Journal of Microbiological Methods 185 (2021) 106226

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Screening for acute disseminated histoplasmosis in HIV disease using urinary antigen detection enzyme immunoassay: A pilot study in Cameroon

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ARTICLE INFO

Keywords:
Histoplasmosis
Cameroon
HIV/AIDS
Diagnosis
Urine antigen

ABSTRACT

Acute disseminated histoplasmosis (ADH) is an AIDS-defining illness and reported in Cameroon, but there are few data about its incidence. Between June and August 2019, we conducted a descriptive cross-sectional study to screen for histoplasmosis in a population of adults with HIV infection, irrespective of their CD4 T-cell counts, using *Histoplasma* urine antigen detection enzyme immunoassay (EIA) and histoplasmin skin test. Of the 138 participants screened, 36 (26%) had detectable antigen in urine, using an OD cut off of 0.045. Skin lesions were present in two (6%) cases. Of 39 patients tested for histoplasmin skin test positivity, one was positive. *Histoplasma* antigenuria was associated with a positive history of chest infection (Odds ratio: 3.632, 95% confidence interval: 1.635–8.071, $p = 0.001$). As 30 (21.7%) of titres were between 0.045 (the current cut off) and 0.25, the cut off may need adjustment in Cameroon, using disease confirmation with alternative, highly sensitive diagnostic approaches such as PCR and bone marrow examination. *H. capsulatum* infection appears to be common among HIV-infected patients attending outpatient clinics at the Buea Regional Hospital. There is an acute need to improve awareness and management of HIV patients with respect to *H. capsulatum* infection.

1. Introduction

Histoplasmosis is an infectious disease acquired through inhalation of spores of a globally distributed dimorphic fungus of the genus *Histoplasma* (CDC, 2018). Human histoplasmosis is caused by two varieties of *Histoplasma* referred to as *Histoplasma capsulatum* var. *capsulatum* (Hcc) and *Histoplasma capsulatum* var. *duboisii* (Hcd), the latter known as African histoplasmosis (Loulergue et al., 2007; Muotoe-Okafor et al., 1996). Acute progressive disseminated histoplasmosis mostly occurs in severely immunocompromised individuals such as those with advanced HIV disease (Nacher et al., 2020). In some countries in Central and Latin America, histoplasmosis is the most common AIDS-defining illness responsible for over 30% of AIDS-related deaths, a figure similar to deaths attributable to tuberculosis (Colombo et al., 2011; Nacher et al., 2020). At the end of 2017, HIV/AIDS was estimated in Cameroon at 510, 000, with 24,000 deaths and 28,000 new infections (WHO, 2018).

The burden of histoplasmosis among HIV patients in sub-Saharan Africa as in Cameroon, has been derived mainly from single case reports and case series (Mandengue et al., 2015; Mandengue, 2012; Zida et al., 2015; Richaud et al., 2014). The lack of sensitive and specific diagnostic tools for histoplasmosis, including antigen detection assays was noted as a limitation in a study where skin biopsies and staining of bronchial biopsies were used as diagnostic techniques (Mandengue et al., 2015). In a recent study from Guatemala, using both respiratory specimens and blood culture, blood PCR and urine antigen, 72% of cases were positive by urine antigen, compared with 8.5% and 36.3% by respiratory specimens and blood culture (Medina et al., 2020).

Most diagnoses of histoplasmosis in Africa are done using microscopy; antigen testing and polymerase chain reaction (PCR) are not available in most countries. Though culture is the gold standard it is invasive (if bone marrow or skin biopsy), insensitive and time consuming (Mandengue et al., 2015; Guimarães et al., 2006). It has been

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https://doi.org/10.1016/j.mimet.2021.106226
Received 5 March 2021; Received in revised form 15 April 2021; Accepted 15 April 2021
Available online 18 April 2021

demonstrated that antigen testing is a good diagnostic technique for histoplasmosis (Nacher et al., 2018). Studies comparing antigen enzyme immunoassay with other techniques in the diagnosis of disseminated histoplasmosis, have shown an overall agreement ranging from 90 to 99% (Persaud et al., 2019; Guimarães et al., 2006; Caceres and Valdes, 2019). Similar performance was seen with the recently launched *Histoplasma* antigen Lateral Flow Assay (Cáceres et al., 2019).

