L'histoplasmose chez le patient infecté par le VIH en Guyane française

Antoine Adenis

Thèse

En vue de l'obtention du grade de

Docteur de l'Université de Guyane

Discipline : *Biologie, Santé, Médecine*

Spécialité : *Recherche clinique, innovation, technologie, santé publique*

Présentée et soutenue publiquement le 21 novembre 2017 par :

ANTOINE ADENIS

L'histoplasmose chez le patient infecté par le VIH en Guyane française

---

JURY

Pierre COUPPIE	PU-PH	Examinateur
Olivier LORTHOLARY	PU-PH	Examinateur
Magalie DEMAR	PU-PH	Examinateur
Pierre TATTEVIN	PU-PH	Rapporteur
Marie-Elisabeth BOUGNOUX	MCU-PH	Rapporteur
Mathieu NACHER	PU-PH	Directeur de Thèse

Ecole Doctorale n°587 : *Diversités, Santé et développement en Amazonie*

RAPPEL DU TITRE

L'histoplasmose chez le patient infecté par le VIH en Guyane française.

TABLE DES MATIERES

RESUME

Les infections fongiques invasives sont responsables d'infections opportunistes majeures, portes d'entrée du stade de l'immunodéficience acquise (SIDA) au cours de l'infection par le virus de l'immunodéficience humaine(VIH). Parmi celles-ci, l'histoplasmose était décrite comme une des principales infections opportunistes et une des premières causes de décès au stade SIDA en Guyane française.

Sur la base d'une revue de la littérature plusieurs travaux scientifiques ont été menés. Le taux d'incidence de l'histoplasmose était estimé à 1,5 pour 100 personnes-années infectées par le VIH. Elle était classée première infection opportuniste au stade SIDA depuis la mise à disposition des trithérapies antirétrovirales en 1997. Les tendances temporelles récentes décrivaient un nombre de cas incidents stabilisé à un niveau élevé et une proportion de décès à un mois inférieure à 10%, divisée par quatre depuis la période 1992-1997. La description de l'influence importante des paramètres climatiques, accompagnée d'une saisonnalité franche, se traduisait par une surincidence significative des cas de coinfection histoplasmose et VIH en fin de saison sèche et début de petite saison des pluies. On pouvait en conclure que le principal processus menant à une maladie symptomatique en zone d'endémie était une nouvelle infection dans l'environnement. Fréquemment confondues au stade avancé de l'infection par le VIH, la comparaison des cas de tuberculose aux cas d'histoplasmose retrouvait des différences notables : un profil clinique respiratoire dans un contexte inflammatoire marqué pour la tuberculose et un profil disséminé accompagné de cytopénies et d'anomalies des tests hépatiques chez des patients plus immunodéprimés pour l'histoplasmose. Dans l'attente de la mise à disposition de méthodes diagnostiques rapides et spécifiques, l'évaluation d'un test commercial de détection sérique de l'antigène galactomananne d'*Aspergillus* sp. était pertinente pour le diagnostic de l'histoplasmose. Dans l'objectif de réduire encore la mortalité liée à l'histoplasmose, une redéfinition des critères de sévérité et le développement d'arguments pour une stratégie thérapeutique empirique à visée antifongique toujours plus agressive étaient proposés.

Les résultats préliminaires de nos projets démontraient la présence (au Suriname et au Guyana) et une incidence, à priori importante voire supérieure à la Guyane française, de l'histoplasmose chez les patients infectés par le VIH du Suriname. A l'échelle de l'Amérique Latine, la mortalité liée à l'histoplasmose était estimée équivalente voire supérieure à celle de la tuberculose chez les patients infectés par le VIH. L'histoplasmose chez les patients infectés par le VIH était jugée négligée, méconnue, responsable de nombreux décès évitables en Amérique Latine. Une initiative est en cours pour l'intégration de cette problématique dans les programmes nationaux et internationaux dans l'objectif de réduire la mortalité liée au stade SIDA de l'infection par le VIH.

ABSTRACT

Invasive fungal infections are responsible for major opportunistic infections among human immunodeficiency virus (HIV) infected patients entering the acquired immunodeficiency syndrome (AIDS) stage. Among them, histoplasmosis was known as one of the first AIDS-defining condition and AIDS-related deaths in French Guiana.

Based on a literature review, we developed several scientific programmes. Histoplasmosis incidence was estimated at 1.5 per 100 person-years HIV-infected. Since 1997 and the availability of the highly active antiretroviral therapy, HIV-associated histoplasmosis was the main AIDS-defining condition. According to recent temporal trends, the high number of incident cases was stable and the proportion of deaths within one month was below 10%, divided four fold since 1992-1997. The influence of climatic parameters was described, along with a clear seasonal pattern, as during the end of the long dry season and the beginning of the short wet season there was a significant increase in the incidence of HIV-associated histoplasmosis cases. This let us conclude that environmental new infections might be the main pathophysiological mechanism leading to symptomatic disease in endemic areas. At the advanced stage of HIV infection tuberculosis and histoplasmosis are often confused for one another. Their comparison found differences with a respiratory clinical picture in a context of pronounced inflammation for tuberculosis and a disseminated disease along with cytopenia and liver test abnormalities in individuals with greater immunosuppression for histoplasmosis. Awaiting rapid and specific diagnostic tools, our evaluation of a commercial test detecting *Aspergilus* sp. galactomannan antigen in serum was relevant for histoplasmosis diagnosis. In a continuous objective of reducing histoplasmosis-related deaths, reframed severity criteria and arguments for a more agressive antifungal therapy empiric strategy were proposed.

The preliminary results of our projects showed the presence of HIV-associated histoplasmosis in Suriname and Guyana and a probably greater incidence compared to French Guiana. In the whole Latin American region, we estimated that AIDS-related histoplasmosis deaths were similar or greater than AIDS-related tuberculosis deaths. HIV-associated histoplasmosis is in fact neglected, largely unknown and responsible for numerous avoidable deaths in Latin America. Following the objective of reducing AIDS-related deaths, an initiative is ongoing with the aim of making HIV-associated histoplasmosis part of national and international programmes.

REMERCIEMENTS

Je souhaite adresser mes sincères et respectueux remerciements aux membres du jury pour leur présence honorable dans l'objectif de juger mon travail.

Au Dr M-E. Bougnoux, je vous remercie d'être rapporteur de ce travail. En votre qualité de spécialiste des infections fongiques et de leur diagnostic, c'est un honneur de vous savoir juge de mon travail et de pouvoir bénéficier de votre éclairage critique.

Au Professeur P. Tattevin, c'est un honneur de vous compter parmi mes rapporteurs. Ayant souvent assisté à vos communications dans divers congrès, j'ai pu apprécier la qualité de vos revues critiques de la littérature sur les maladies infectieuses. J'espère humblement que mes travaux pourront satisfaire à la perspicacité entrevue.

Au Professeur O. Lortholary, je vous remercie d'apporter votre expertise à la revue critique de mes travaux. En votre qualité de spécialiste des infections fongiques et de responsable adjoint du centre national de référence des mycoses invasives et antifongiques, votre jugement et vos conseils seront précieux à l'évaluation de mes travaux et à la définition des orientations d'avenir.

Au Professeur P. Couppié, avec tout le respect que je vous porte, c'est toujours un plaisir de vous compter parmi mes juges. Depuis mes premiers pas sur cette thématique au lit du malade et jusqu'à ce jour, j'ai toujours pu compter sur la pertinence de vos avis critiques, votre disponibilité, votre bienveillante attention et votre participation active aux évolutions que nous donnons à ce sujet qui vous tient à cœur.

Au Professeur M. Demar, je suis très honoré de vous compter parmi les juges de mes travaux pour la première fois. Votre double qualité de clinicienne en maladies infectieuses et biologiste spécialiste en parasitologie et mycologie, votre connaissance fine du terrain guyanais, votre analyse critique du fond et de la forme, permettront de juger de la pertinence des travaux visant à faire le pont entre le lit du malade et la paillasse, pour plus d'efficacité et de qualité dans la prise en charge des patients.

Au Professeur M. Nacher, directeur de ce travail de thèse, je souhaiterais adresser mes plus sincères et chaleureux remerciements. Sans ta brillante et géniale ambition, ton talent en épidémiologie clinique, ton soutien bienveillant et ton humour, je ne pense pas que le limougeaud que je suis en serait arrivé là.

Je voudrais également remercier mes collègues de l'équipe EA 3593 écosystèmes amazoniens et pathologie tropicale (EPaT), du centre d'investigation clinique Antilles Guyane (Inserm CIC 1424), les interlocuteurs et investigateurs du centre hospitalier de Cayenne ainsi que l'ensemble des partenaires de nos projets, sans qui rien de tout ce qui est exposé dans ce manuscrit ne serait possible.

A mes amis de toujours qui se reconnaîtront, merci pour ce lien indéfectible et les moments partagés, qui sont une de mes principales sources d'inspiration et de motivation !

A ma famille, mes parents, mon frère et les siens, merci de toujours m'entourer dans ces aventures universitaires répétées, qui ne cessent jamais, même pendant les vacances en famille !

A Fany, Jules et Émilie, je vous embrasse comme je vous aime.

LISTE DES FIGURES

LISTE DES TABLEAUX

ABREVIATIONS

AFD : Agence Française de Développement

ANRS-Inserm : Agence Nationale de Recherche sur le Sida et les hépatites virales – Agence autonome de l'Institut National de la Santé et de la Recherche Médicale

ARS : Agence Régionale de Santé

ARV : Traitement antirétroviral

ASTMH : American Society of Tropical Medicine and Hygiene

CD4 : Lymphocyte T CD4+

CDC : Centers for Disease Control and prevention

CDC-MDB : Centers for Disease Control and prevention - Mycotic Diseases Branch

CIB : Corporación para Investigaciones Biológicas

CIC AG : Centre d'Investigation Clinique Antilles Guyane

CISIH : Centre d'Information et de Suivi de l'Immunodéficience Humaine

Cnam : Conservatoire national des arts et métiers

COREVIH : COordination RÉgionale de lutte contre le VIH

DFA : Département Français d'Amérique

ELISA : Enzyme-Linked Immunosorbent Assay

EORTC / MSG : Consensus group from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group

FEDER-FSE : Fonds Européen de DÉveloppement Régional - Fonds Social Européen

FHDH : French Hospital Database on HIV

HAART : Highly Active Antiretroviral Therapy

H. capsulatum : Histoplasma capsulatum

IC95% : Intervalle de confiance à 95%

IDR : IntraDermoRéaction

IGRAs : Interferon-Gamma Release Assays

INSEE : Institut Nationale de la Statistique et des Etudes Economiques

Inserm : Institut National de la Santé et de la Recherche Médicale

InVS : Institut de Veille Sanitaire

LACEN-AP : Laboratório Central de Saúde Pública do Amapá

LDH : Lacticodeshydrogénase

LFA : Lateral Flow Assay

OMS : Organisation Mondiale de la Santé

ONUSIDA : Programme de l'Organisation des Nations Unies pour la lutte contre le VIH

OPS-OMS : Organisation Panaméricaine de la Santé – Bureau régional de l'OMS

PEPFAR : President's Emergency Plan for AIDS Relief

PMSI : Programme de Médicalisation des Systèmes d'Information

PS-OMS : Échelle de Performance Status de l'OMS

RIGI : Registre d'issue de Grossesse Informatisé

RT-PCR : Real-Time Polymerase Chain Reaction

SAMU-SMUR : Service d'Aide Médicale Urgente - Service Mobile d'Urgence et de Réanimation

SIDA : Syndrome de l'ImmunoDéficience acquise

SRIS : Syndrome Inflammatoire de Reconstitution Immunitaire

STRonGER : Strengthening Transdisciplinary Research on Infectious and Emerging Diseases in French Guiana: linking fieldwork, benchside and bedside

TCC : Programme of Technical Cooperation among countries

TGO : Aspartate aminotransférase

TGP : Alanine aminotransférase

USA : United States of America

VIH : Virus de l'Immunodéficience Humaine

INTRODUCTION GENERALE

Considérant la population mondiale, les infections fongiques touchent environ un milliard d'individus, dont environ 11,5 millions d'infections potentiellement mortelles et plus de 1,5 million de décès chaque année (1).

Au cours des deux dernières décennies, il y a eu des progrès significatifs dans le développement des méthodes diagnostiques des infections fongiques et la mise à disposition de nouvelles molécules antifongiques. Toutefois, à l'échelle mondiale, la plupart des individus concernés par les infections fongiques ne bénéficient pas de ces progrès. Ainsi, dans les pays disposant d'un système de soins dit « développé », le plus souvent dans des pays à haut niveau de revenus, les infections fongiques sont diagnostiquées et traitées, même si elles restent parfois sous-diagnostiquées ou identifiées tardivement lors d'autopsies. Dans les autres pays du monde, à niveaux de revenus faible ou intermédiaire, le manque de formation des personnels de santé, l'absence d'outils diagnostics d'infections fongiques et l'indisponibilité de molécules antifongiques maintiennent une morbidité et une mortalité associées aux infections fongiques à des niveaux élevés et inacceptables (1).

