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1 **A complementary tool for management of disseminated**
2 ***Histoplasma capsulatum* var. *capsulatum* infections in AIDS**
3 **patients**

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26
27 **RUNNING TITLE:** A complementary tool for histoplasmosis management

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30 **ABSTRACT**

31
32 In South America, disseminated histoplasmosis due to *Histoplasma capsulatum* var.

33 *capsulatum* (*H. capsulatum*), is a severe and frequent opportunistic infection in AIDS

34 patients. In areas outside the USA where specific-*Histoplasma* antigen detection is not

35 available, the diagnosis is difficult. With the galactomannan antigen (GM) detection, a test

36 commonly used for invasive aspergillosis diagnosis, there is a cross-reactivity with *H.*

37 *capsulatum* that can be helpful for the diagnosis of histoplasmosis. The aim of this study was

38 to evaluate the GM detection for the diagnosis of disseminated histoplasmosis in AIDS

39 patients. The performance of the GM detection was evaluated with serum collected in French

40 Guiana where *H. capsulatum* is highly endemic. Sera from AIDS patients with disseminated

41 histoplasmosis occurring from 2002 to 2009 and from control HIV-positive patients without

42 histoplasmosis were tested with the GM detection and *Histoplasma*-specific antibody

43 detection (IEP). In 39 AIDS patients with proven disseminated histoplasmosis, the sensitivity

44 of the *Histoplasma* IEP was only 35.9% and was linked to the TCD4+ lymphocyte level. For

45 the GM detection, the sensitivity (Se) was 76.9% and specificity (Sp) was 100% with the

46 recommended threshold for aspergillosis diagnosis (0.5). The test was more efficient with a

47 threshold of 0.4 (Se: 0.82 [95% CI: 0.66-0.92], Sp: 1.00 [95% CI: 0.86-1.00], LR+: >10, LR-:

48 0.18). This study confirms that the GM detection can be a surrogate marker for the diagnosis

49 of disseminated histoplasmosis in AIDS patients in endemic areas where *Histoplasma* EIA is

50 not available.

1
2
3 **KEYWORDS :** histoplasmosis; AIDS; *Histoplasma capsulatum*; Diagnosis; Platelia;
4 galactomannan; antibody; cross-reactivity
5
6

7 **INTRODUCTION**

8

9 Histoplasmosis caused by *Histoplasma capsulatum* var. *capsulatum* (*H. capsulatum*) is
10 endemic in the United States, in several countries of Central and South America and in
11 scattered areas of Asia and Africa (Kauffman, 2007). Disseminated histoplasmosis is a severe
12 illness that occurs almost exclusively in immunosuppressed patients, particularly in patients
13 with acquired immunodeficiency syndrome (AIDS). In French Guiana, histoplasmosis is the
14 most frequent opportunistic infection in HIV-infected patients and the first cause of AIDS-
15 related death (Couppie et al., 2004; Huber et al., 2008; Lewden et al., 2004). Even if isolation
16 of *Histoplasma* from cultures is the reference procedure for histoplasmosis diagnosis
17 (Kauffman, 2008), it can take weeks and is positive in only 50-70% of cases
18 (Sathapatayavongs et al., 1983). Serological methods based on specific antibody detection can
19 be performed rapidly but usually give false negative results in AIDS patients (Tobon et al.,
20 2005). In this context, circulating specific-*Histoplasma* antigen detection (*Histoplasma* EIA)
21 represents a useful option for diagnosis but this method is often unavailable in the majority of
22 endemic areas outside the USA (Connolly et al., 2007; Hage et al., 2011).

23 The Platelia *Aspergillus* enzyme immunoassay (EIA) is a ready-to-use test which detects
24 galactofuranose-containing side chains of galactomannan. This test is commonly used for the
25 diagnosis of invasive aspergillosis, particularly in solid organ transplant recipients or patients
26 with haematological malignancies (Aquino et al., 2007), but cross-reactivity exists with many
27 other fungi (Aquino et al., 2007; Dalle et al., 2005; Desoubeaux et al., 2014; Giacchino et al.,
28 2006; Huang et al., 2007; Van Der Veer et al., 2012; Xavier et al., 2009) in particular *H.*
29 *capsulatum* (Narreddy and Chandrasekar, 2008; Wheat et al., 2007). Galactomannan antigen
30 (GM) detection can lead to a false-positive diagnosis of aspergillosis in these
31 immunocompromised patients who have histoplasmosis (Jones et al., 2009; Vergidis et al.,
32 2012). In areas outside the USA where *Histoplasma* EIA is not available, this cross-reactivity
33 with the galactomannan antigen could be helpful for the diagnosis of disseminated
34 histoplasmosis (Pineau et al., 2010; Ranque et al., 2007; Riviere et al., 2012). GM detection
35 could be particularly interesting in HIV-infected patients because of the low incidence of
36 invasive aspergillosis in this population compared to patients with organ transplantation or
37 hematological malignancies (Desoubeaux et al., 2014).