We hypothesized that *Histoplasma capsulatum* infection may be much more common and yet largely an undiagnosed disease among outpatient HIV infected patients in our setting compared to incidence rates reported in severely immunocompromised, hospitalised advanced HIV disease patients elsewhere in Africa and in the Americas. The aim of our study was therefore to determine the prevalence of *Histoplasma capsulatum* infection and associated risk factors in HIV-infected patients attending the Buea Regional Hospital. We utilised a new CE marked urine antigen assay which detects *Histoplasma* galactomannan and had excellent agreement with a well-recognised standard assay (Persaud et al., 2019). To our knowledge, the assay has never been used in Africa before. As such we sought to assess its potential utility with particular attention to the appropriateness of the manufacturer's cut-off, in our population.

2. Patients and methods

2.1. Study design and setting

A descriptive cross-sectional study was conducted from June to August 2019 among HIV outpatients recruited in the Buea Regional Hospital. The hospital comprises approximately twelve units with around three hundred hospital beds, serving the Buea municipality (200,000 inhabitants in 2012 (CDP, 2012)) and its environs. The HIV unit manages more than two thousand files annually, with an average attendance of 150 patients and three admissions of patients with advanced HIV per month.

2.2. Study participants and data extraction

HIV-infected outpatients were randomly recruited regardless of their CD4 cell count, viral load, antiretroviral therapy (ART) status for *Histoplasma* antigen testing. HIV-infected outpatients with, a CD4 count greater than 350 cells/mm^3 were recruited for histoplasmin skin testing. Socio-economic and demographic data; known risk factors associated with *Histoplasma* infections were obtained through administration of questionnaires. Clinical and laboratory data such as time of HIV diagnosis, viral load, and antifungal therapy were obtained from patient records. CD4 counts were not available for the majority of the patients.

2.3. Exclusion criteria

Patients receiving antifungal treatment and those who did not give their consent were excluded from the study.

2.4. Laboratory procedure

2.4.1. Antigen enzyme immunoassay

Urine samples (5 mL) for *Histoplasma* antigen testing were collected aseptically following universal precautions and transported to the laboratory where they were stored at −20 °C until tested. Specimens were brought to room temperature prior to testing and were analysed in duplicates using the Optimum Imaging Diagnostics (OIDx) Histoplasma Antigen sandwich EIA (Scarborough, ME., USA), following the manufacturer's instructions.

2.4.2. Histoplasmin skin test

histoplasmin reagents used in this study were obtained from the laboratory of Dr. Conchita Toriello laboratory of the National Autonomous University of Mexico (UNAM) where it was prepared according to previous studies (Toriello et al., 1993; Toriello et al., 1991). Reagents were stored at 4 to 8 °c upon reception until used.

Skin test was performed by injection of 0.1 ml of the antigen intradermally in the volar surface of any of the arms using a short, beveled 24 to 26-gauge needle. The test was read after 48 h of the inoculation, by measuring with a ruler the diameter of the visible and palpable induration produced at the injection point. An induration equal to or above 5 mm was considered as a positive test (LIFE, 2018; Oladele et al., 2018).

2.5. Data analysis

Data were entered in MS Excel and analysed in SPSS version 21. Prevalence of *H. capsulatum* infection was expressed in charts. Association between *Histoplasma* infection and sociodemographic and risk factors, correlation between viral load (Log) and optical density was investigated using univariate and multivariate analysis at 95% confidence interval. $P \leq 0.05$ was considered as statistically significant.

2.6. Ethics and institutional review

Ethical clearance for this study was obtained from the Faculty of Health Science Institutional Review Board (FHS-IRB) of the University of Buea and administrative clearances were obtained from the delegation of Public Health for the South West region and from the Regional Hospital of Buea. All patient details were anonymised; identifying codes were used throughout the study.