En dépit de ces progrès récents, les experts du monde de la mycologie médicale s'accordent pour qualifier la mycologie médicale et les infections fongiques humaines comme « négligées » socialement et politiquement (1). Il y a cinquante ans on parlait déjà de l'iceberg de la mycologie médicale (2). En effet, si les autorités sanitaires sous-estiment l'impact en termes de morbidité et de mortalité de la plupart des infections fongiques, les acteurs institutionnels ou privés de la recherche biomédicale y répondent par un manque d'intérêt et de faibles investissements ou financements proposés dans les appels à projets.

En mycologie médicale, il convient de distinguer : les mycoses cosmopolites (rencontrées sur tous les continents ou toutes les latitudes) des mycoses dites tropicales ou endémiques (spécifiques à certaines zones bioclimatiques) ; les mycoses externes (cutanées et sous-cutanées) des mycoses profondes ou systémiques ou disséminées (le plus souvent dans des contextes particuliers, notamment d'immunosuppression des individus). Les mycoses cutanées ou cutanéomuqueuses, groupe le plus commun d'infections fongiques par leurs fréquences de survenue, n'engagent généralement pas le pronostic vital des patients mais peuvent être à l'origine de séquelles visibles et socialement gênantes. La plupart des infections fongiques profondes, disséminées ou invasives, sont

sévères et requièrent un haut niveau de compétences médicales (clinique, diagnostique et thérapeutique). En effet, les infections fongiques invasives peuvent affecter l'ensemble des tissus ou organes et sont souvent difficiles à diagnostiquer, voire confondues avec d'autres maladies, plus communes ou plus fréquemment observées dans certaines zones bioclimatiques. Elles sont probablement responsables d'un plus grand nombre de décès au cours de l'infection par le Virus de l'immunodéficience humaine (VIH) que la tuberculose et les infections bactériennes réunies (1).

Ainsi, en préambule à la présentation des travaux et projets de recherche, nous décrirons quelques généralités sur les infections fongiques invasives chez le patient infecté par le VIH puis, plus particulièrement sur l'histoplasmose, avant d'envisager en détails le contexte et le plan de travail.

1. Généralités et état des connaissances à propos des infections fongiques invasives chez le patient infecté par le VIH

Les infections fongiques invasives sont responsables d'infections opportunistes majeures au cours de l'infection par le VIH, comme autant de portes d'entrée pour le Syndrome d'Immunodéficience Acquise (SIDA), stade évolutif tardif et terminal de l'infection par le VIH. Pourvoyeuses d'une morbidité et d'une mortalité importantes, elles constituent un enjeu de santé publique majeur. Toutefois, le niveau de connaissance et les moyens de la lutte contre ces maladies ne sont pas à la hauteur des enjeux, notamment dans le cas de la coinfection histoplasmose et VIH, objet de ce travail de thèse.

L'article de revue de la littérature ci-dessous décrit l'épidémiologie et les progrès récents de la prise en charge (diagnostique et thérapeutique) des quatre principales infections fongiques invasives chez le patient infecté par le VIH : pneumocystose, cryptococcose, talaromycose (anciennement pénicilliose) et histoplasmose.

ARTICLE 1 : Fungal infections in HIV/AIDS.

Author: Limper, Andrew H., **Adenis, Antoine**, Le, Thuy and Harrison, Thomas S.

Year: 2017 **Journal:** The Lancet Infectious Diseases

ISSN: 1473-3099 **DOI:** 10.1016/S1473-3099(17)30303-1

Abstract: Fungi are major contributors to the opportunistic infections that affect patients with HIV/AIDS. Systemic infections are mainly with Pneumocystis jirovecii (pneumocystosis), Cryptococcus neoformans (cryptococcosis), Histoplasma capsulatum (histoplasmosis), and Talaromyces (Penicillium) marneffei (talaromycosis). The incidence of systemic fungal infections has decreased in people with HIV in high-income countries because of the widespread availability of antiretroviral drugs and early testing for HIV. However, in many areas with high HIV prevalence, patients present to care with advanced HIV infection and with a low CD4 cell count or re-present with persistent low CD4 cell counts because of poor adherence, resistance to antiretroviral drugs, or both. Affordable, rapid point-of-care diagnostic tests (as have been developed for cryptococcosis) are urgently needed for pneumocystosis, talaromycosis, and histoplasmosis. Additionally, antifungal drugs, including amphotericin B, liposomal amphotericin B, and flucytosine, need to be much more widely available. Such measures, together with continued international efforts in education and training in the management of fungal disease, have the potential to improve patient outcomes substantially.

URL: http://dx.doi.org/10.1016/S1473-3099(17)30303-1

Fungal infections 1

Fungal infections in HIV/AIDS

Andrew H Limper, Antoine Adenis, Thuy Le, Thomas S Harrison

Fungi are major contributors to the opportunistic infections that affect patients with HIV/AIDS. Systemic infections are mainly with *Pneumocystis jirovecii* (pneumocystosis), *Cryptococcus neoformans* (cryptococcosis), *Histoplasma capsulatum* (histoplasmosis), and *Talaromyces* (*Penicillium*) *marneffei* (talaromycosis). The incidence of systemic fungal infections has decreased in people with HIV in high-income countries because of the widespread availability of antiretroviral drugs and early testing for HIV. However, in many areas with high HIV prevalence, patients present to care with advanced HIV infection and with a low CD4 cell count or re-present with persistent low CD4 cell counts because of poor adherence, resistance to antiretroviral drugs, or both. Affordable, rapid point-of-care diagnostic tests (as have been developed for cryptococcosis) are urgently needed for pneumocystosis, talaromycosis, and histoplasmosis. Additionally, antifungal drugs, including amphotericin B, liposomal amphotericin B, and flucytosine, need to be much more widely available. Such measures, together with continued international efforts in education and training in the management of fungal disease, have the potential to improve patient outcomes substantially.

Lancet Infect Dis 2017

Published Online July 31, 2017 http://dx.doi.org/10.1016/S1473-3099(17)30303-1

See Online/Series http://dx.doi.org/10.1016/S1473-3099(17)30304-3, http://dx.doi.org/10.1016/S1473-3099(17)30309-2, http://dx.doi.org/10.1016/S1473-3099(17)30306-7, http://dx.doi.org/10.1016/S1473-3099(17)30316-X, http://dx.doi.org/10.1016/S1473-3099(17)30442-5, http://dx.doi.org/10.1016/S1473-3099(17)30443-7, and http://dx.doi.org/10.1016 S1473-3099(17)30308-0

See Online/Comment http://dx.doi.org/10.1016/S1473-3099(17)30319-5

This is the first in a Series of eight papers about fungal infections

Mayo Clinic College of Medicine, Rochester, MN, USA (A H Limper MD); **Inserm CIC 1424, Centre d'Investigation Clinique Antilles Guyane, Centre Hospitalier de**

Introduction

Fungi contribute greatly to opportunistic infections in patients with late-stage HIV infection. *Pneumocystis jirovecii* is the most common cause of respiratory infection and *Cryptococcus neoformans* the most common cause of CNS infection in patients with AIDS across large parts of the world. *Histoplasma capsulatum* (especially common in parts of the Americas) and *Talaromyces* (formerly *Penicillium*) *marneffei* (endemic in south and southeast Asia) are thermally dimorphic fungi that cause disseminated infections.

In this Series paper, we review the epidemiology and progress in diagnosis and therapy for these four major systemic fungal pathogens in patients with HIV/AIDS. We cite the most relevant recent papers, but additional supplementary references are available online, organised by section (appendix).

Although we focus on these major infections, other fungi are also important in patients with HIV/AIDS. *Coccidioides* spp especially affect patients with AIDS in the Americas and *Emmonsia* sp in South Africa.[1,2] *Candida* spp commonly cause mucosal, oral, vaginal, and oesophageal infections in patients with stage 3 and 4 HIV disease, and fungal skin and nail infections are major causes of morbidity in HIV-infected individuals. However, mucosal candida infections usually readily respond to azole antifungal treatment and immune reconstitution with antiretroviral therapy (ART). In the era of ART, recurrent azole-resistant *Candida* spp infections are rare.

With widespread availability of ART and earlier testing and treatment for HIV, the incidence of systemic fungal infections has decreased in people living with HIV in high-income countries, although room for improvement remains.[3] By contrast, in many regions with high HIV prevalence, particularly sub-Saharan Africa, there is little evidence for a substantial decrease in cases.[4] Many patients present with advanced HIV and with a low CD4 cell count.[5] Additionally, enrolment data from cryptococcal meningitis trials show that, although the total number of cases was stable over time, half or more of patients with cryptococcal meningitis had taken ART[6] but had persistent low CD4 cell counts due to problems of retention in care and or ART resistance. Thus, further efforts to address the problem of fungal infections through rapid point-of-care diagnostics for these major fungal pathogens and global access to antifungal drugs are needed as an integral part of an effective response to the HIV pandemic.

Pneumocystis pneumonia

Epidemiology

Pneumocystis pneumonia has emerged as a major cause of infection in those with HIV/AIDS, and is estimated to

Key messages

- Incidence of systemic fungal infections in patients with HIV/AIDS has decreased in many resource-rich areas after the introduction of antiretroviral therapy and earlier diagnosis and treatment of infection
- In many resource-limited settings incidence is not yet decreasing due to continued late diagnosis and challenges with retention in HIV care
- New PCR-based assays can distinguish colonisation from infection with pneumocystis
- Measurement of cerebrospinal fluid pressure is essential in cryptococcal meningitis, and management of raised cerebrospinal fluid pressure through careful therapeutic lumbar punctures reduces mortality
- In large parts of the world, HIV-related histoplasmosis is often neglected, undiagnosed, or misdiagnosed as tuberculosis, because of poor access to current diagnostics
- The intersection with HIV has transformed *Talaromyces marneffei* from a rare human pathogen to a major cause of HIV-associated death in southeast Asia; amphotericin B was shown to be superior to itraconazole as initial treatment in a large randomised trial
- Novel, affordable, point-of-care diagnostics for pneumocystis, histoplasmosis, and talaromycosis, and wider access to effective antifungals are urgently needed to reduce the burden of HIV-associated fungal infections in resource-limited settings

Cayenne, Cayenne, France (A Adenis MD); **Equipe EA 3593, Ecosystèmes Amazoniens et Pathologie Tropicale, Université de Guyane, Cayenne, France** (A Adenis); **Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam** (T Le MD); **Hawaii Centre for AIDS, University of Hawaii at Manoa, Honolulu, HI, USA** (T Le); **and Institute of Infection and Immunity, St George's, University of London, London, UK** (Prof T S Harrison FRCP)

Correspondence to: Prof Thomas S Harrison, Institute of Infection and Immunity, St George's, University of London, London SW17 0RE, UK
tharriso@sgul.ac.uk

See Online for appendix

cause more than 400 000 cases worldwide every year.[7] Many of these patients are undiagnosed or diagnosed late, particularly in resource-limited settings. The mortality of pneumocystis pneumonia ranges from 10% to 30% or higher, depending on the patient population, comorbidities, and whether the diagnosis is made early.[8,9] Although the incidence has been reduced by implementation of ART, pneumocystis pneumonia continues to be a problem in patients who are unaware that they are infected with HIV and in those with ART failure or who stop taking ART.[10]

Pathogenesis

Pneumocystis spp are members of the Ascomycetous fungi. Each mammalian species can harbour at least one unique species of the *Pneumocystis* genus[11]—eg, *P jirovecii* infecting human beings and *Pneumocystis carinii* infecting rats. Serological and epidemiological data indicate that most people are exposed and transiently infected with *P jirovecii* early in life.[12] With healthy immune responses, this early infection is effectively cleared. However, during periods of immune suppression such as in patients with HIV who have CD4 counts lower than 200 cells per µL, the organism proliferates, leading to life-threatening pneumonia. CD4 immunity is essential for long-term control and memory responses to this fungus; contributing immunity is provided by innate immune responses, CD8 cells, and B-lymphocytes. In the absence of effective CD4-based immunity, innate inflammatory responses promote the accumulation of inflammatory cells, including neutrophils and CD8 lymphocytes, which strongly contribute to lung injury. Appreciation of this innate immune response has led to the use of adjunctive anti-inflammatory corticosteroids in moderate-to-severe pneumocystis pneumonia.[13] Evidence suggests that infection can be acquired from other infected individuals. Therefore, whenever feasible, immunosuppressed patients should not be directly exposed to individuals with active pneumocystis pneumonia.[14,15]

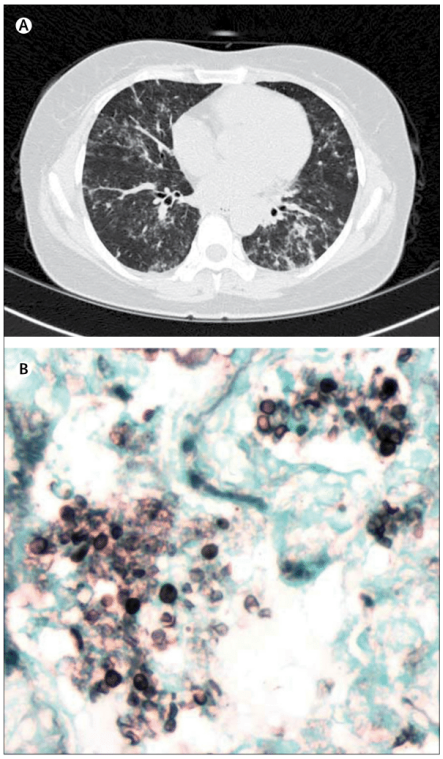


Figure 1: **Features of *Pneumocystis jirovecii* pneumonia in patients with HIV**
CT image of a 41 year old woman presenting with cough and dyspnoea (A). Imaging shows mixed alveolar and interstitial infiltrates, which were diagnosed as being due to *Pneumocystis jirovecii* pneumonia on bronchoalveolar lavage. Methenamine silver staining demonstrates typical pneumocystis organisms clustered in alveolar exudates (B).

