Annales Africaines de Médecine
Cas clinique

Lymphadenitis due to *Histoplasmosis capsulatum* var. *capsulatum:* Unawareness or rare occurrence in the Democratic Republic of Congo? Unusual original report with first immunohistochemical phenotyping of the fungus

Lymphadénite à histoplasma capsulatum var capsulatum. Méconnaissance ou rareté en République Démocratique du Congo ? Cas clinique inhabituel avec caractérisation du fungus par immunohistochimie

Nestor Muyulu Pakasa[1,3], Eyamba Ajebo[1,2†], Guy-Gerard Nyuki Kingebeni[1,3], Djibril Nyessi[1], Gregory Jouvion[4]

Corresponding author
Nestor Muyulu Pakasa, *MD*, *PhD*
Courriel : nestorpks2@gmail.com ;
nestor.pakasa@unikin.ac.cd

Résumé

Nous décrivons le premier cas d'une lymphadénite spécifique histoplasmique à *Histoplasma capsulatum var capsulatum*. Il s'agissait d'une patiente de 55 ans originaire du village Yabaondo dans l'ancienne Province Orientale, actuellement Province de la Tshopo. Son état général était satisfaisant excepté un amaigrissement et la présence de nombreux ganglions cervicaux gauches de taille variable, présumés tuberculeuses (TB). La biopsie a montré un remaniement de l'architecture folliculaire remplacée par une prolifération de gros macrophages bourrés des spores d'*H. capsulatum var capsulatum*. L'immunohistochimie utilisant un anticorps spécifique maison a été réalisé à l'Institut Pasteur de Paris et a confirmé l'identité moléculaire du microorganisme. La rareté des cas d'histoplasmose à *H. capsulatum* en RD Congo nous a motivé à rapporter ce cas inédit afin d'attirer l'attention et de contribuer à la préparation de la lutte contre cette entité émergente/reémergente négligée. L'Histoplasmose à *H. capsulatum var capsulatum* doit être evoquée dans la demarche de diagnostic differentiel d'une lymphadénopathie en milieu tropical.

Mots-clés: Lymphadénite, Histoplasmose, *capsulatum*, Yabaondo, RD Congo

Reçu le 6 avril 2020
Accepté le 11 mai 2020

1 Department of Pathology. Kinshasa University Hospital. P.O Box 822. Kinshasa XI
† in memoriam
2 Division of Nephropathology, Uropathology and Neglected Tropical Diseases. Dept of Pathology, University hospital of Kinshasa.
3 Human Histopathology & Animal Models, Department Infection & Epidemiology Pasteur Institute, Paris

Summary

We have described herein the first lymphadenitis due to *Histoplasma capsulatum var capsulatum* with molecular characterization of the pathogen. The patient was a 55-year-old female from a remote village of Yabaondo in the former Province Orientale, currently Tshopo Province, Democratic Republic of Congo (DRC) who presented with multiple swollen left neck lymph nodes. She reported weight loss (undefined) but was otherwise healthy, and a presumptive diagnosis of tuberculosis (TB) was postulated. The biopsy specimen yielded plenty yeast cells of *H. capsulatum var capsulatum* on Hematoxylin-Eosin routine staining. The molecular identity of the fungus was confirmed by immunohistochemistry at the Pasteur Institute of Paris. The rarity of reported cases of *H. capsulatum var capsulatum* in DRC prompted us to report this unique case to generate awareness and preparedness of this emerging/reemerging neglected tropical fungal infection against outbreaks. *H. capsulatum var capsulatum* needs to be considered in the work-up of lymphadenopathies in tropical environment.

Keywords: Lymphadenitis, histoplasmosis, *capsulatum*, Yabaondo, DRC.

Received: April 6th, 2020
Accepted: May 11th, 2020

Introduction

Although in Africa the classic *Histoplasmosis capsulatum* var *capsulatum* is believed to coexist with *H. capsulatum* var *duboisii*, only one non-HIV pediatric case has been recorded in the recent literature from DR Congo in 1982 at the General Referral Hospital of Kinshasa (formerly Mama Yemo General Hospital) compared to the commonest *H. duboisii* or African Histoplasmosis clade. Authors report the first adult case of lymphadenitis due to *H. capsulatum var capsulatum* and hope to generate awareness and preparedness on this neglected tropical fungal infection.