38 Given the rarity of histoplasmosis in Europe, preliminary studies on GM detection for this
39 diagnosis were only conducted on a limited number of samples (Ranque et al., 2007). Thus,
40 the aim of our study was to evaluate more widely the GM detection for the diagnosis of
41 disseminated histoplasmosis in AIDS patients. This evaluation was carried out in a *H.*
42 *capsulatum* endemic area (French Guiana) to obtain a larger cohort of patients.
43
44

45 **PATIENTS AND METHODS**

46

47 **Study population**

48 Disseminated histoplasmosis cases occurring in AIDS patients followed in the Cayenne
49 hospital (French Guiana) were selected from 2002 to 2009. Among this cohort, all patients for
50 whom sera were collected at the time of diagnosis of histoplasmosis (± 10 days=baseline) and
51 available for analysis (stored at -20°C) were included in the study. HIV-patients were

1 considered as histoplasmosis cases, if there was microbiological evidence of *Histoplasma*
2 infection (positive direct examination and/or culture and/or polymerase chain reaction (Simon
3 et al., 2010) for *Histoplasma capsulatum*). Thirty HIV-positive patients who had never been
4 in an endemic area for *Histoplasma* were included as negative controls. For these controls, no
5 critical antibiotics (Piperacillin-tazobactam), polyvalent immunoglobulins or dialysis were
6 present when GM detection was performed.

7 8 **GM detection and *Histoplasma*-specific antibody detection**

9 Sera stored at -20°C were tested with the Platelia Aspergillus EIA (BioRad, France) in
10 accordance with the manufacturer's specifications. Briefly, after a pretreatment (6 min,
11 120°C), 50 µl of serum was added to 50 µl of conjugate before being incubated at 37°C for 90
12 min. After washing, chromogen substrate solution was added and the plates were dark-
13 incubated for 30min. After the addition of stop solution, the optical density (OD) was
14 determined at 450nm (reference filter 620/630nm). Samples were considered as positive when
15 the galactomannan index (GMI) was ≥ 0.5 (De Pauw et al., 2008).

16 In parallel, an immunoelectrophoretic assay for *Histoplasma*-specific antibody detection (IEP)
17 was performed according to manufacturer's instruction (Beckman Paragon, France). Briefly,
18 3µL of *Histoplasma* antigen (Laboratoire Méridian, France) were electrophoresed (20min,
19 100V; Beckman Paragon, France) on ready-to-use Hydragel-IEP-Plus gels (Sebia, France).
20 After a 24h-incubation with serum, the gel was washed, placed under a press, stained with
21 acid violet, washed again and finally dried out. The test was considered as positive if at least
22 one precipitation line was detected by visual observation.

23 24 **Analysis and Statistical Methods**

25 Data were analyzed with SIGMA Stat software (2.03) using the Mann-Whitney rank sum test
26 for a two-group comparison and the χ^2 test for patient characteristic comparison.
27 Relationships between two variables were analyzed by Spearman rank order correlation test.
28 Values were reported as the median and interquartile range IQR [25%; 75%]. Analysis of
29 Receiver Operator Characteristic (ROC) curves was performed to determine the cut-off for
30 positivity. A comparison was considered statistically significant if the p value was ≤ 0.05 .

31 32 33 **RESULTS**

34 35 **Patient Characteristics**

36 Between 2002 and 2009, 39 AIDS patients diagnosed for disseminated histoplasmosis and
37 with an available concomitant serum were included in the study (Table 1). There was no
38 significant difference in the median age or sex ratio between case and control patients.
39 Diagnosis of histoplasmosis was obtained mainly on hematology (48.7%) or digestive
40 (28.2%) samples. Thirty seven patients (94.9%) had a positive culture for *Histoplasma*
41 *capsulatum*. For two patients, a rapid and extensive development of *Candida albicans* on the
42 culture did not allow the growth of *Histoplasma* but PCR and direct examination were both
43 positive, confirming the diagnosis of histoplasmosis. All the index case and control patients
44 were negative for the diagnosis of *Aspergillus* infection (culture and anti-*Aspergillus* antibody
45 detection).

46 47 **Anti-*Histoplasma* antibody detection**

48 For AIDS-patients with disseminated histoplasmosis, the sensitivity (Se) of *Histoplasma* IEP
49 was only 35.9% [95% CI: 21.7-52.8] while the specificity (Sp) was 100% [95% CI: 85.9-100]
50 (Table 1). The sensitivity was linked to the TCD4+ lymphocyte level as the counts of these
51 cells was statistically higher in patients with positive *Histoplasma*-specific antibody detection

1 than in patients with negative serology (26 [5; 37] vs 84 [77; 90]) (p=0.007; Mann-Whitney
2 rank sum test). On the contrary, there was no statistical difference for TCD8+ lymphocyte
3 levels (data not shown).