Diagnosis

Most patients with pneumocystis pneumonia present with cough and progressive dyspnoea (initially on exertion) and pulmonary infiltrates on chest radiograph or lung imaging (figure 1A). Definitive diagnosis relies on identification of *P jirovecii* organisms in respiratory secretions or bronchoalveolar lavage samples (figure 1B). Experienced observers can identify the organisms using Wright-Giemsa stained smears. However, tinctorial methods including methenamine silver, Papanicolau, cresyl echt violet, and Calcofluor white staining assists in rapid identification of organisms.[16] Fluorescence staining with monoclonal antibodies further increases sensitivity to approximately 95% when applied to bronchoalveolar lavage samples.[9]

Over the past decade, many laboratories have switched to identification using PCR. Nested PCR has been useful to identify colonisation as well as clinical infection, whereas, single-copy real-time PCR assays have been devised to rapidly identify patients with clinical infection, rather than colonisation.[17,18] In expensive point-of-care diagnostic strategies, which are technically less demanding, are needed for regions with limited resources.[7] Work is ongoing to develop relevant pneumocystis antigen-detection systems and thermocycler independent amplification strategies that can be used in such settings.

β-D-glucan assays in the serum can be useful as a screening tool and adjunct to diagnosis because they have good sensitivity in patients with HIV and pneumocystis pneumonia. Although β-D-glucan testing does cross react with the glucans released from other fungi, high levels of β-D-glucan (>100 pg/mL) in patients with HIV in the appropriate clinical setting does provide evidence to support starting therapy.[19]

Vast regions of the world including sub-Saharan Africa, Asia, and South America do not have ready access to laboratory facilities equipped for the diagnosis of pneumocystis pneumonia, rendering the true burden and impact of this infection under-recognised. For instance, when modern diagnostic methods were applied, unsuspected pneumocystis infection was found to be the cause of up to 7% of all severe pneumonia in children younger than 5 years in a hospital in southern Mozambique.[20] In regions with few laboratory facilities, the diagnosis of pneumocystis is often made on clinical grounds. Although such an approach is pragmatic, it lacks specificity, which is necessary to rapidly focus the appropriate antibiotic therapy towards pneumocystis when it is present.

Treatment

The mainstay of therapy is intravenous co-trimoxazole (sulfamethoxazole-trimethoprim) with the trimethoprim dosed at 15–20 mg/kg per day and sulfamethoxazole at 75–100 mg/kg per day. This is given in four equally divided doses for 21 days. For patients with milder disease, and once the disease is under control, therapy can be safely switched from intravenous to oral administration. It is useful to monitor therapeutic trough levels to avoid toxicity and ensure benefit, although this is infrequently done.[13] Often, clinical improvement is not noted for up to 1 week. Patients who cannot tolerate co-trimoxazole are usually treated with the combination of primaquine and clindamycin or pentamidine. Atovaquone is generally reserved for those with milder disease.[13] Efavirenz significantly reduces atovaquone concentrations. Only co-trimoxazole is present on the WHO Model List of Essential Medicines for pneumocystis pneumonia, with the alternative drugs appearing on the complementary list and for other indications.

Adjunctive corticosteroid therapy is given to patients with moderate to severe pneumocystis pneumonia, as defined by a room air PaO_2 lower than 70 mm Hg or alveolar–arterial oxygen gradient higher than 35 mm Hg. For these individuals, prednisone is provided at 40 mg twice a day on days 1–5, 40 mg once a day on days 6–10, and then 20 mg once a day up to day 21.[21] In children younger than 13 years, prednisone can be given at 1 mg/kg twice a day on days 1–5, 0·5 mg/kg twice a day on days 6–10, and finally 0·5 mg/kg once a day on days 11–21.[22]

Prevention

Appropriate pneumocystis prophylaxis is essential to prevent the often-lethal infection. Patients who have CD4 counts lower than 200 cells per µL or a history of oropharyngeal candidiasis should receive prophylaxis with one double-strength co-trimoxazole tablet three times a week or one single-strength co-trimoxazole tablet once a day.[23] Co-trimoxazole in low-income and middle-income countries has additional benefits by reducing early mortality from malaria, reducing anaemia, and improving growth in children.[24] Alternatives for patients who cannot tolerate co-trimoxazole include dapsone, atovaquone, or dapsone with pyrimethamine and leucovorin.[13] Dapsone should be use cautiously in individuals with glucose-6-phosphate dehydrogenase deficiency.[13] Generally, prophylaxis can be discontinued when CD4 counts are consistently higher than 200 cells per µL for longer than 3 months. An effective pneumocystis vaccine is not yet available, but pre-clinical primate studies support the potential efficacy of such an approach for patients with HIV.[25]

Cryptococcosis

Epidemiology

Latest estimates suggest that HIV-associated cryptococcal meningitis accounts for 150 000–200 000 deaths per year. These deaths occur mostly in sub-Saharan Africa where the associated mortality remains at around 70% at 3 months. Most HIV-associated infections are caused by *C neoformans*, although in Botswana, up to 30% of infections are with *Cryptococcus gattii*. Patients with cryptococcal meningitis present with headache and fever, with a median duration of 2 weeks between symptom onset and first presentation.[26] Many patients develop nausea, vomiting, diplopia due to cranial nerve VI palsies, and reduced visual acuity related to raised cerebrospinal fluid pressure. If untreated, symptoms progress to abnormal mental status, reduced conscious level, seizures, and finally coma.

Diagnosis

Traditionally, diagnosis has relied on lumbar puncture. A cerebrospinal fluid India ink preparation is positive in 70–90% of patients with HIV-associated infections,[26] and the remainder of patients can be reliably diagnosed by cryptococcal antigen (CrAg) detection or culture. However, headache is non-specific and lumbar puncture is frequently delayed, especially in resource-limited settings, until such time as the prognosis is poor. Development of a point-of-care lateral flow test for detection of CrAg is a major advance.[27] Antigen is present in blood (serum, plasma, or whole-blood finger-prick sample) before the development of symptoms and the test is highly specific and more sensitive than previous latex agglutination assays. The test enables earlier diagnosis, even in primary care settings, and screening of patients in hospitals in high-prevalence areas. It also makes feasible screening and pre-emptive therapy as a strategy to prevent the development of meningitis after HIV diagnosis and before starting ART in those with low CD4 cell counts.[28]

Antifungal therapy

The gold-standard antifungal therapy for cryptococcal meningitis is the combination of amphotericin B deoxycholate (D-AmB; 0·7–1·0 mg/kg per day) and

For the **WHO Model Lists of Essential Medicines** see http://www.who.int/medicines/publications/essentialmedicines/en

flucytosine (100 mg/kg per day in four divided doses) for the initial 2 weeks, followed by fluconazole (400–800 mg per day for 8 weeks, and 200 mg per day thereafter) for a minimum of 1 year and until immune reconstitution.[29–31] Addition of flucytosine was associated with a 40% reduction in mortality compared with D-AmB alone.[32] Pre-hydration with normal saline and pre-emptive replacement of potassium and magnesium is recommended to mitigate the toxicities of D-AmB,[30] but anaemia remains an important problem where transfusion capacity is limited. Liposomal amphotericin B (L-AmB) at 3–6 mg/kg per day is as effective as and better tolerated than D-AmB.[33] Studies are ongoing to assess whether L-AmB could be used in intermittent high doses, as for leishmaniasis, to provide a convenient and cost-effective induction treatment. With care taken to adjust doses in case of renal impairment, flucytosine is generally well tolerated in this patient population for 2 weeks.[34]

Complications

Raised cerebrospinal fluid pressure caused by a blockage of cerebrospinal fluid reabsorption at the level of the arachnoid granulations is common in cryptococcal meningitis, with about a quarter of patients having a pressure greater than 35 cm H_2O.[35] If untreated, high pressure is associated with increased mortality,[35] but increasing evidence points to the effectiveness of careful therapeutic lumbar punctures.[26,36,37] Only the most severely ill patients might require a temporary lumbar drain or ventricular shunt.[38,39] High cerebrospinal fluid pressure can develop in the second and third weeks of treatment, despite effective sterilisation of the cerebrospinal fluid, meaning that a lumbar puncture should be repeated if symptoms persist or recur.

Recommendations are to start ART between 4 weeks and 6 weeks after starting antifungal therapy. This schedule is based on the findings of studies suggesting 3 days or 8 days is too soon[40,41] and 6 weeks or later is probably unnecessarily late. Given that in many centres, half of cryptococcal meningitis cases now occur in ART-experienced patients, it is prudent to only switch to second-line ART in those thought to have ART resistance or re-start ART in those who have discontinued taking ART, after 4 weeks of antifungal therapy. Cryptococcal meningitis in patients with ART failure needs to be distinguished from patients with unmasking cryptococcal immune reconstitution inflammatory syndrome (IRIS)[42] (in which presentation is precipitated by starting ART) and in whom ART should be continued. Frequent clinical review is needed given some overlapping toxic effects of antifungal and antiretroviral drugs.

Paradoxical cryptococcal IRIS occurs in 15–20% of patients who are treated for cryptococcal meningitis. These patients respond to treatment but later have a recurrence of symptoms, at a median of around 1 month after starting ART.[43] In patients re-presenting with a recurrence of symptoms, cerebrospinal fluid pressure should be measured and managed, because raised pressure is common in cryptococcal IRIS. Re-introduction of induction antifungal therapy can be considered while cerebrospinal fluid culture results are pending and while alternative diagnoses are actively pursued. If cryptococcal IRIS remains the likely diagnosis, and the patient is deteriorating, then short courses of corticosteroids have been used successfully. By contrast, corticosteroids given with initial antifungal therapy are harmful.[6] Although cryptococcal IRIS can be life-threatening, related mortality should be lower than recorded in earlier series because of increased awareness and more prudent timing of ART.

Prevention

The lateral flow CrAg test has made feasible a screen and pre-emptive fluconazole treatment strategy to prevent the development of meningitis in patients with low CD4 cell counts. 3–8% of individuals with a CD4 count lower than 100 cells per µL usually test positive on CrAg screening of blood samples.[44] In a retrospective study from Cape Town, patients testing positive for CrAg had a high (28%) chance, without treatment, of developing meningitis in the first year of ART, whereas, of those who tested negative and were started promptly on ART, none went on to develop meningitis.[28] Subsequent modelling suggested such a strategy could be highly cost-effective,[44,45] and screening has been endorsed in WHO guidelines[30] and introduced in South Africa and elsewhere. Prospective studies are underway, with one report demonstrating that such screening and pre-emptive fluconazole, combined with ART adherence support, led to a 28% reduction in mortality in patients with late-stage HIV in the first year of ART.[46] However, further work is needed to optimise the treatment of CrAg-positive patients. Despite the success of fluconazole therapy, these patients still have higher mortality compared with those who are CrAg negative,[46,47] and of some concern there have been reports of decreased susceptibility to fluconazole in some areas.[48] Some patients, even though asymptomatic or minimally symptomatic, have evidence of meningitis if they agree to a lumbar puncture, and this risk is related to blood antigen titre.[47] Studies are planned to ascertain if those with a high antigen titre in blood would benefit from more aggressive antifungal therapy.