Methods

A lymph node specimen fixed in 10% formalin was accessioned and archived in our laboratory at the Department of Pathology, Kinshasa University Hospital. The biopsy was processed per standard technics for histopathology and stained with Hematoxylin and Eosin (HE). The diagnosis of *H. capsulatum* was made. Recently, we retrieved the block and performed additional Periodic Acid-Schiff (PAS), Grocott Methenamine Silver (GMS) special staining as well as ancillary immunohistochemistry (IHC) using an anti *H. capsulatum var capsulatum* monoclonal antibody developed at the Pasteur Institute, Paris.

Case report

The patient was a 55-year-old female from a remote rural area of Yabaondo rural hospital (Tshopo province, formerly a district of Oriental Province, map figure 1).

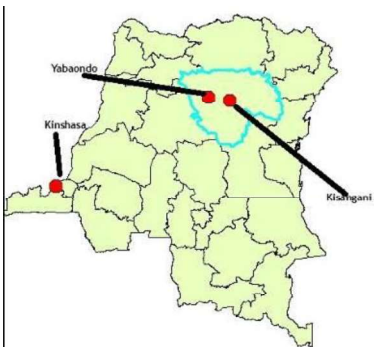

Figure1. Location of Yabaondo in Province of Tshopo (capital: Kisangani), Isangi District. Democratic Republic of Congo

She presented with multiple swollen left neck lymph nodes filled with purulent material clinically presumed for tuberculosis (TB). She reported weight loss but was otherwise healthy. No other pertinent symptoms like fever, pain, vomiting, cough, pulmonary disease were recorded. Pus culture was reported negative. Initial microscopic examination of the specimen revealed an encapsulated lymph node parenchyma (Figure 2a). The normal follicular pattern has been completely replaced by a proliferation of numerous large macrophages filled with plenty of small rounded to ovoid encapsulated yeasts consistent with *H. capsulatum* var *capsulatum* (*Hcc*) (Figure 2b) with areas of homogenous caseating-like necrosis (Figure 2c).

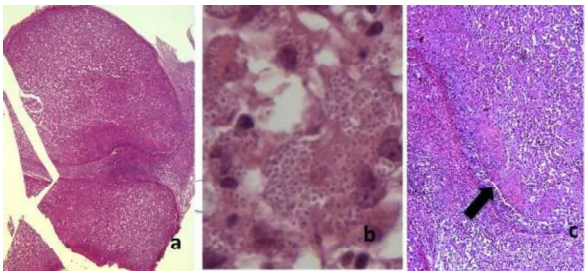

Figure 2. Photomicrographs of *H. capsulatum* lymphadenitis: (a) Low magnification of an encapsulated lymph node showing homogenous pattern with effacement of normal follicular architecture (HE); (b) macrophages filled with tear-drop shaped encapsulated yeasts of *Hcc* (HEx25) (c) area of eosinophilic necrosis (arrow) (HE x10)

Recently, the paraffine block was retrieved and brought to France for reevaluation and additional histochemical staining with HE, periodic Schiff acid (PAS), Grocott methenamine silver (GMS) or Gomori-Grocott (Fig.3a) and Fontana Masson at Pasteur Institute of Paris. The results confirmed the fungus *Hcc*, while immunohistochemistry (IHC, Figure 3b, c) yielded strong positivity to *Hcc*. The microorganisms measured roughly 4 µm in diameter consistent with *Hcc*. These whole results were validated by the molecular mycology unit of the French "Centre National de Référence des Mycoses Invasives et Antifongiques" (CNRMA).