4 5 **GM detection for diagnosis of histoplasmosis**

6 Galactomannan indexes (GMI) were significantly higher in HIV-positive patients with
7 histoplasmosis compared to *Histoplasma*-uninfected ones (Table 1). With the recommended
8 threshold for invasive aspergillosis diagnosis (0.5), the sensitivity was 76.9% [95%
9 confidence intervals (95% CI): 60.3-88.3] and the specificity was 100% [95% CI: 85.9-100]
10 for histoplasmosis diagnosis. The TCD4+ or TCD8+ lymphocytes counts were not
11 statistically different between groups of patients with positive or negative GMI (data not
12 shown). Moreover, the GMI level was not correlated with TCD4+ or TCD8+ lymphocyte
13 counts in HIV-positive patients with histoplasmosis (data not shown).

14 The area under the curve was 0.963 on the ROC curve (Fig. 1). Two other thresholds (0.4 and
15 0.35) appeared to be potentially more interesting than the recommended threshold 0.5, with
16 sensitivities of 82.1% [95% CI: 65.9-91.9] and 87.2% [95% CI: 71.8-95.1] and specificities of
17 100% [95% CI: 85.9-100] and 93.3% [95% CI: 76.5-98.8] respectively. Positive likelihood
18 ratios (LR+) were 13.08 [95%CI: 3.41-50.2], +∞ [95%CI: non-calculable], +∞ [95% CI: non-
19 calculable] for thresholds of 0.35; 0.4; 0.5 respectively and negative likelihood ratios (LR-)
20 were 0.14 [95% CI: 0.06-0.31], 0.18 [95% CI: 0.09-0.35] and 0.23 [95% CI: 0.13-0.41] for
21 thresholds of 0.35; 0.4; 0.5 respectively.

22 Taking account of this information, the threshold 0.4 seems to be the most relevant (Se:
23 82.1% [95% CI: 65.9-91.9], Sp: 100% [95% CI: 85.9-100], LR+: +∞ [95% CI: non-
24 calculable], LR-: 0.18 [95% CI: 0.09-0.35]). Coupling galactomannan and anti-*Histoplasma*
25 antibody detection did not significantly improve the overall diagnosis performance (Se:
26 82.1% [95% CI: 65.9-91.9], Sp: 100% [95% CI: 85.9-100]).

27 28 29 **DISCUSSION**

30
31 In areas outside the USA where *Histoplasma* EIA is not available, the diagnosis of
32 disseminated histoplasmosis is often difficult to obtain in a timely manner consistent with the
33 life-threatening character of this disease: culture takes a long time, direct examination is of
34 poor sensitivity and *Histoplasma*-specific PCR is not available in most centers. As shown by
35 others (Tobon et al., 2005; Wheat, 2006), we confirmed that detection of anti-*Histoplasma*
36 specific antibodies had a low sensitivity (35.9%) in AIDS patients. It is now well-established
37 that CD4+T-cell depletion due to HIV was responsible for an IL-7-dependant alteration of B-
38 lymphocyte responses (Moir and Fauci, 2009). This phenomenon could explain the CD4+T
39 cell-dependent decrease in anti-*Histoplasma* antibody production observed in our study
40 among AIDS patients with histoplasmosis.

41 In contrast, we found that the GM detection performed well (Se: 76.9%, Sp: 100%). However,
42 the threshold used for *Aspergillus* (0.5) could be lowered to 0.4 to increase the sensitivity (Se
43 82.1%) without decreasing the specificity. Keeping the recommended threshold for
44 aspergillosis diagnosis (0.5) would lead to a decrease in sensitivity of 5.2%. Unlike antibody
45 levels, GMI were not influenced by TCD4+ lymphocyte counts. For GM detection, Ranque *et*
46 *al.* already reported a sensitivity of about 73% in 11 patients with pulmonary histoplasmosis
47 and 100% in 6 HIV-positive patients (Ranque et al., 2007). Thus, in *Histoplasma* endemic
48 areas where *Histoplasma* EIA is not available, this quick and easy-to-perform technique might
49 be a powerful alternative for the diagnosis of disseminated histoplasmosis in HIV-positive
50 patients.