Histoplasmosis

Epidemiology

Histoplasmosis is caused by *Histoplasma capsulatum*, a thermally dimorphic ascomycete.[49] The mould form is distributed worldwide in moist and enriched soils containing bird droppings or bat guano.[50] Autochthonous cases of HIV-associated histoplasmosis have been described on five continents, the Americas accounting for most cases, notably the central eastern USA and Latin America.[51] However, HIV-associated histoplasmosis is more widespread than was previously thought and is probably neglected, undiagnosed, or misdiagnosed as

tuberculosis.[52] In endemic areas, histoplasmosis occurs in 2–25% of patients with HIV/AIDS and represents the first AIDS-defining infection in up to 50–75% of patients with HIV.[53] Mortality rates range between 10% and 60%, depending on whether the diagnosis is made by experienced physicians with adequate infrastructure and access to antifungals other than fluconazole.[54,55]

Pathogenesis

Soil disruption aerosolises microconidia or mycelial fragments, which are inhaled and, at body temperature, convert into yeasts in the lungs.[50] Infection might also develop when, years after the primary infection, quiescent organisms are reactivated during immunosuppression.[56] Once in the lungs, *H capsulatum* survives phagocytosis in macrophages, facilitating its dissemination throughout the mononuclear phagocyte system. An early robust proinflammatory response (Th1/Th17) is required to control *H capsulatum* growth.[57] Hence, people with HIV are at higher risk of disseminated histoplasmosis, an AIDS-defining disease, which is lethal if left untreated.[50]

Environmental exposures to bird droppings or bat guano and history of exposure to chicken coops are associated with an increased risk of histoplasmosis.[58] Host factors such as low CD4 count lower than 200 cells per µL, nadir CD4 count lower than 50 cells per µL, CD8 count lower than 50 cells per µL, absence of ART or systemic antifungal therapy, the first 6 months of ART, history of herpes simplex virus infection, and male sex are independently associated with histoplasmosis in people with HIV infection.[59]

Clinical features and diagnosis

In AIDS, histoplasmosis usually presents as a disseminated disease (>95% of patients). All organs and tissues can be involved. Fever, fatigue, and weight loss are almost universal. Cough and dyspnoea are the most frequent localising symptoms, in association with diffuse radiological infiltrates, usually with a miliary reticulonodular pattern (figure 2A).[53] Abdominal pain and diarrhoea are frequent and reflective of colonic ulcerations (figure 2C). Lymph node enlargement, hepatosplenomegaly, and mucocutaneous manifestations are diagnostic clues.[53] Lactate dehydrogenase, liver enzymes (a higher concentration of aspartate aminotransferase than alanine aminotransferase), and ferritin elevation, with or without pancytopenia and haemophagocytosis syndrome, should lead to further investigations.[51] Usually subacute (1–2 months), the disease evolution varies from latency to 10–20% fulminant severe forms (septic shock

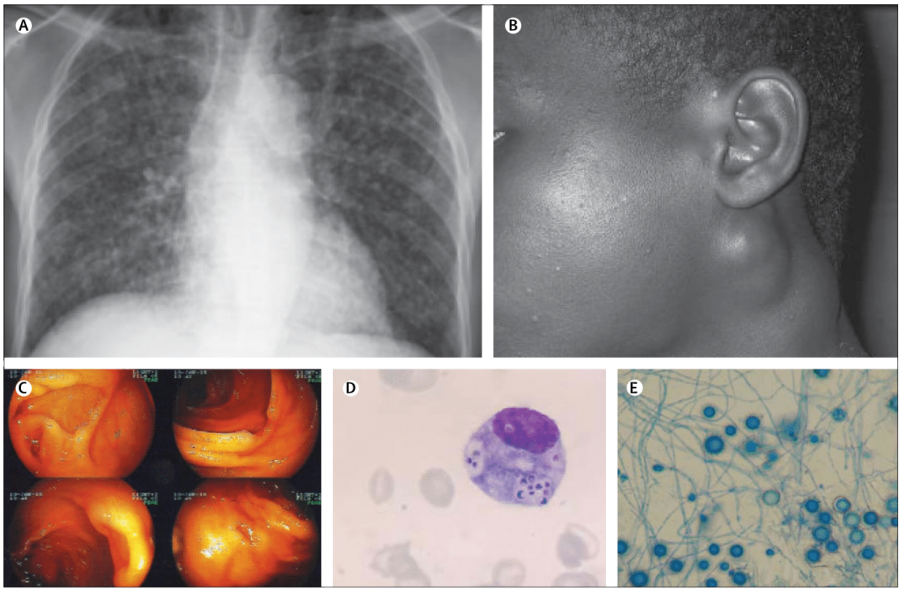


Figure 2: **Features of histoplasmosis in patients with HIV**
Chest radiograph showing a diffuse interstitial reticulonodular pattern in a patient with histoplasmosis (A). Photograph of a lymph node enlargement in histoplasmosis (B). Photographs of colonic ulcerations in a patient with histoplasmosis (C). Giemsa-stained bone marrow smear showing the yeast phase of *Histoplasma capsulatum* (D). Lactophenol blue-stained bone marrow culture showing the mould form of *H capsulatum* (E). Used with permission of P Couppié (A, B,C) and C Aznar (D, E) from Cayenne General Hospital, France.

with multiorgan failure), mainly in late presenters, with fatality rates reaching 50–70%.[51,53]

Direct examination with special staining (May-Grünwald Giemsa, periodic acid–Schiff, and Grocott-Gömöri's methenamine-silver) and culture of all tissues or body fluids at room temperature are the gold standard methods for diagnosis.[60] Bone marrow aspiration, blood culture, and tissue biopsies are all useful.[51] Direct examination is rapid, but culture, which requires a biosafety level 3 laboratory, takes a median of 2 weeks, and maximum of 6 weeks. For both methods, sensitivity varies with sample type, disease severity, and operator experience.[51] Antibody detection in cerebrospinal fluid is of most interest for the diagnosis of neuromeningeal forms.[61] Although useful molecular tools are being developed, their place in care and treatment is still evolving.[51] Detection of *H capsulatum* antigen is among the most sensitive and rapid means to diagnose disseminated histoplasmosis in AIDS.[62] The non-invasive reference method in the USA has been a polyclonal quantitative *Histoplasma* spp antigen radio-immunoassay in urine, blood, and bronchoalveolar lavage, but it is unavailable in other endemic areas.[51] However, a new commercially available monoclonal ELISA detecting galactomannan antigen in urine is being made widely available; with promising results.[62,63] It is noteworthy that the highest yield is achieved through the combination of several diagnostic methods.

Therapy

In moderately severe to severe cases, intravenous L-AmB (3–4 mg/kg per day) is recommended for 2 weeks or until clinical improvement.[64] An alternative lipid formulation of amphotericin B at the same dosage might be preferred over D-AmB (0·7 mg/kg per day) if L-AmB is unavailable. Continued treatment with oral itraconazole (200 mg three times a day for 3 days, followed by 200 mg twice a day) is given for at least 1 year.[64] Patients with non-severe cases can be treated with itraconazole alone.[64] Prevention is based on recommendations for workers at risk, and long-term suppressive therapy with itraconazole 200 mg a day as primary (only in the USA) or secondary prophylaxis.[65] ART should be started promptly, within the month after antifungal therapy initiation.[64] Nevirapine and efavirenz are moderate inducers of the CYP3A4 enzyme, and reduce the concentration of itraconazole.[66] However, both itraconazole and its major metabolite hydroxyitraconazole are equally active antifungal drugs, and it is unclear whether itraconazole dose adjustment is needed. In view of these interactions, a randomly obtained serum level of at least 1·0 µg/mL of itraconazole is recommended after 2 weeks of therapy.[64,67]

Morbidity and mortality from histoplasmosis have increased, largely attributable to the spread of HIV.[68] Although thousands are dying of a treatable disease, histoplasmosis is off the radar of international organisations involved in the fight against HIV/AIDS and tuberculosis.[69] Hence, the global burden of histoplasmosis remains unknown and access to rapid and simple diagnostics and effective antifungals remains a challenge in low-income and middle-income countries.[69,70]

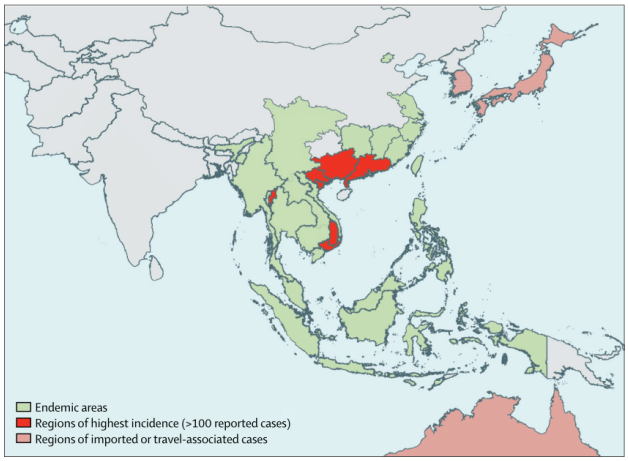


Figure 3: **Geographical distribution of *Talaromyces marneffei* infection**

Talaromycosis (formerly penicilliosis)

Epidemiology

T marneffei causes a life-threatening mycosis affecting primarily immunocompromised residents and travellers in southeast Asia, southern China, and northeastern India (figure 3).[71,72] The intersection with HIV has transformed *T marneffei* from a rare human pathogen to a major cause of HIV-associated death, second only to tuberculosis and cryptococcosis or pneumocystis pneumonia in Thailand and Hong Kong.[73,74] In Vietnam, talaromycosis makes up 4–11% of AIDS-related admissions[75,76] and is the second most common cause of bloodstream infections after cryptococcosis.[77] ART has led to a decline in incidence, but talaromycosis remains a major problem in people with undiagnosed and untreated HIV infection. Increasingly talaromycosis occurs outside endemic regions because of increased migration and international travel[78] and a rise in use of chemotherapy and immunosuppression.[79]

Ecology and transmission

Pathogen reservoirs and disease acquisition are being elucidated. The bamboo rat is the only non-human host of *T marneffei* with a high frequency of asymptomatic infection.[80,81] Isolates from bamboo rat and human beings have similar or identical genotypes.[80,81] However, there is no evidence of direct bamboo-rat-to-human transmission; instead, occupational exposure to plants and animals has been associated with human

infection.[82] This association is recently confirmed in a large case-control study from Vietnam, which additionally revealed that residents from or travellers to highland areas are at increased risk.[83] Incidence increases 30–50% during the rainy months,[76,84] and can be predicted by humidity levels.[85,86] *T marneffei* has been isolated from bamboo rat faeces and soil samples within bamboo rat burrows,[87,88] and *T marneffei* DNA detected in elephant-associated soil.[89] Collectively, these epiecological data suggest an interplay among multiple environmental reservoirs involving soil, plants, and animals, in which bamboo rats may be exploited by *T marneffei* to expand its biomass and biogeography. Infections in people probably occur through inhalation of *T marneffei* conidia. The incubation period is estimated to be 1–3 weeks in acute disease, whereas reactivation disease can occur many years after exposure in immunocompromised hosts.

Clinical features and outcomes

Most infections with talaromycosis occur in patients with CD4 counts lower than 100 cells per µL. Patients typically develop disseminated disease with fever, weight loss, hepatosplenomegaly, lymphadenopathy, and respiratory and gastrointestinal abnormalities. Papulonecrotic skin lesions are present in 60–70% of patients (figure 4A). Common laboratory findings include anaemia, thrombocytopenia, and elevated transaminases.[71,75,76] Concomitant opportunistic infections are common, particularly tuberculosis and salmonella infections.[75,76]

Case fatality rates in treated patients vary, from 10% in Thailand and Hong Kong[71,90] to 33% in China and Vietnam,[76,85,91] reflecting differences in time to diagnosis and access to ART. Some clinical and laboratory predictors of mortality include older age, shorter duration of symptoms, dyspnoea, absence of fever or skin lesions, thrombocytopenia, and increased lactate dehydrogenase concentrations.[75,76]

T marneffei-associated IRIS has been reported; commonly occurring as unmasking IRIS in patients starting ART. Skin lesions can be atypical, including erythematous nodules, verrucous lesions, or erythematous plaques.[92,93] Prospective studies that define incidence, risk, and impact of talaromycosis IRIS are needed. Continuation of ART, antifungal therapy, and cautious use of non-steroidal anti-inflammatory medications are the main therapeutic approaches.

Diagnosis

Presumptive diagnosis can be made based on microscopic findings of intramacrophage and extramacrophage yeast organisms in smears of skin lesions, lymph node, or bone marrow aspirate (figure 4B). Definitive diagnosis is made by pathogen isolation from clinical specimens demonstrating thermal dimorphism (figure 4C). Culture can be slow (3–14 days), resulting in diagnostic delay and raised mortality, particularly in patients without skin lesions.[73,76] PCR-based assays have been developed for rapid diagnosis;[94,95] however, the sensitivities are insufficient (range 60–70%) to be considered widely applicable. Recently developed ELISA to detect *T marneffei* antigen appears to be more sensitive than blood culture and other diagnostic methods,[96,97] and should be further evaluated for clinical application. Point-of-care tests are urgently needed.