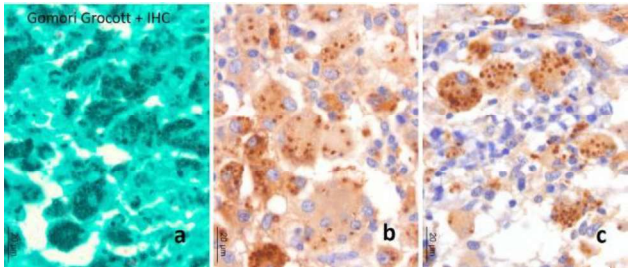

Figure 3. Photomicrographs of ancillary technics. Plenty intracellular yeast microorganisms (a) within

macrophages (Grocott-Gomori), (b) in multinucleate giant cells (arrow) and (c) in macrophages, with dense core positive staining. (IHC, PAP stain)

Discussion

We have reported the first lymphadenitis due to *Hcc* from the DRC. The etiologic fungus molecular phenotype was confirmed by an IHC assay. Histoplasmosis is a disease caused by an infection by a fungus known as *H. capsulatum*, a thermally dependent dimorphic fungus which is common in the environment, most frequently in association with bird or bat droppings. The fungus takes a mycelial form at room temperature and transforms to a yeast form in host tissue or at 35°-38°C in certain culture media. Some people also refer to the disease as "cave disease", Darling disease, classic histoplasmosis, or mainly as North American histoplasmosis (1-2). Phylogenetic studies of *H. capsulatum* have identified at least eight clades, which correspond to different geographic regions. The African clade includes those isolates previously classed as *H. capsulatum var. duboisii* (*Hcd*) due to *H duboisii* as well as those classed as *H. capsulatum var. capsulatum* due to *Hc*c and *H. capsulatum var. farciminosum* (3). Histoplasmosis has predominantly a rural or village distribution and its occurrence is related to exposure to soils enriched by the fecal material of chicken, other birds and bat's guano. Indeed, *Histoplasma* has been isolated from soil of chicken houses, caves, hollow trees and barnyard considered therefore as reservoirs of infection (1-2). Infection occurs after inhalation of conidia from the dust generated when soil is disturbed. The clinical presentation of histoplasmosis is highly dependent on two major factors – the size of the inoculum, and the immune status of the host. In some respects, the relationship between histoplasmosis infection and immune competence is analogous to primary TB; infants, elderly and immune compromised patients are likely to develop a (rapidly) progressive pulmonary and systemic infection that is often fatal, but in most immune competent adults, the primary infection is self-limited, and indeed is often not diagnosed in endemic regions. All age-groups are affected with a maximum incidence in the second decade (1-2). Males are more affected than females. *Hcc* is a disease of the mononuclear phagocytic system (MPS) previously known as the reticuloendothelial system. When aerosolized spores reach the respiratory tract, they are phagocytosed by alveolar macrophages; after development of intracellular yeast forms and reproduction by budding, hematogenous dissemination can lead to the involvement of lymph nodes and other organs followed by occurrence of foci of granulomatous inflammation (4). In 1982, a single pediatric case of *Hcc* was reported in Kinshasa. The 4-year-old immunocompetent girl originated from the rainy forest village Bokote (former Equator province) and had a generalized disease (5). The patient under consideration in this study was an adult female with unknown HIV status although the patient was seen before the HIV/AIDS pandemic era (1976). Her disease was limited to the neck lymph nodes and was probably misdiagnosed and mishandled as TB. It is likely that she acquired the disease through her daily chores in the woods or farming. There is no existing knowledge about *Hcc* in Yabaondo. Recent personal telephone interviews among former and/or current physicians having worked in Yabaondo yielded complete lack of knowledge about this pathology. The initial clinical assumption was TB, like in most cases of lymphadenopathies in this country with purulent material mimicking caseous necrosis. No further case of *Hcc* was found in the files of the Department of pathology up to now. Recent studies from Southern America revealed that implementation of a training course increased the diagnosis of *Hcc* in Colombia (6). It is likely that implementing similar training courses could enhance recognition of *Hcc* in several parts of DR Congo as part of preparedness against severe