3. Results

A total of 138 participants were recruited with a mean age of 43.7 years (SD: 12.18) (Table 1). The majority of the participants was aged between 31 and 40 years (30.4%), female (73.2%), single (43.5%), farmer (25.4%), and (54.3%) had primary school level of education. *H. capsulatum* antigen was detected in 36 patients (26.1% (95%CI 18.8–33.4)), with skin lesions present in two (6%) cases. The OD median value was 0.036 and the OD cut-off value for positivity was 0.045 and positive titres ranged from 0.045 to 0.418. If the cut off was moved to 0.1, 20 (14.5%) would be positive and if moved to 0.250, then only 6 (8%) would be positive (Fig. 1).

The relative antigen detection rates were not affected by age, sex, occupation and educational level (Table 1). Multiple other possible environmental and sociological factors were investigated for a link to a positive antigen test. Exposure to chickens, other birds or bats was not linked to antigen positivity (Table 2). Likewise, some other exposures

Table 1
Sociodemographic characteristics of participants and association with Histoplasma infection ($P \leq 0.05$).

		n (%)	Positive antigen test (%)	*P* value
Aall patients		138	36 (26.1)	
Age group	20–30	19 (14)	6 (32)	0.85
	31–40	42 (30)	9 (21)	
	41–50	38 (28)	10 (26)	
	51–60	25 (18)	8 (32)	
	>60	14 (10)	3 (21)	
Sex	Female	101 (73)	28 (28)	0.47
	Male	37 (27)	8 (22)	
	Not defined	00 (00)	00(00)	
Occupation	Business	35 (25)	9 (26)	0.47
	Farmer	35 (25)	7 (20)	
	Other	64 (47)	20 (31)	
	Student	4 (3)	00 (00)	
Education	Primary	75 (54)	20 (27)	0.77
	Secondary	47 (34)	13 (28)	
	University	16 (12)	3 (19)	
Marital status	Married	55 (40)	13 (24)	0.81
	Single	60 (43)	16 (27)	
	Widow	23 (17)	7 (30)	

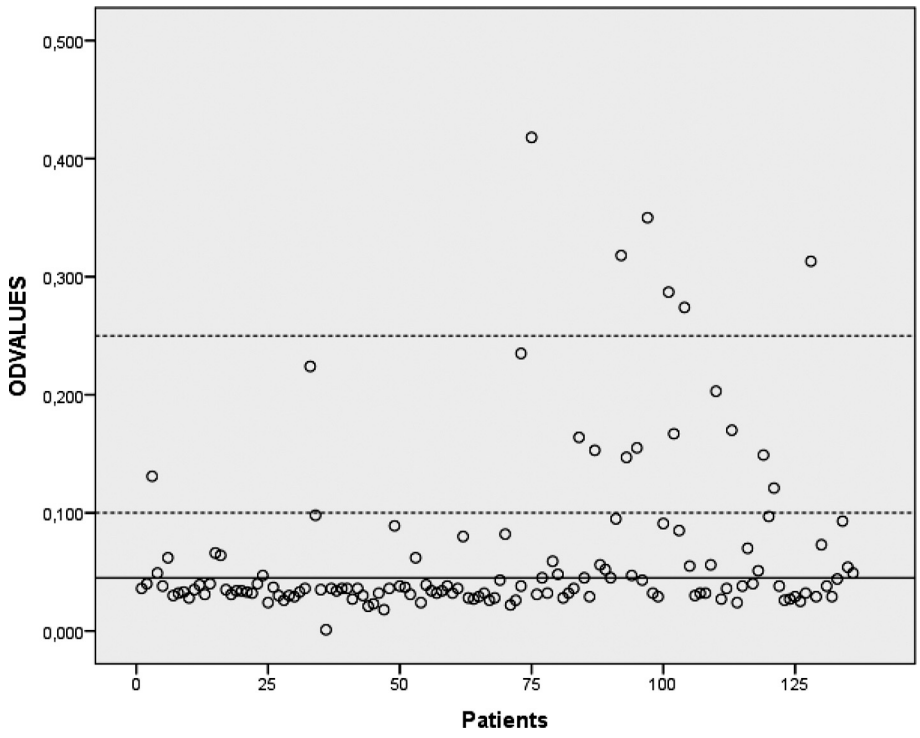

Fig. 1. Distribution of optical densities on a scatter plot. First line (full): current cut-off, second and third lines (dotted): tentative cut-off for Cameroon.