Antifungal therapy and prevention

D-AmB and itraconazole are effective drugs in talaromycosis, whereas *T marneffei* is resistant to fluconazole.[75,76,90,91] The IVAP multicentre trial comparing D-AmB and itraconazole induction therapy reported mortality after 6 months of 11·3% and 21·0%, respectively (absolute risk difference 9·7%, 95% CI 2·8–16·6; p=0·006).[98] L-AmB and voriconazole are also effective; however, these drugs are not available in resource-limited settings. International guidelines recommend D-AmB (0·6–1·0 mg/kg per day) for 2 weeks, followed by itraconazole 400 mg per day for 10 weeks, with itraconazole 200 mg per day continued until CD4 counts are higher than 100 cells per µL for at least 6 months.[99] Primary

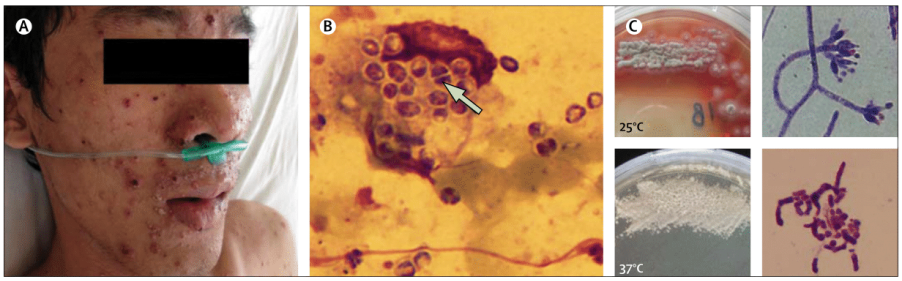


***Figure 4*: Features of talaromycosis in patients with HIV**
Photograph of skin lesions in a patient with talaromycosis (A). Giemsa-stained touch skin smear showing oval-shape yeast organisms inside and outside of a ruptured macrophage (B). The arrowhead highlights the midline septum in a dividing yeast cell characteristic of *Talaromyces marneffei*. Morphology of *T marneffei* colonies and *T marneffei* cells grown at 25°C and at 37°C on Sabouraud agar medium (C).

Search strategy and selection criteria

We identified references for this Series paper through searches of PubMed for articles published from January 1, 1980, to June 30, 2016, by use of the terms "pneumocystis, PCP, *P carinii*, *P jirovecii*, cryptococcal meningitis, cryptococcosis, *C neoformans*, *C gattii*, histoplasmosis, *Histoplasma capsulatum*, *Penicillium marneffei*, penicilliosis, *Talaromyces marneffei*, talaromycosis, fungal infection, mycoses, AND (HIV OR AIDS)". We identified relevant articles published before these dates and abstracts through searches of our records. We reviewed articles resulting from these searches and relevant references cited in those articles. Articles published in English, French, and Spanish were included.

prophylaxis with itraconazole reduces invasive fungal infections in patients with CD4 counts lower than 200 cells per μL;[100] however, the strategy has not been widely adopted in Asia because of concerns about costs, toxic effects, drug resistance, and interactions.

Conclusion

Serious fungal infections continue to contribute considerably to HIV/AIDS-related mortality worldwide. For pneumocystosis, talaromycosis, and particularly histoplasmosis, affordable, rapid point-of-care diagnostic tests, as have been developed for cryptococcosis, are urgently needed. Antifungal drugs, including D-AmB, L-AmB, and flucytosine, need to be made much more widely available.[101] D-AmB and flucytosine have been recently added to the WHO Model List of Essential Medicines. Such measures, together with continued international efforts for education and training in the management of fungal disease, have the potential to greatly improve patient outcomes.

Contributors

All authors planned the scope of the review. AHL, TSH, AA, and TL wrote the first drafts of the sections on pneumocystis, cryptococcosis, histoplasmosis, and talaromycosis, respectively. All authors contributed to and edited the final manuscript.

Declaration of interests

TSH received an investigator award from Gilead Sciences, diagnostic tests for research from ImmunoMycologics, honoraria from Pfizer, and serves on the advisory board for Viamet. AA spoke for Janssen Pharmaceutical Companies of Johnson & Johnson (in 2016) and received diagnostic tests for research from the Centers for Disease Control and Prevention Mycotic Diseases Branch and from IMMY (ImmunoMycologics). AHL and TL report no competing interests.

Acknowledgments

TL is supported by the Hawaii Center for AIDS in the US and by the Joint Global Health Trial Grant funded by the Wellcome Trust, the Medical Research Council, and the Department for International Development in the UK. AA is supported by an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA; reference ANR-10-LABX-0025), by the Agence Nationale de Recherche sur le SIDA et les hépatites virales—Agence autonome de l'Institut National de la Santé et de la Recherche Médicale (ANRS-Inserm number 12260) and by the European Union (PO FEDER 2007–2013, number Présage: 31362, Guyane Française).

References

1 Nguyen C, Barker BM, Hoover S, et al. Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis. *Clin Microbiol Rev* 2013; **26:** 505–25.

2 Schwartz IS, Govender NP, Corcoran C, et al. Clinical characteristics, diagnosis, management, and outcomes of disseminated emmonsiosis: a retrospective case series. *Clin Infect Dis* 2015; **61:** 1004–12.

3 Buchacz K, Lau B, Jing Y, 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.

4 Jarvis JN, Boulle A, Loyse A, et al. High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. *AIDS* 2009; **23:** 1182–83.

5 Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002–2013: a meta-analysis. *Clin Infect Dis* 2015; **60:** 1120–27.

6 Beardsley J, Wolbers M, Kibengo FM, et al. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. *N Engl J Med* 2016; **374:** 542–54.

7 Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* 2012; **4:** 165rv13.

8 Thomas CF, Jr., Limper AH. Current insights into the biology and pathogenesis of pneumocystis pneumonia. *Nat Rev Microbiol* 2007; **5:** 298–308.

9 Thomas CF Jr, Limper AH. Pneumocystis pneumonia. *N Engl J Med* 2004; **350:** 2487–98.

10 Lopez-Sanchez C, Falco V, Burgos J, et al. Epidemiology and long-term survival in HIV-infected patients with *Pneumocystis jirovecii* pneumonia in the HAART era: experience in a university hospital and review of the literature. *Medicine* 2015; **94:** e681.

11 Cushion MT. Are members of the fungal genus *Pneumocystis* (a) commensals; (b) opportunists; (c) pathogens; or (d) all of the above? *PLoS Pathog* 2010; **6:** e1001009.

12 Pifer LL, Hughes WT, Stagno S, Woods D. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. *Pediatrics* 1978; **61:** 35–41.

13 Limper AH, Knox KS, Sarosi GA, et al. An official American Thoracic Society Statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011; **183:** 96–128.

14 Chave JP, David S, Wauters JP, Van Melle G, Francioli P. Transmission of *Pneumocystis carinii* from AIDS patients to other immunosuppressed patients: a cluster of *Pneumocystis carinii* pneumonia in renal transplant recipients. *AIDS* 1991; **5:** 927–32.

15 Keely SP, Baughman RP, Smulian AG, Dohn MN, Stringer JR. Source of *Pneumocystis carinii* in recurrent episodes of pneumonia in AIDS patients. *AIDS* 1996; **10:** 881–88.

16 Krajicek BJ, Limper AH, Thomas CF Jr. Advances in the biology, pathogenesis and identification of pneumocystis pneumonia. *Curr Opin Pulm Med* 2008; **14:** 228–34.

17 Morris A, Kingsley LA, Groner G, Lebedeva IP, Beard CB, Norris KA. Prevalence and clinical predictors of pneumocystis colonization among HIV-infected men. *AIDS* 2004; **18:** 793–98.

18 Wilson JW, Limper AH, Grys TE, Karre T, Wengenack NL, Binnicker MJ. *Pneumocystis jirovecii* testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. *Diagn Microbiol Infect Dis* 2011; **69:** 145–52.

19 Onishi A, Sugiyama D, Kogata Y, et al. Diagnostic accuracy of serum 1,3-beta-D-glucan for *Pneumocystis jiroveci* pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. *J Clin Med* 2012; **50:** 7–15.

20 Lanaspa M, O'Callaghan-Gordo C, Machevo S, et al. High prevalence of *Pneumocystis jirovecii* pneumonia among Mozambican children <5 years of age admitted to hospital with clinical severe pneumonia. *Clin Microbiol Infect* 2015; **21**: 1018 e9–e15.

21 Ewald H, Raatz H, Boscacci R, Furrer H, Bucher HC, Briel M. Adjunctive corticosteroids for *Pneumocystis jiroveci* pneumonia in patients with HIV infection. *Cochrane Database Syst Rev* 2015; **4**: CD006150.

22 Bye MR, Cairns-Bazarian AM, Ewig JM. Markedly reduced mortality associated with corticosteroid therapy of *Pneumocystis carinii* pneumonia in children with acquired immunodeficiency syndrome. *Arch Pediatr Adolesc Med* 1994; **148:** 638–41.

23 Masur H, Brooks JT, Benson CA, et al. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Updated Guidelines from the Centers for Disease Control and Prevention, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; **58:** 1308–11.

24 Church JA, Fitzgerald F, Walker AS, Gibb DM, Prendergast AJ. The expanding role of co-trimoxazole in developing countries. *Lancet Infect Dis* 2015; **15:** 327–39.

25 Kling HM, Norris KA. Vaccine-induced immunogenicity and protection against pneumocystis pneumonia in a nonhuman primate model of HIV and pneumocystis coinfection. *J Infect Dis* 2016; **213:** 1586–95.

26 Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis* 2014; **58:** 736–45.

27 Jarvis JN, Percival A, Bauman S, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin Infect Dis* 2011; **53:** 1019–23.

28 Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis* 2009; **48:** 856–62.

29 Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious Diseases Society of America. *Clin Infect Dis* 2010; **50:** 291–322.

30 WHO Guidelines Approved by the Guidelines Review Committee. Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: World Health Organization, 2011.

31 Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004; **363:** 1764–67.

32 Day JN, Chau TT, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med* 2013; **368:** 1291–302.

33 Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis* 2010; **51:** 225–32.

34 Loyse A, Dromer F, Day J, Lortholary O, Harrison TS. Flucytosine and cryptococcosis: time to urgently address the worldwide accessibility of a 50-year-old antifungal. *J Antimicrob Chemother* 2013; **68:** 2435–44.

35 Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups. *Clin Infect Dis* 2000; **30:** 47–54.

36 Rolfes MA, Hullsiek KH, Rhein J, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. *Clin Infect Dis* 2014; **59:** 1607–14.

37 Bicanic T, Brouwer AE, Meintjes G, et al. Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. *AIDS* 2009; **23:** 701–06.

38 Macsween KF, Bicanic T, Brouwer AE, Marsh H, Macallan DC, Harrison TS. Lumbar drainage for control of raised cerebrospinal fluid pressure in cryptococcal meningitis: case report and review. *J Infect* 2005; **51:** e221–24.

39 Fessler RD, Sobel J, Guyot L, et al. Management of elevated intracranial pressure in patients with cryptococcal meningitis. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **17:** 137–42.

40 Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa. *Clin Infect Dis* 2010; **50:** 1532–38.

41 Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* 2014; **370:** 2487–98.

42 Longley N, Harrison TS, Jarvis JN. Cryptococcal immune reconstitution inflammatory syndrome. *Curr Opin Infect Dis* 2013; **26:** 26–34.

43 Bicanic T, Meintjes G, Rebe K, et al. Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. *J Acquir Immune Defic Syndr* 2009; **51:** 130–34.

44 Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010; **51:** 448–55.

45 Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. *PLoS One* 2013; **8:** e69288.

46 Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. *Lancet* 2015; **385:** 2173–82.

47 Longley N, Jarvis JN, Meintjes G, et al. Cryptococcal antigen screening in patients initiating ART in South Africa: a prospective cohort study. *Clin Infect Dis* 2016; **62:** 581–87.

48 Smith KD, Achan B, Hullsiek KH, et al. Increased antifungal drug resistance in clinical isolates of *Cryptococcus neoformans* in Uganda. *Antimicrob Agents Chemother* 2015; **59:** 7197–204.

49 Brandt ME, Gómez BL, Warnock D. Histoplasma, blastomyces, coccidioides, and other dimorphic fungi causing systemic mycoses In: Versalovic J, Carroll KC, Funke G, et al, eds. Manual of Clinical Microbiology. 10th edn. Washington DC: American Society for Microbiology, 2011: 1902–18.

50 Woods JP. Revisiting old friends: Developments in understanding *Histoplasma capsulatum* pathogenesis. *J Microbiol* 2016; **54:** 265–76.

51 Adenis AA, Aznar C, Couppie P. Histoplasmosis in HIV-infected patients: a review of new developments and remaining gaps. *Curr Trop Med Rep* 2014; **1:** 119–28.

52 Nacher M, Adenis A, Aznar C, et al. How many have died from undiagnosed human immunodeficiency virus-associated histoplasmosis, a treatable disease? Time to act. *Am J Trop Med Hyg* 2013; **90:** 193–94.

53 Wheat J. Endemic mycoses in AIDS: a clinical review. *Clin Microbiol Rev* 1995; **8:** 146–59.

54 Peigne V, Dromer F, Elie C, Lidove O, Lortholary O. Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. *Am J Trop Med Hyg* 2011; **85:** 934–41.

55 Adenis A, Nacher M, Hanf M, et al. HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority. *PLoS Negl Trop Dis* 2014; **8:** e3100.

56 Hanf M, Adenis A, Couppie P, Carme B, Nacher M. HIV-associated histoplasmosis in French Guiana: recent infection or reactivation? *AIDS* 2010; **24:** 1777–78.

57 Kroetz DN, Deepe GS. The role of cytokines and chemokines in *Histoplasma capsulatum* infection. *Cytokine* 2012; **58:** 112–17.

58 Hajjeh RA, Pappas PG, Henderson H, et al. Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. *Clin Infect Dis* 2001; **32:** 1215–20.

