AG, Slagle DC, et al. False-positive Aspergillus galactomannan assay in solid organ transplant recipients with histoplasmosis. *Transpl Infect Dis* 2012;**14**:213–17. doi:10.1111/j.1399-3062.2011.00675.x.
[25] Azar MM, Hage CA. Laboratory diagnostics for histoplasmosis. *J Clin Microbiol* 2017;**55**:1612–20. doi:10.1128/JCM.02430-16.
[26] Falci DR, Monteiro AA, Braz Caurio CF, Magalhães TCO, Xavier MO, Basso RP, et al. 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. *Open Forum Infect Dis* 2019;**6**:ofz073. doi:10.1093/ofid/ofz073.
[27] Cachera L, Adenis A, Guarmit B, Rabier S, Couppié P, Djossou F, et al. Invasive fungal infections in persons living with HIV in an Amazonian context: French Guiana, 2009–2019. *J Fungi (Basel)* 2021;**7**:421. doi:10.3390/jof7060421.
[28] Nabet C, Imbert S, Normand AC, Blanchet D, Chanlin R, Becker P, et al. Unexpected mould diversity in clinical isolates from French Guiana and associated identification difficulties. *Med Mycol* 2020:myaa091. doi:10.1093/mmy/myaa091.
[29] Colombo AL, Tobón A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol* 2011;**49**:785–98. doi:10.3109/13693786.2011.577821.
[30] Nacher M, Couppié P, Epelboin L, Djossou F, Demar M, Adenis A. Disseminated Histoplasmosis: fighting a neglected killer of patients with advanced HIV disease in Latin America. *PLoS Pathog* 2020;**16**:e1008449. doi:10.1371/journal.ppat.1008449.
[31] Caceres DH, Echeverri Tirado LC, Bonifaz A, Adenis A, Gomez BL, Flores CL, et al. Current situation of endemic mycosis in the Americas and the Caribbean. Proceedings of the first international meeting on endemic mycoses of the Americas (IMEMA). *Mycoses* 2022;**65**:1179–87. doi:10.1111/myc.13510.