that could be relevant such as hunting, fruit trees nearby, construction *etc.* were not apparently linked to a positive antigen test result. Of 39 patients tested for histoplasmin skin test positivity, one was positive (Fig. 2); she also had a positive urine antigen test (OD =0.054) and had some recent health issues of an undetermined nature.

A prior chest infection was strongly linked to a positive antigen test result, 44.2% with antigen positive reported this compared with 17.9% who did not (OR 3.63 (1.64–8.07, $p = 0.001$) (Table 2). The duration of the chest infection was not apparently important. There was no association between the prevalence of *H. capsulatum* infection and the viral load ($P = 0.07$) and no correlation between viral load and the quantity of *H. capsulatum* antigen ($P = 0.371$, $r = -0.138$). Only 10 patients were experiencing virologic failure which is viral load greater than 1000 RNA copies/mL of plasma.

4. Discussion

HIV infection is a state of immunosuppression that exposes the patients to several opportunistic infections such as *Histoplasma* infection, which has been documented as AIDS-related illness (Huber et al., 2008). In this pilot study aimed at screening for histoplasmosis among HIV-infected patients attending a large outpatient clinic in Cameroon, we found a very high prevalence of positive *H. capsulatum* antigen test at 26.1%. The assay we used had 99% agreement when compared to the MiraVista EIA, which is extensively used in the USA (Persaud et al., 2019). The assay detects *Histoplasma* galactomannan, which can also be detected by the *Aspergillus* galactomannan antigen ELISA (Iriart et al., 2014). The assay also detects Blastomyces antigen, a rare infection in Africa (Maphanga et al., 2020). However, some false positive tests in our population are likely, and using a higher cut off may be appropriate. Galactomannan is found in food and may be absorbed, thus causing false positive results. Many patients with HIV have diarrhoea and impaired small bowel function and their guts could be 'leaky', as seen in post-chemotherapy patients (Mennink-Kersten et al., 2005; Letscher-Bru et al., 1998). In internal studies done by the manufacturer, and clinical studies (Persaud et al., 2019), *Blastomyces* antigen can cross-react and this fungus is occasionally a cause of disease in southern Africa. Cryptococcal antigen may also cross-react, although the urinary concentration of cryptococcal antigen is usually very low. Another explanation to this high prevalence may be the cross-reactivity of anti-Galactomannan antibody EB-A2 with galactofuranosyl, a component commonly found in lipoteichoic acid of *Bifidobacterium bifidum* subsp. *Pennsylvanicum* and in *H. capsulatum* (Mennink-Kersten et al., 2005). Moreover, *Bifidobacterium* spp. are common within the human gastrointestinal tract (Mennink-Kersten et al., 2005). It may be appropriate to use a modified interpretation in the range of 0.45 to 0.25 as 'low level of antigen detected', as a way of signalling uncertainty about a definitive diagnosis of disseminated histoplasmosis.

This antigen prevalence is higher than the 13% obtained in Yaoundé based on culture and biopsy (Mandengue et al., 2015). In our study a more sensitive diagnostic tool (EIA) was used. Moreover, in the study of Mandengue et al. (2015), patients at different stages of HIV may have been studied which may have led to a different prevalence. It is not also in accordance with the burden of histoplasmosis in Cameroon reported by Mandengue and Denning, 2018 (3%), which is far lower than we obtained in this study. The prevalence obtained here also differs from the study of Vantilcke et al. (2014) where it was 37.5%. Vantilcke et al conducted their study in a confirmed hyperendemic area (French Guiana) on symptomatic patients suspected of having *Histoplasma* infection, but in this study HIV patients were randomly selected.