59 Nacher M, Adenis A, Blanchet D, et al. Risk factors for disseminated histoplasmosis in a cohort of HIV-infected patients in French Guiana. *PLoS Negl Trop Dis* 2014; **8:** e2638.

60 De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; **46:** 1813–21.

61 Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. *Clin Infect Dis* 2005; **40:** 844–52.

62 Theel ES, Harring JA, Dababneh AS, Rollins LO, Bestrom JE, Jespersen DJ. Reevaluation of commercial reagents for detection of *Histoplasma capsulatum* antigen in urine. *J Clin Microbiol* 2015; **53:** 1198–203.

63 Caceres DH, Scheel CM, Tobón AM, et al. Validation in Colombia of a commercial antigen detection test for the diagnosis of progressive disseminated histoplasmosis. 19th Congress of the International Society for Human and Animal Mycology; Melbourne, VIC, Australia; May 4–8, 2015. Abstract 545.

64 Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. 2013.

65 Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007; **45:** 807–25.

66 Jaruratanasirikul S, Sriwiriyajan S. Pharmacokinetic study of the interaction between itraconazole and nevirapine. *Eur J Clin Pharmacol* 2007; **63:** 451–56.

67 Huet E, Hadji C, Hulin A, Botterel F, Bretagne S, Levy Y. Therapeutic monitoring is necessary for the association itraconazole and efavirenz in a patient with AIDS and disseminated histoplasmosis. *AIDS* 2008; **22:** 1885–86.

68 Homei A, Worboys M. Fungal disease in Britain and the United States, 1850–2000, mycoses and modernity. Nov 15, 2013 edn. Manchester, UK: Palgrave Macmillan, 2013.

69 Neglected histoplasmosis in Latin America group. Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations. *AIDS* 2016; **30:** 167–70.

70 Murphy RA, Gounder L, Manzini TC, Ramdial PK, Castilla C, Moosa MY. Challenges in the management of disseminated progressive histoplasmosis in human immunodeficiency virus-infected patients in resource-limited settings. *Open Forum Infect Dis* 2015; **2:** ofv025.

71 Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. Disseminated *Penicillium marneffei* infection in southeast Asia. *Lancet* 1994; **344:** 110–13.

72 Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev* 2006; **19:** 95–110.

73 Supparatpinyo K, Chiewchanvit S, Hirunsri P, Uthammachai C, Nelson KE, Sirisanthana T. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1992; **14:** 871–74.

74 Wong KH, Lee SS, Chan KC, Choi T. Redefining AIDS: case exemplified by *Penicillium marneffei* infection in HIV-infected people in Hong Kong. *Int J STD AIDS* 1998; **9:** 555–56.

75 Larsson M, Nguyen LH, Wertheim HF, et al. Clinical characteristics and outcome of *Penicillium marneffei* infection among HIV-infected patients in northern Vietnam. *AIDS Res Ther* 2012; **9:** 24.

76 Le T, Wolbers M, Chi NH, et al. Epidemiology, seasonality, and predictors of outcome of AIDS-associated *Penicillium marneffei* infection in Ho Chi Minh City, Viet Nam. *Clin Infect Dis* 2011; **52:** 945–52.

77 Nga TV, Parry CM, Le T, et al. The decline of typhoid and the rise of non-typhoid salmonellae and fungal infections in a changing HIV landscape: bloodstream infection trends over 15 years in southern Vietnam. *Trans R Soc Trop Med Hyg* 2012; **106:** 26–34.

78 Antinori S, Gianelli E, Bonaccorso C, et al. Disseminated *Penicillium marneffei* infection in an HIV-positive Italian patient and a review of cases reported outside endemic regions. *J Travel Med* 2006; **13:** 181–88.

79 Chan JF, Chan TS, Gill H, et al. Disseminated infections with *Talaromyces marneffei* in non-AIDS patients given monoclonal antibodies against CD20 and kinase inhibitors. *Emerg Infect Dis* 2015; **21:** 1101–06.

80 Gugnani H, Fisher MC, Paliwal-Johsi A, Vanittanakom N, Singh I, Yadav PS. Role of *Cannomys badius* as a natural animal host of *Penicillium marneffei* in India. *J Clin Microbiol* 2004; **42:** 5070–75.

81 Cao C, Liang L, Wang W, et al. Common reservoirs for *Penicillium marneffei* infection in humans and rodents, China. *Emerg Infect Dis* 2011; **17:** 209–14.

82 Chariyalertsak S, Sirisanthana T, Supparatpinyo K, Praparattanapan J, Nelson KE. Case-control study of risk factors for *Penicillium marneffei* infection in human immunodeficiency virus-infected patients in northern Thailand. *Clin Infect Dis* 1997; **24:** 1080–86.

83 Le T, Jonat B, Kim Cuc NT, et al. The exposure and geospatial risk factors for AIDS-associated penicilliosis in Vietnam. Conference on Retroviruses and Opportunistic Infections; Seattle, WA, USA; Feb 23–26, 2015.

84 Chariyalertsak S, Sirisanthana T, Supparatpinyo K, Nelson KE. Seasonal variation of disseminated *Penicillium marneffei* infections in northern Thailand: a clue to the reservoir? *J Infect Dis* 1996; **173:** 1490–93.

85 Hu Y, Zhang J, Li X, et al. *Penicillium marneffei* infection: an emerging disease in mainland China. *Mycopathologia* 2012; **175:** 57–67.

86 Bulterys PL, Le T, Quang VM, Nelson KE, Lloyd-Smith JO. Environmental predictors and incubation period of AIDS-associated *Penicillium marneffei* infection in Ho Chi Minh City, Vietnam. *Clin Infect Dis* 2013; **56:** 1273–79.

87 Chariyalertsak S, Vanittanakom P, Nelson KE, Sirisanthana T, Vanittanakom N. *Rhizomys sumatrensis* and *Cannomys badius*, new natural animal hosts of *Penicillium marneffei*. *J Med V Med* 1996; **34:** 105–10.

88 Huang X, He G, Lu S, Liang Y, Xi L. Role of *Rhizomys pruinosus* as a natural animal host of *Penicillium marneffei* in Guangdong, China. *Microb Biotechnol* 2015; **8:** 659–64.

89 Pryce-Miller E, Aanensen D, Vanittanakom N, Fisher MC. Environmental detection of *Penicillium marneffei* and growth in soil microcosms in competition with *Talaromyces stipitatus*. *Fungal Ecol* 2008; **1:** 49–56.

90 Wu TC, Chan JW, Ng CK, Tsang DN, Lee MP, Li PC. Clinical presentations and outcomes of *Penicillium marneffei* infections: a series from 1994 to 2004. *Hong Kong Med J* 2008; **14:** 103–09.

91 Son VT, Khue PM, Strobel M. Penicilliosis and AIDS in Haiphong, Vietnam: evolution and predictive factors of death. *Med Mal Infect* 2014; **44:** 495–501.

92 Saikia L, Nath R, Hazarika D, Mahanta J. Atypical cutaneous lesions of *Penicillium marneffei* infection as a manifestation of the immune reconstitution inflammatory syndrome after highly active antiretroviral therapy. *Indian J Dermatol Venereol Leprol* 2010; **76:** 45–48.

93 Sudjaritruk T, Sirisanthana T, Sirisanthana V. Immune reconstitution inflammatory syndrome from *Penicillium marneffei* in an HIV-infected child: a case report and review of literature. *BMC Infect Dis* 2012; **12:** 28.

94 Lu S, Li X, Calderone R, et al. Whole blood nested PCR and real-time PCR amplification of *Talaromyces marneffei* specific DNA for diagnosis. *Med Mycol* 2016; **54:** 162–68.

95 Hien HT, Thanh TT, Thu NT, et al. Development and evaluation of a real-time polymerase chain reaction assay for the rapid detection of *Talaromyces marneffei* MP1 gene in human plasma. *Mycoses* 2016; **59:** 773–80.

96 Wang YF, Cai JP, Wang YD, et al. Immunoassays based on *Penicillium marneffei* Mp1p derived from *Pichia pastoris* expression system for diagnosis of penicilliosis. *PLoS One* 2011; **6:** e28796.

97 Prakit K, Nosanchuk JD, Pruksaphon K, Vanittanakom N, Youngchim S. A novel inhibition ELISA for the detection and monitoring of *Penicillium marneffei* antigen in human serum. *Eur J Clin Microbiol Infect Dis* 2016; **35:** 647–56.

98 Le T, Kinh NV, Cuc NTK, et al. A trial of itraconazole or amphotericin B for HIV-associated talaromycosis. *N Engl J Med* 2017; **376:** 2329–40.

99 Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009; **58:** 1–207.

100 Chariyalertsak S, Supparatpinyo K, Sirisanthana T, Nelson KE. A controlled trial of itraconazole as primary prophylaxis for systemic fungal infections in patients with advanced human immunodeficiency virus infection in Thailand. *Clin Infect Dis* 2002; **34:** 277–84.

101 Loyse A, Thangaraj H, Easterbrook P, et al. Cryptococcal meningitis: improving access to essential antifungal medicines in resource-poor countries. *Lancet Infect Dis* 2013; **13:** 629–37.

2. Généralités et état des connaissances à propos de l'histoplasmose chez le patient infecté par le VIH

2.1. Synthèse historique de cent ans de découvertes et de redécouvertes à propos de l'histoplasmose

C'est en 1905 que Samuel Taylor Darling, anatomopathologiste américain en poste sur le canal de Panama, fait la description princeps d'un cas fatal d'histoplasmose disséminée chez un homme originaire de la Martinique (3). A l'autopsie, les lésions macroscopiques évoquent une tuberculose disséminée, mais en microscopie optique il découvre de nombreux petits corps encapsulés dans le cytoplasme des cellules réticuloendothéliales du foie, de la rate et des ganglions. Il leur donne le nom d'*Histoplasma capsulatum* car ils évoquent un « *Plasmodium* encapsulé » dans le cytoplasme des histiocytes (3). Par la suite, en 1912, Da Rocha Lima est le premier à suggérer que l'agent pathogène découvert par Darling n'est pas un protozoaire mais plutôt une levure (4). Par ailleurs, le terme encapsulé est inexact, cette levure n'ayant pas de capsule à proprement parler, mais persiste encore aujourd'hui (5).

Ainsi, depuis cette description princeps, le principal diagnostic différentiel de l'histoplasmose au plan clinique est représenté par les maladies granulomateuses telle que la tuberculose ou la sarcoïdose et, au plan de l'examen direct en microscopie optique, les formes amastigotes de parasitoses (*Leishmania* spp et *Trypanosoma* spp.) ou autres levures (*C. glabrata* et *T. marneffei*) (6).

Dans les années 1920, au décours de la découverte de la tombe de Toutankhamon par Howard Carter et Lord Carnavon, plusieurs décès de membres de l'équipe de fouilles ont été attribués à l'histoplasmose. Toutefois, aucun arguments clinico-biologiques chez les personnes décédées n'a pu étayer cette hypothèse étiologique et justifier l'emballement médiatique qualifiant l'histoplasmose de « Malédiction des Pharaons ». Par ailleurs, *H. capsulatum* ne fut jamais identifié dans le sol de la vallée des rois (7).

Les années 1930 à 1940 sont marquées par des avancées importantes, majoritairement décrites aux USA, dans la compréhension du pathogène et sur son importance au plan de la santé publique.

En 1934, suite au premier diagnostic d'histoplasmose disséminée du vivant d'un enfant américain par Dodd et Tompkins, De Monbreun révèle le caractère dimorphique d'*H. capsulatum* (une phase mycélienne saprophyte à température ambiante sur milieu de Sabouraud et parasitaire après culture à 37°C sur un milieu complexe à base de sang) et conclut que la forme saprophyte est probablement

présente dans l'environnement (8, 9). En 1945, Parsons et Zarafonetis rapportent sept nouveaux cas post mortem d'histoplasmose et font une revue mondiale de 71 cas autopsiques connus (10). En 1949, après de longues investigations sur le site de camp Gruber, Oklahoma, où une épidémie d'histoplasmose avait été décrite chez des soldats américains, Emmons met en évidence pour la première fois la forme saprophyte d'*H. capsulatum* dans l'environnement (11, 12). Prélude au diagnostic sérologique de l'histoplasmose, Tenenberg et Furcolow, en 1948, mettent en évidence chez l'animal l'existence d'une réponse anticorps (13).