epidemics that may occur like in several parts in world. Remarkably, the most encountered Histoplasma clade in DRC remains the *H. capsulatum var. duboisii* (7-8). Histopathology remains the gold standard for the diagnosis of the fungus. Morphologic defining features of the etiologic microorganism remain the size of the yeasts (1-3 µm vs 7-15 µm for *Hcd*) and the MPS exclusive composition of the granulomatous inflammatory infiltrate. The Infectious Diseases Society of America has developed guidelines for the treatment of histoplasmosis using Ketoconazole or Itraconazole^{R} (9). *Hcc* is the most common cause of fungal respiratory infection globally. While most infections are mild, 10% of cases can be life-threatening such as inflammation of the pericardium and fibrosis of major blood vessels (10).

Conclusion

To the best of our knowledge, this is the first case of lymphadenitis due to *Hcc* in DRC with ancillary molecular identification of the fungus. Generating the awareness is incredibly important because the condition is nowadays largely unknown, misdiagnosed, and mishandled. Finally, *Hcc* should be considered in the workup of most lymphadenopathies in tropical environment.

Acknowledgements
The contributing authors would like to appreciate the map expert drawing from Pastor Gauthier Mangiala (available on https://www.linkedin.com/in/gauthier-mangiala-a21790153/)

Disclosure/Conflict of Interest
The authors declare they have no conflict of interests.

Contributing authors
All the authors have contributed equally. The paper is published in memoriam of Dr E. AJEBO who supervised the first biopsy examination.

References

1. Dubois A, Janssens PG, Brutsaert P, Vanbreuseghem R. A case of African histoplasmosis; with a mycological note on *Histoplasma duboisii* n. sp. [in French]. *Ann Soc Belg Med Trop*. 1952; **32**:569–584.
2. Oladele RO, Ayanlowo OO, Richardson MD, Denning DW. Histoplasmosis in Africa: An emerging or a neglected disease? *PLoS Negl Trop Dis* 2018 ; **12** (1) : e0006046.
3. Bauder B., Kuu bber-Heiss A., Steineck T., Kuttin E. S., Kaufman L.: Granulomatous skin lesions due to histoplasmosis in a badger (*Meles meles*) in Austria. *Medical Mycology* 2000; **38**, 249–253.
4. Ashbee H. R., Evans E. G., Viviani M. A. Dupont B., Chryssanthou E., *et al.* Histoplasmosis. Histoplasmosis in Europe: Report on an epidemiological survey from the European Confederation of Medical Mycology Working Group. *Medical Mycology* 2008; **46**: 57-65.
5. Lamey B., Parisien G. Forme disséminée d'histoplasmose à *Histoplasma capsulatum* chez une enfant zaïroise. *Médecine tropicale* 1982;**5** : 557-559.
6. Diego H. Caceres, Alejandra Zuluaga, Karen Arango-Bustamante, Catalina de Bedout, Ángela Maria Tobón, Ángela Restrepo, Beatriz L. Gómez, Luz Elena Cano, and Ángel González*. Implementation of a Training Course Increased the Diagnosis of Histoplasmosis in Colombia. *Am. J. Trop. Med. Hyg.*2015; **93** (3):662–667.doi:10.4269/ajtmh.15-0108
7. Pakasa MN, Nsiangana Z: Histoplasmosis due to Histoplasma Duboisii. Five new cases. Possible association with Schistosoma Mansoni. *J. Mycol. Med* 1991; **1:306**-309.
8. Pakasa N., Biber A., Nsiangana S., Imposo D., Sumaili E., Muhindo H., Buitrago M.J., Barshack I., Schwartz E. African Histoplasmosis in HIV-Negative Patients, Kimpese, Democratic Republic of the Congo. *Emerg. Infect. Dis* 2018; **24**:2068–2070. doi: 10.3201/eid2411.180236.
9. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Kauffman CA, *et al.* Infectious Diseases Society of America. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007; **45** (7):807-825.

10. Friedman D. Z. P., Schwartz I. S. Emerging fungal infections: new patients, new patterns, and new pathogens. *J. Fungi* 2019; **5** (3): E67. 10.3390/jof5030067.