In contrast, only one of 39 (2.5%) patients had a positive skin test to histoplasmin. This percentage is lower than the one of Oladele and colleagues in 2018 where it was 4.4%. However, Oladele et al. study was on a larger and more distributed sample size, and included healthy participants who are more susceptible to produce intradermic reactions. It is also lower compared to the study of Muotoe-Okafor and colleagues done in 1996 which revealed a 10.6% positivity. Their study was carried out in the vicinity of a natural focus of *Histoplasma capsulatum* var. *duboisii* compared to the present study that was in the urban setting.

Assessment of history of chest infection showed that only 31.2% of our participants have had chest infection in the past. However out of the 21.6% that were positive to *H. capsulatum* antigen testing, 52.8% had past chest infection. A previous pulmonary infection was strongly associated with *Histoplasma* antigenuria ($P = 0.001$). The chance of

Table 2
Risk factors associated with *H. capsulatum* infection ($P \leq 0.05$).

		n (%)	Positive antigen test	*P*-value	OR (CI)
Owning poultry	No	125 (90.6)	34 (27.2)	0.28	
	Yes	13 (9.4)	2 (15.4)		
Presence of Poultry in the quarter	No	91 (65.9)	22 (24.2)	0.47	1.30 (0.42–2.46)
	Yes	47 (34.1)	14 (29.8)		
Distance of poultry from the house	1	11 (8.0)	5 (45.5)		0.58
	2	10 (7.2)	3 (42.9)		
	3	4 (2.9)	1 (25)		
	4	1 (0.8)	0		
	5	4 (2.9)	2 (50)		
	>5	17 (12.3)	3 (17.6)		
Local chickens in the vicinity of home	No	35 (25.3)	9 (25.7)	0.95	1.02 (0.42–2.46)
	Yes	103 (74.6)	27 (26.2)		
Warehouse close to home or place of work	No	124 (89.9)	32 (25.8)	0.52	
	Yes	14 (10.1)	4 (28.6)		
Working in a forested region	No	104 (75.4)	26 (25)	0.61	1.25 (0.52–2.95)
	Yes	34 (24.6)	10 (29.4)		
Contact with hunters	No	125 (90.6)	32 (25.6)	0.68	1.29 (0.37–4.68)
	Yes	13 (9.4)	4 (30.8)		
Travelled to areas with caves	No	117 (84.8)	29 (24.8)	0.41	1.51 (0.55–4.12)
	Yes	21 (15.2)	7 (33.3)		
History of working or living in areas with lots of birds	No	87 (63)	20 (23)	0.49	
	Yes	51 (37)	16 (31.4)		
House or place of work place with lots of fruit trees	No	93 (67.4)	21 (22.6)	0.17	1.71 (0.78–3.76)
	Yes	45 (32.6)	15 (33.3)		
Heavy construction sites near home or place of work	No	114 (82.6)	28 (24.6)	0.37	1.53 (0.59–9.97)
	Yes	24 (17.4)	8 (33.3)		
Past self injection	No	134 (97.1)	35 (26.1)	0.72	0.94 (0.09–9.36)
	Yes	4 (2.9)	1 (25)		
Past chest infection	No	95 (68.8)	17 (17.9)	0.001	3.63 (1.63–8.07)
	Yes	43 (31.2)	19 (44.2)		
Duration of chest infection	<3 months	18 (13)	6 (33.3)	0.27	2.00 (0.57–6.95)
	>6 months	26 (18.8)	13 (50)		
Prior antifungal therapy	No	136 (98.6)	35 (25.7)	0.26	1.02 (0.97–1.08)
	Yes	2(1.4)	1 (50)		

those not having past chest infection testing positive was 3 times higher than those with past chest infection (OR: 3.632, CI: 1.635–8.071) This outcome is different from a study done in Nigeria where a similar questionnaire was used (Oladele et al., 2018). In that study there was no association between past chest infection and *Histoplasma* infection. It is possible that treatment of a past chest infection included an antifungal agent which could have cleared the infection if present. Just 10 (8.1%)