Au cours de cette période, le corps médical prend conscience de l'intérêt qu'il faut porter à cette pathologie, à présent nommée « Histoplasmose », qualifiée de rare mais rapidement fatale (14). Le développement concomitant de la mycologie médicale et l'utilisation d'isolats antigéniques d'*H. capsulatum* (issus des travaux de De Monbreun) pour réaliser des intradermoréactions (IDR) à l'histoplasmine, vont révéler la distribution de l'histoplasmose aux Etats-Unis d'Amérique (USA) (7). En effet, avec le développement de l'IDR à la tuberculine et de la radiographie pulmonaire, associées dans le dépistage de masse de la tuberculose, les médecins américains font face à un nombre important d'individus ayant une anergie tuberculinique associée à des nodules pulmonaires radiologiques. Ces lésions radiographiques étant alors considérées comme spécifiques de la tuberculose infection, les médecins restent perplexes (7). C'est Palmer qui, en 1945, au décours d'une campagne nationale d'IDR à l'histoplasmine aux Etats-Unis d'Amérique, démontre qu'un nombre important de ces tests positifs prédomine dans certaines régions comme les vallées des fleuves Ohio et Mississipi. Il conclut également que les calcifications pulmonaires radiologiques attribuées à la tuberculose chez des individus ayant un test cutané tuberculinique négatif sont probablement dues à l'histoplasmose, celle-ci survenant par petites épidémies (15, 16). L'histoplasmose passe donc du statut de pathologie isolée rapidement fatale, à celui d'une pathologie bénigne très répandue et rarement létale aux USA. Palmer pose ainsi les bases de nombreux travaux qui révéleront des diagnostics d'histoplasmose chez des personnes pour lesquelles un diagnostic de tuberculose avait été porté à tort (7, 17-19).

Les années 1950 à 1960 s'accompagnent d'un vif intérêt à l'échelle mondiale pour l'étude de l'histoplasmose, comme en atteste l'augmentation rapide du nombre de publications scientifiques (Figure 1) (7). Cette période est marquée par les premières estimations du niveau de prévalence de l'histoplasmose en population générale hors Amérique du Nord et la confirmation des hypothèses formulées concernant les facteurs de risque d'exposition environnementale à *H. capsulatum.*

Ainsi, en 1952, Zeidberg montre la présence plus fréquente d'*H. capsulatum* dans le sol autour des poulaillers ou d'autres sources de déjections aviaires (20). Puis, Emmons, en 1958, établit l'association entre la présence de chauve-souris et d' *H. capsulatum* dans l'environnement (21).

La disponibilité croissante d'histoplasmine de bonne qualité autorise la mise en œuvre d'enquêtes de prévalence à grande échelle. C'est Mochi et Edwards qui, alors en poste à l'Organisation Mondiale de la Santé (OMS) au sein du bureau de recherche sur la tuberculose, publient successivement deux revues de la littérature sur la distribution mondiale de l'histoplasmose, base à l'établissement par Edwards de la première carte mondiale de répartition des IDR positifs à l'histoplasmine (Figure 2) (22, 23).

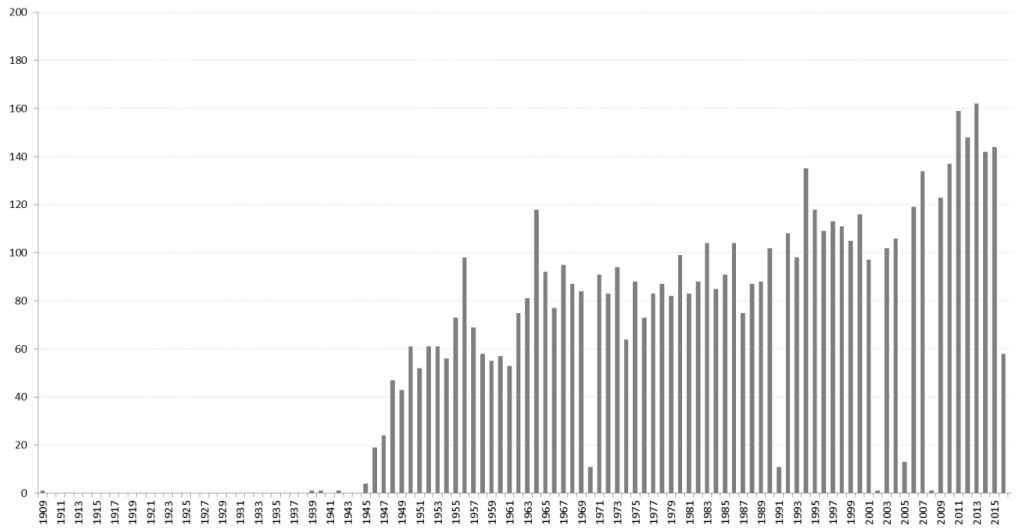
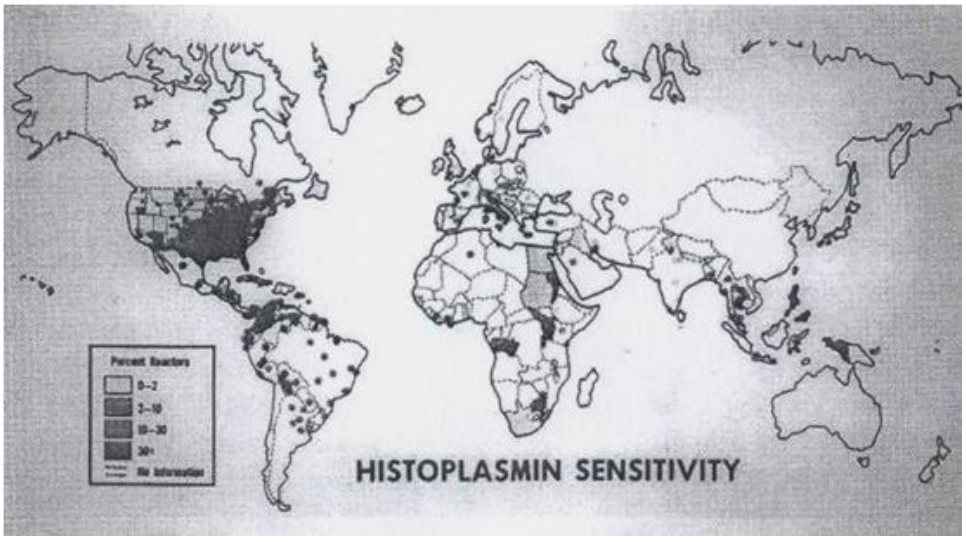


Figure 1 : Nombre de publication scientifiques référencées par années entre 1909 et 2016 (d'après une requête Pubmed avec le terme « Histoplasmosis », le 16/05/2016).



Source : Histoplasmosis: Proceedings of the second national conference. Springfield, Ill: Thomas. 1971.

Figure 2 : **Carte mondiale de la répartition des tests cutanés positifs à l'histoplasmine selon P.Q. Edwards, 1956.**

Les années 1970 sont marquées par la synthèse des connaissances à propos de l'histoplasmose avec l'établissement de cartes de distribution de la prévalence par continents, le développement d'une classification (Figure 3) et de stratégies de prise en charge thérapeutique basées sur des essais cliniques antérieurs et des séries de cas publiées (14).

TABLE 1. A classification of histoplasmosis.

Normal Host
- Mild exposure
  - Usual asymptomatic primary infection
  - Occasional symptomatic primary infection (young children)
  - Asymptomatic reinfection
- Heavy exposure
  - Acute pulmonary histoplasmosis
    - Primary type
    - Reinfection type

Abnormal Host
- Opportunistic infection
  - Disseminated histoplasmosis (immune defect)
  - Chronic pulmonary histoplasmosis (structural defect)
- Excessive fibrotic response to healing primary infection
  - Histoplasmoma
  - Mediastinal fibrosis or collagenosis

Figure 3 : Classification des formes cliniques d'histoplamsose selon le statut de l'hôte par R.A. Goodwin, 1980 (14)

A cette époque, les connaissances sur la prévalence et l'incidence de l'histoplasmose sont importantes comparées aux autres infections fongiques profondes, bien qu'appartenant à ce qu'Ajello qualifiait d' « iceberg de la mycologie médicale » (2). Sans être une maladie à déclaration obligatoire, l'histoplasmose est décrite sur les cinq continents avec une fréquence variable. Si 25 pays rapportent des études avec une prévalence supérieure à 10% en population générale, la partie Centre-Est des USA est considérée comme la zone d'hyperendémicité avec : jusqu'à 90% d'exposés à partir de 15 ans, 200 000 cas symptomatiques et 74 décès estimés par an, sans compter les 95% d'individus non symptomatiques nouvellement infectés tous les ans (Figure 4) (2, 24).

Figure 4 : Répartition géographique de la fréquence de réaction positive cutanée à l'histoplasmine parmi 275 558 recrue de l'U.S. Navy, âgées de 17 à 21 ans et ayant résidé toute leur vie dans le même comté (25)

Le terme « histoplasmose disséminée » apparaît dans les classifications, accompagné de la notion de déficit immunitaire (inné ou acquis essentiellement iatrogène) comme condition quasi nécessaire à son développement et à l'évolution systématique vers le décès en l'absence de traitement. Une synthèse des corrélations entre les spectres anatomopathologiques et cliniques est proposée par Goodwin (Figure 5) (14).

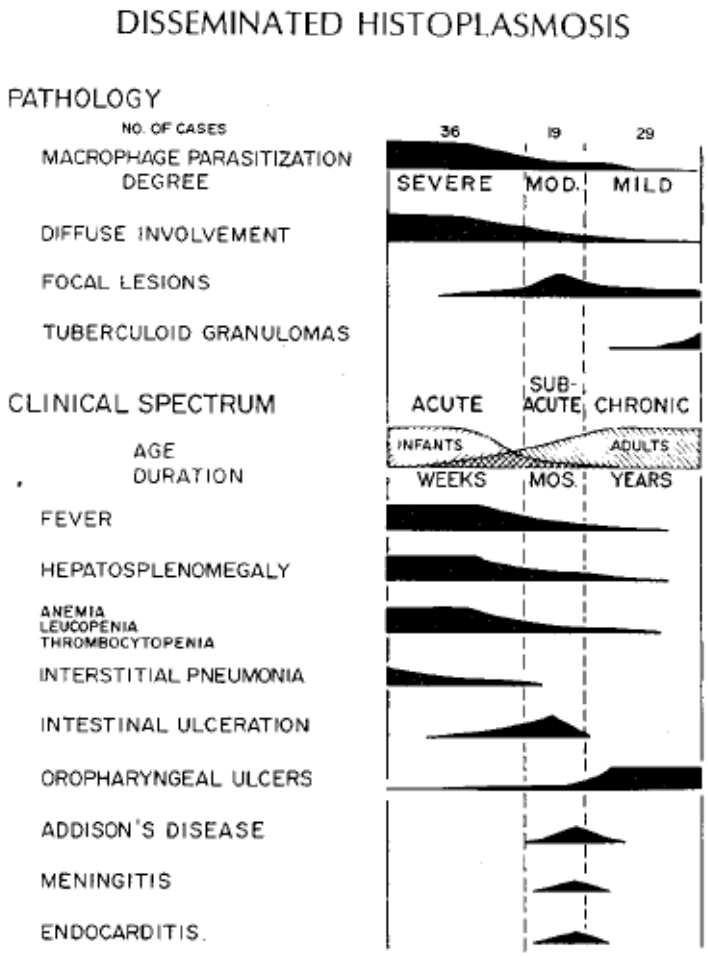


Figure 5 : Schéma des corrélations observées entre les spectres anatomopathologiques et cliniques de l'histoplasmose disséminée (25)

Des années 1980 à nos jours, la morbidité et la mortalité imputables à l'histoplasmose (aigüe ou disséminée) a augmenté du fait d'un accroissement du nombre de personnes immunodéprimées. Si les immunosuppresseurs et autres biothérapies utilisés pour la prise en charge de patients transplantés ou atteints de maladies inflammatoires chroniques ont largement contribué à cet accroissement, la part la plus importante est attribuable à l'émergence et à la diffusion globale de l'infection par le VIH (26).

Les premiers cas d'histoplasmose disséminée associée à un SIDA, chez des patients identifiés rétrospectivement comme infectés par le VIH, sont décrits en 1982 à Indianapolis **(27)**. Et, ce n'est

qu'en 1987 que les Centers for Disease Control and Prevention (CDC) inscrivent l'histoplasmose disséminée (autre localisation, isolée ou associée à une atteinte pulmonaire ou ganglionnaire cervicale ou médiastinale) à la liste des infections opportunistes définissant le stade SIDA chez les patients infectés par le VIH (28).

Depuis, l'accroissement du nombre de cas d'histoplasmose disséminée, rapportés dans la littérature comme associés à une infection par le VIH, s'est accompagné d'avancées diagnostiques et thérapeutiques notables.

Au plan du diagnostic, des méthodes performantes non invasives (échantillons de sang et d'urines principalement) de détection d'antigène d'*H. capsulatum* ont été développées mais peu diffusées car non commercialisées hors USA (29). De nombreuses publications envisagent différentes méthodes de biologie moléculaire comme des outils utiles. Toutefois, leur place dans la prise en charge est incertaine et aucun kit n'est commercialisé (30). Au plan thérapeutique, des essais cliniques ont été menés, principalement en Amérique du Nord, chez des patients infectés par le VIH, pour la prise en charge des formes sévères (31) et non sévères (32) ou la prévention primaire (33) de l'histoplasmose disséminée. A noter que le niveau de preuve des recommandations actuelles pour la prise en charge thérapeutique reste intermédiaire, les essais cliniques publiés n'ayant été réalisés qu'une seule fois dans leur grande majorité.