1 In two other studies (Wheat et al., 2007; Xavier et al., 2009), the sensitivity of the GM
2 detection was about 48% and 67% in histoplasmosis diagnosis but these studies were not only
3 limited to AIDS patients and the clinical presentation was not specified (e.g., fungemia *versus*
4 non-disseminated disease). As the GM detection cross-reactivity seems to occur with high
5 levels of *Histoplasma* antigens (Wheat et al., 2007), the sensitivity could be better in AIDS
6 patients with disseminated disease because of a high *Histoplasma* burden (Ranque et al.,
7 2007). For this reason, it seems to be essential to reserve the GM detection for disseminated
8 histoplasmosis diagnosis in AIDS patients on the basis of epidemiological, clinical, and
9 laboratory arguments. Moreover, as the incidence of invasive aspergillosis in HIV-positive
10 patients is generally <0.5% (Tong et al., 2009), the risk of misdiagnosis with aspergillosis is
11 low. The positivity of the GM detection seems to be also very useful in the diagnosis and the
12 monitoring of African histoplasmosis due to *Histoplasma capsulatum* var *duboisii* (Therby et
13 al., 2014). Similarly, the cross-reactivity of the GM detection exists with *Cryptococcus*
14 (Dalle et al., 2005) but the incidence of this infection was lower in French Guiana with about
15 0.25 per 100 HIV/AIDS patients-years (Debourgogne et al., 2011). Nevertheless, an important
16 limitation of this test concerns its significant cost. Moreover, the test requires a large number
17 of controls which does not make its use consistent with small series. In South America, where
18 histoplasmosis should be considered as neglected disease, the price of this test does not easily
19 allow its use outside the rich countries.

20 GM detection could also be very helpful for histoplasmosis diagnosis outside endemic areas
21 in HIV-positive travellers but the PPV and NPV should be reconsidered because of a lower
22 prevalence of the disease in this context. However, the contribution of this test should be
23 lower for histoplasmosis diagnosis in immunocompetent patients, especially compared to the
24 detection of specific anti-*Histoplasma* antibodies that immunocompetent patients are able to
25 synthesize.

26 The results of this study are somewhat limited by the retrospective design and the size of the
27 cohort even if, to our knowledge, this is the largest study concerning GM detection
28 specifically performed on AIDS patients with disseminated histoplasmosis. Moreover, the
29 impact of serum storage at -20°C is unknown on the performance of the GM detection test.
30 However, it is usually believed that long-term storage may rather decrease galactomannan
31 levels (Aquino et al., 2007) which would imply a higher sensitivity with fresh serum.

32 In conclusion, this study confirms that GM detection can be very helpful for the diagnosis of
33 disseminated histoplasmosis in AIDS patients, particularly in endemic areas where
34 *Histoplasma* EIA is not available.

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42 133.
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1 **TABLES**

2 **Table 1: Patients baseline characteristics, galactomannan and *Histoplasma*-specific**
 3 **antibody detection in AIDS patients with or without histoplasmosis**

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	AIDS patients		P value
	with histoplasmosis	without histoplasmosis	
Total, No.	39	30	
Age, median IQR [25% ;75%], years	43 [38;50]	46 [37;53]	0.565 ^a
Male / Female patients, No.	23/16	20/10	0.513 ^b
Samples for histoplasmosis diagnosis, No. (%):			
- Digestive (colon, liver, esophagus biopsies)	11 (28.2%)	-	-
- Pulmonary (BAL, bronchial aspiration)	6 (15.4%)	-	-
- Cerebral (CSF)	1 (2.6%)	-	-
- Haematologic (Blood, Bone marrow)	19 (48.7%)	-	-
- Others (ganglion biopsy)	2 (5.1%)	-	-
Histoplasmosis diagnosis method, No./total available data (%):			
- Direct examination	15/37 (40.5%)	-	-
- Culture	37/39 (94.9%)	-	-
- Specific PCR	19/20 (95.0%)	-	-
Patients with positive specific antibodies, No. (%):	14 (35.9%)	0 (0.0%)	<0.001 ^b
Patients with positive GMI \geq 0.5 (GM detection), No. (%):	30 (76.9%)	0 (0.0%)	<0.001 ^b
GMI (GM detection), median IQR [25% ;75%]	1.38 [0.52;4.8]	0.12 [0.08;0.21]	<0.001 ^a
Patients with positive GMI (GMI \geq 0.5) or antibodies, No. (%):	32 (82.1%)	0 (0.0%)	<0.001 ^b

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 6 ^a calculated by Mann-Whitney rank sum test; ^b calculated by χ^2 test
 7 BAL: broncho-alveolar lavage, CSF: Cerebro-spinal fluid

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1 **FIGURE LEGEND**

2 **Fig 1: Performance of GM detection according to the threshold**

3 Receiver Operating Characteristics (ROC) curve for determination of cut-off and assay
4 sensitivity (Se) and specificity (Sp). The thresholds 0.35, 0.4 and 0.5 are shown on the ROC
5 curve with a triangle, circle and square, respectively.

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GMI threshold	Performance	
	Se (%)	Sp (%)
0.10	100	36.7
0.20	94.9	70,0
0.30	87.2	90,0
0.35	87.2	93.3
0.40	82.1	100
0.50	76.9	100
0.60	66.7	100
2.00	33.3	100
15.00	0,0	100

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