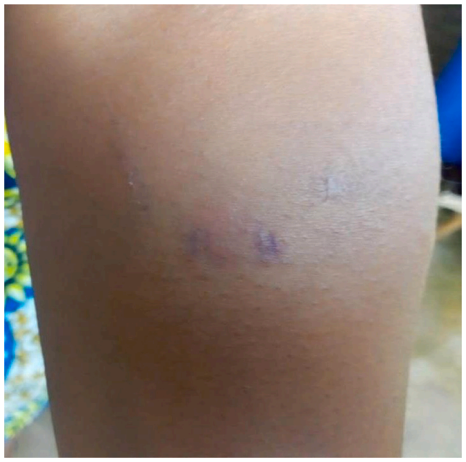

Fig. 2. Histoplasmin skin positive test result (palpable induration >5 mm produced at the injection point).

of the participants were experiencing HIV virologic failure, the majority having suppressed viral load with no association found between the occurrence of *Histoplasma* antigenuria and the viral load ($P = 0.07$). This is similar to the study of Boigues et al. (2018) where viral load was not associated with the outcome of disseminated histoplasmosis. The weak negative correlation between optical density and viral load (Log) was not significant ($P = 0.371$).

The main limitation of this study was uncertainty about the optimal cut off in our setting in Cameroon. This will require concurrent alternative tests for disseminated histoplasmosis to be done, including PCR, which had a 63% sensitivity compared to urine antigen detection in Guatemala (Medina et al., 2020) or bone marrow examination. Also, incomplete patient records in some of the participants and a lack of CD4 counts limit the conclusions. In addition, the study should be repeated in other HIV centres and towns of the country because of the microfoci occurrence of histoplasmosis.

In conclusion, *H. capsulatum* infection is probably common among non-hospitalised HIV-infected patients attending the Buea Regional Hospital. A need to improve awareness and management of HIV patients with respect to *H. capsulatum* infection to limit the dissemination of the disease is clear. Screening for disseminated histoplasmosis may need to be extended to include apparently stable HIV-infected patients attending outpatient clinics.

Challenges

Histoplasmin skin test survey could not be conducted to the end and many of those who participated could not come back or be traced the following days due to the ongoing political crisis in the South West Region of Cameroon.

Author contributions

Kuate Ngouanom, David Denning and Christine Mandengue: conceptualization.
Kuate Ngouanom, David Denning: funding acquisition
Kuate Ngouanom: investigation
Nyasa Raymond: supervision
Tendongfor Nicholas: data analysis
Kuate Ngouanom and Felix Bongomin: writing-original draft
All authors: writing-review and editing

Declaration of Competing Interest

The authors whose names are listed immediately below certify that

they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgments

The authors wish to thank Geoff Piasio and Optimum Imaging Diagnostics (OIDx) company, for providing test reagents used in this study. The authors also thank Dr. Conchita Toriello of the Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico for the histoplasmin skin test reagents. The authors also extend their regards to the staff of the Buea Regional Hospital and of the Biotechnology Unit of the university of Buea. Our thanks also go to HIV positive patients attending clinics at the Buea Regional Hospital. David Denning is partially funded by NIHR Manchester Biomedical Research Centre. Support was also provided by the Medical Research Council, London, UK (Newton grant MR/P017622/1).

References

Boigues, B.C., Paniago, A.M., Lima, G.M., Nunes, M., Uehara, S.N., 2018. Clinical outcomes and risk factors for death from disseminated histoplasmosis in patients with AIDS who visited a high-complexity hospital in Campo Grande, MS, Brazil. Rev. Soc. Bras. Med. Trop. 51 (2), 155–161. https://doi.org/10.1590/0037-8682-0369-2017.

Caceres, D.H., Valdes, A., 2019. Histoplasmosis and tuberculosis co-occurrence in people with advanced HIV. J. Fungi 5 (3), 73. https://doi.org/10.3390/jof5030073.

Cáceres, D.H., Gómez, B.L., Tobón, A.M., Chiller, T.M., Lindsley, M.D., 2019. Evaluation of a Histoplasma antigen lateral flow assay for the rapid diagnosis of progressive disseminated histoplasmosis in Colombian patients with AIDS. Mycoses 00, 1–6. https://doi.org/10.1111/myc.13023.

Centers for Disease Control and Prevention, 2018. Histoplasmosis. https://www.cdc.gov/fungal/diseases/histoplasmosis/index.html. Accessed the 01/12/2018.

Colombo, A.L., Tobón, A., Restrepo, A., Queiroz-Telles, F., Nucci, M., 2011. Epidemiology of endemic systemic fungal infections in Latin America. Med. Mycol. 1–14. https://doi.org/10.3109/13693786.2011.577821.

Communal Development Plan (CDP), 2012. Buea Communal Development Plan. https://www.pndp.org/documents/04_CDP_Buea.pdf. Accessed the 18/09/2020.

Guimarães, A.J., Nosanchuk, J.D., Zancopé-Oliveira, R.M., 2006. Diagnosis of histoplasmosis. Braz. J. Microbiol. 37 (1), 1–13. https://doi.org/10.1590/S1517-83822006000100001.

Huber, F., Nacher, M., Aznar, C., Pierre-Demar, M., El Guedj, M., Vaz, T., et al., 2008. Aids-related *Histoplasma capsulatum* Var. Capsulatum infection: 25 Years' experience of French Guiana. Aids 22 (9), 1047–1053. https://doi.org/10.1097/QAD.0b013e3282ffde67.

Iriart, X., Blanchet, D., Menard, S., Lavergne, R-A., Chauvin, P., Adenis, A., et al., 2014. A complementary tool for management of disseminated Histoplasma capsulatum var. capsulatum infections in AIDS patients. Int. J. Med. Microbiol. 304 (8), 1062–1065. https://doi.org/10.1016/j.ijmm.2014.07.016.

Leading International Fungal Education, 2018. Fungal Infection. http://www.life-worldwide.org/fungal-diseases/skin-testing-endemic.

Letscher-Bru, V., Cavalier, A., Pernot-Marino, E., Koenig, H., Eyer, D., Waller, J., et al., 1998. *Aspergillus* galactomannan antigen detection with Platelia. J. Med. Mycol. 8, 112–113.

Loulergue, P., Bastides, F., Baudouin, V., Chandenier, J., Mariani-Kurkdjian, P., Dupont, B., et al., 2007. Literature review and case histories of Histoplasma capsulatum var. duboisii infections in HIV-infected patients. Emerg. Infect. Dis. 13 (11), 1647–1652. https://doi.org/10.3201/eid1311.070665.

Mandengue, E.C., 2012. A case of disseminated Histoplasmosis detected in peripheral blood smear staining revealing AIDS at terminal phase in a female patient from Cameroon. Case Rep. Med. 2012, 1–3. https://doi.org/10.1155/2012/215207.

Mandengue, C., Denning, D., 2018. The burden of serious fungal infections in Cameroon. J. Fungi 4 (2), 44. https://doi.org/10.3390/jof4020044.

Mandengue, C.E., Ngandjio, A., Atangana, P.J., 2015. Histoplasmosis in HIV-infected persons, Yaoundé, Cameroon. Emerg. Infect. Dis. 21 (11), 2094–2096. https://doi.org/10.3201/eid2111.150278.

Maphanga, T.G., Birkhead, M., Muñoz, J.F., Allam, M., Zulu, T.G., Cuomo, C.A., et al., 2020. Human blastomycosis in South Africa caused by *Blastomyces percursus* and *Blastomyces emzantsi* sp. nov., 1967 to 2014. J. Clin. Microbiol. 58, e01661-19 https://doi.org/10.1128/JCM.01661-19.

Medina, N., Alastruey-Izquierdo, A., Mercado, D., Bonilla, O., PÃ©rez, J.C., Aguirre, L., et al., 2020. Comparative performance of the laboratory assays used by a Diagnostic Laboratory Hub for opportunistic infections in people living with HIV. AIDS 34 (11), 1625–1632. https://doi.org/10.1097/QAD.0000000000002631.

Mennink-Kersten, M.A., Ruegebrink, D., Klont, R.R., Warris, A., Gavini, F., Op den Camp, H.J., et al., 2005. Bifidobacterial lipoglycan as a new cause for false-positive platelia Aspergillus enzyme-linked immunosorbent assay reactivity. J. Clin. Microbiol. 43 (8), 3925–3931. https://doi.org/10.1128/JCM.43.8.3925-3931.2005.

Muotoe-Okafor, F.A., Gugnani, H.C., Gugnani, A., 1996. Skin and serum reactivity among humans to histoplasmin in the vicinity of a natural focus of Histoplasma capsulatum var. duboisii. Mycopathologia 134 (2), 71–74. https://doi.org/10.1007/BF00436867.

Nacher, M., Blanchet, D., Bongomin, F., Chakrabarti, A., Couppie, P., Demar, M., et al., 2018. Histoplasma capsulatum antigen detection tests as an essential diagnostic tool for patients with advanced HIV disease in low- and middle-income countries: a systematic review of diagnostic accuracy studies. PLoS Negl. Trop. Dis. 12 (10), e0006802 https://doi.org/10.1371/journal.pntd.0006802.

Nacher, M., Couppié, P., Epelboin, L., Djossou, F., Demar, M., Adenis, A., 2020. Disseminated Histoplasmosis: fighting a neglected killer of patients with advanced HIV disease in Latin America. PLoS Pathog. 16 (5), e1008449 https://doi.org/10.1371/journal.ppat.1008449.

Oladele, R.O., Toriello, C., Ogunsola, F.T., Ayanlowo, O.O., Foden, P., Fayemiwo, A.S., et al., 2018. Prior subclinical histoplasmosis revealed in Nigeria using histoplasmin skin testing. PLoS One 13 (5), e0196224. https://doi.org/10.1371/journal.pone.0196224.

Persaud, S.P., Lawton, T., Burnham, C.-A.D., Anderson, N.W., 2019. Comparison of urine antigen assays for the diagnosis of *Histoplasma capsulatum* infection. J. Appl. Lab. Med. https://doi.org/10.1373/Jalm.2018.028910.

Richaud, C., Chandesris, M.-O., Lanternier, F., Benzaquen-Forner, H., Garcia-Hermoso, D., Picard, C., et al., 2014. Imported African Histoplasmosis in an Immunocompetent Patient 40 Years after Staying in a Disease-Endemic Area. Am. J. Trop. Med. Hyg. 91 (5), 1011–1014. https://doi.org/10.4269/ajtmh.13-0731.

Toriello, C., Arjona-Rosado, L.C., Díaz-Gómez, M.L., Taylor, M.L., 1991. Efficiency of crude and purified fungal antigens in serodiagnosis to discriminate mycotic from other respiratory diseases. Mycoses 34, 133–140. https://doi.org/10.1111/j.1439-0507.1991.tb00634.x.

Toriello, C., Jiménez-Montiel, J.A., Reyes-Montes, M.R., Taylor, M.L., 1993. Two-dimensional immunoelectrophoresis of histoplasmin and a purified polysaccharide-protein antigen of *Histoplasma capsulatum*. Mycopathologia 122 (1), 7–13. https://doi.org/10.1007/bf01103703.

Vantilcke, V., Boukhari, R., Jolivet, A., Vautrin, C., Misslin, C., Adenis, A., Nacher, M., 2014. Fever in hospitalized HIV-infected patients in Western French Guiana: first think Histoplasmosis. Int. J. STD AIDS 25 (9), 656–661. https://doi.org/10.1177/0956462413516299.

World Health Organization, 2018. HIV country profile. Cameroon: HIV Country Profile: 2017. http://cfs.hivci.org/country-factsheet.html#. Accessed 05/12/2018.

Zida, A., Niamba, P., Barro-Traoré, F., Korsaga-Somé, N., Tapsoba, P., Briegel, J., Guiguemdé, R.T., 2015. Disseminated histoplasmosis caused by Histoplasma capsulatum var. duboisii in a non-HIV patient in Burkina Faso: case report. J. Mycol. Méd. 25 (2), 159–162. https://doi.org/10.1016/j.mycmed.2015.03.002.