A l'ère des trithérapies antirétrovirales accompagnées de politiques de dépistage et de prise en charge précoces de l'infection par le VIH, on observe une tendance significative à la baisse de la part des hospitalisations pour histoplasmose et infection par le VIH en Amérique du Nord (Figure 6).

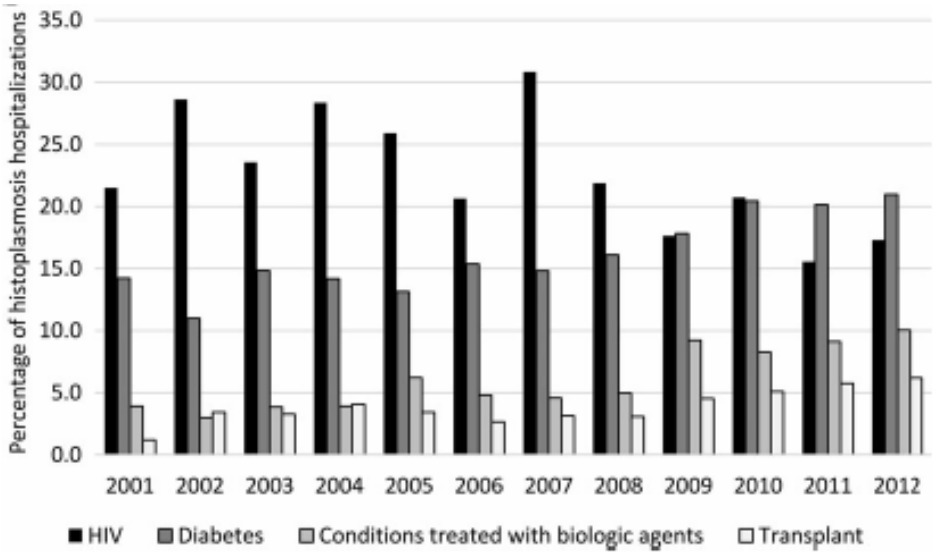


Figure 6 : Répartition de la fréquence annuelle (%) des comorbidités associées au diagnostic d'histoplasmose au décours d'une hospitalisation, USA, 2001-2012 (34)

On observe également une tendance significative à la baisse de la mortalité attribuable à la coinfection histoplasmose et VIH dans certains centres de référence hors Amérique du Nord (34-36).

Toutefois, en 2017, ces évolutions favorables ne sont pas la règle pour la grande majorité des patients infectés par le VIH vivants en zone d'endémie (37-39). L'histoplasmose reste une infection opportuniste majeure au cours de l'infection par le VIH dont la réalité des coûts humains et économiques en termes de santé publique sont toujours largement méconnus et probablement sous-estimés (34, 38).

Depuis 2013 et la publication d'une série de cas de patients coinfectés histoplasmose et VIH dans la vallée du fleuve chinois Yang-Tsé-Kiang, tout se passe comme si certains auteurs, pour la plupart nord-américains, redécouvrent que l'histoplasmose existe en dehors des Amériques et plus particulièrement de la région d'hyperendémicité de la vallée du fleuve Ohio (40-42). Ceci, alors même que des études menées dans les années 1950 à 1960 décrivent des prévalences importantes de tests cutanés positifs à l'histoplasmine en Asie (24), dans des régions qui ne sont pas indemnes d'épidémies d'infection par le VIH de nos jours.

Ainsi, dans les zones d'endémie ou non pour l'histoplasmose, principalement en dehors des USA, de très nombreux défis restent à relever pour améliorer l'un ou l'ensemble des paramètres suivants : la connaissance de l'épidémiologie locale de l'histoplasmose, la prise de conscience du corps médical de l'existence et du poids de la maladie, la mise à disposition de tests diagnostiques rapides et simples, la mise à disposition de traitement antifongiques efficaces à prix abordables, une prise en charge optimale de l'infection par le VIH et notamment une diminution importante de la part des personnes dépistées tardivement (CD4<200/mm3).

Des initiatives sont en cours pour faire face à ces défis (43-45). Certaines actions et collaborations engagées dans le cadre de ce travail de thèse s'envisagent humblement comme une tentative de réponse à certains de ces défis.

2.2. Etat des connaissances à propos de l'histoplasmose chez le patient infecté par le VIH

Comme nous venons de le décrire, l'histoplasmose est une infection fongique profonde décrite sur les cinq continents avec des zones qualifiées de haute et basse endémicité. Communément observable par les connaisseurs, elle est qualifiée de première infection fongique respiratoire au monde par certains auteurs. Toutefois, le poids de l'histoplasmose en terme de santé

publique diffère selon que la personne est immunodéprimée ou non. Chez l'immunocompétent, l'histoplasmose est le plus souvent asymptomatique et spontanément résolutive, à l'origine d'une faible morbi-mortalité. Chez l'immunodéprimé, en présence notamment d'un déficit de l'immunité cellulaire congénital ou acquis, l'histoplasmose est à l'origine d'une morbidité et d'une mortalité importantes. A tous les âges de la vie, elle est le plus souvent fatale en l'absence de traitement approprié. C'est pourquoi, suite aux premiers cas décrits chez des patients infectés par le VIH, sa forme extrapulmonaire ou disséminée a été inscrite sur la liste des infections opportunistes définissant le stade SIDA.

Avec la diffusion de la pandémie à VIH, la morbidité et la mortalité imputables à l'histoplasmose ont augmenté de façon importante au cours des dernières décennies. En comparaison aux autres causes d'immunodépression, les patients infectés par le VIH paient probablement le plus lourd tribu à cette infection fongique. Car, Indépendamment de la mise à disposition des trithérapies antirétrovirales, l'histoplasmose chez les patients infectés par le VIH reste à ce jour un problème de santé publique méconnu dans de nombreux pays en zone d'endémie et notamment en Amérique Latine. L'histoplasmose y serait responsable de plusieurs dizaines de milliers de décès depuis le début de l'épidémie à VIH, plus particulièrement dans la région amazonienne et le plateau des Guyanes.

L'histoplasmose chez le patient infecté par le VIH constitue un problème de santé publique qu'il convient d'étudier.

L'article de revue de la littérature ci-dessous s'envisage comme un état des lieux des connaissances, des problématiques et des avancées récentes sur les aspects épidémiologiques, diagnostiques (cliniques et biologiques) et thérapeutiques de l'histoplasmose chez le patient infecté par le VIH. Par rapport à l'ARTICLE 1, précedemment cité dans ce manuscrit, cette revue est une description plus exhaustive et détaillée de la thématique de travail : étape indispensable à la compréhension globale de la problématique de l'histoplasmose au cours de l'infection par le VIH.

Pour mémoire, ce travail de revue de la littérature avait été réalisé au cours de ma première année de doctorat et présenté oralement à l'occasion de la 33ème Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse, 21-22 novembre 2013, Paris, France (Annexe 1).

ARTICLE 2 : Histoplasmosis in HIV-infected patients: A review of new developments and remaining gaps.

Author: Adenis, A. A., Aznar, C. and Couppie, P.

Year: 2014 **Journal:** Current tropical medicine reports

ISSN: 2196-3045 (Print) **DOI:** 10.1007/s40475-014-0017-8

Abstract: Histoplasma capsulatum is responsible for histoplasmosis, a fungal disease with worldwide distribution that can affect both immunocompromised and imunocompetent individuals. During the highly active antiretroviral therapy (HAART) era, morbidity and mortality due to histoplasmosis remained a public heatlh problem in low-income and high-income countries. The true burden of HIV-associated histoplasmosis is either not fully known or neglected since it is not a notifiable disease. Progress has been made in DNA patterns of strains and understanding of pathogenesis, and hopefully these will help identify new therapeutic targets. Unfortunately, histoplasmosis is still widely mistaken for multidrug-resistant tuberculosis, leading to numerous avoidable deaths, even if they are easily distinguishable. The new diagnostic tools and therapeutics developments have still not been made available in most endemic regions. Still, recent developments are promising because of their good clinical characteristics and also because they will be commercially available and affordable. This review of published data and gaps may help define and guide future research.

URL: https://doi.org/10.1007/s40475-014-0017-8

Curr Trop Med Rep (2014) 1:119–128
DOI 10.1007/s40475-014-0017-8

TROPICAL MYCOSIS (M NACHER, SECTION EDITOR)

Histoplasmosis in HIV-Infected Patients: A Review of New Developments and Remaining Gaps

Antoine A. Adenis · Christine Aznar · Pierre Couppié

Published online: 28 March 2014

Abstract *Histoplasma capsulatum* is responsible for histoplasmosis, a fungal disease with worldwide distribution that can affect both immunocompromised and imunocompetent individuals. During the highly active antiretroviral therapy (HAART) era, morbidity and mortality due to histoplasmosis remained a public heatlh problem in low-income and high-income countries. The true burden of HIV-associated histoplasmosis is either not fully known or neglected since it is not a notifiable disease. Progress has been made in DNA patterns of strains and understanding of pathogenesis, and hopefully these will help identify new therapeutic targets. Unfortunately, histoplasmosis is still widely mistaken for multidrug-resistant tuberculosis, leading to numerous avoidable deaths, even if they are easily distinguishable. The new diagnostic tools and therapeutics developments have still not been made available in most endemic regions. Still, recent developments are promising because of their good clinical characteristics and also because they will be commercially available and affordable. This review of published data and gaps may help define and guide future research.

A. A. Adenis (✉)
Inserm CIC 1424, Centre d'Investigation Clinique Antilles-Guyane, Centre Hospitalier de Cayenne, avenue des flamboyants, BP 6006, 97 300 Cayenne, France
e-mail: antoine.adenis@gmail.com

A. A. Adenis · C. Aznar · P. Couppié
UAG EA 3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, France

C. Aznar
Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier de Cayenne, Cayenne, France

P. Couppié
Service de Dermatologie Vénérologie,, Centre Hospitalier de Cayenne, Cayenne, France

Keywords HIV · AIDS · Histoplasmosis · *Histoplasma* · Tropical medicine · Fungal disease · Tropical mycosis

Introduction

Histoplasma capsulatum (*H. capsulatum*) is the etiologic agent of histoplasmosis, a fungal disease that can affect both immunocompromised and imunocompetent individuals. It was first described in 1905 by S.T. Darling, concerning a case that looked like disseminated tuberculosis in a patient originated from Martinique who was working at building the Panama Canal [1].

Since then, histoplasmosis was described on five continents, with high and low endemicity areas. Thus, epidemiologic, clinical, paraclinical and therapeutic data are available and have led to numerous publications by reference centers, based on autochthonous cases in endemic areas [2–7] or imported cases in non-endemic areas [8–10].

Labeled as the first respiratory fungal infection by some authors, the public health burden of histoplasmosis differs depending on whether the person is immunocompromised or not. Among immunocompetent persons, apart from histoplasmin test campaigns, few serologic prevalence studies have been conducted in the general population. However, some authors estimate that hundred of thousands cases occur every year in the Ohio and Mississipi River regions. Mostly asymptomatic and spontaneously self-limited, histoplasmosis causes low morbidity and mortality among immunocompetent patients [11].

Among immunocompromised patients, in the presence of an acquired or congenital cellular immunity deficiency, histoplasmosis is responsible for an important morbidity and mortality. At all ages, it is mostly fatal in the absence of appropriate treatment. Following the first cases described in 1982 in human immunodeficiency virus (HIV) infected patients in the

USA, its extrapulmonary or disseminated form became an acquired immunodeficiency syndrome (AIDS)-defining infection in 1987 [12, 13].

During the last decades, morbidity and mortality from histoplasmosis have increased because of the increase in the number of immunocompromised persons. Although immunosuppressors used in transplant patients or chronic inflammatory diseases contribute to this increase, the greater part of the problem is mostly attributable to the spread of the HIV pandemic. [14]. The availability of highly active antiretroviral therapy (HAART) and lipid formulations of amphotericin B, the increased awareness of the problem by some teams, and the development of rapid, noninvasive, diagnostic methods have led to a major impact on mortality by histoplasmosis [15]. This progress was so spectacular that some authors no longer consider histoplasmosis a problem in the course of the HIV infection, considering instead that research should focus on other causes of immunosuppression associated with histoplasmosis [11, 16].

However, these developments are heterogeneous, and some authors consider HIV-associated histoplasmosis a neglected disease, notably in South America [17]. Evolving under the radar of health authorities and research institutions, it is thought to have caused several thousand deaths in the Amazon region alone since the beginning of the epidemic [15].

Independently of the availability of HAART, histoplasmosis in HIV-infected patients remains a public health problem that is overlooked in numerous countries in endemic areas, notably in South America. To tackle this problem, there are important needs in terms of epidemiological knowledge, health professionals becoming aware of it, diagnostic development and availability of effective treatments at affordable costs [17].

This review will consider the recent developments and gaps regarding the epidemiological, diagnostic (clinical and biological) and therapeutic aspects of histoplasmosis in HIV-infected patients. The objective is to describe the current situation in order to guide future research.

Taxonomy and Molecular Epidemiology

Historically, *H. capsulatum* was considered to be divided into three varieties based on morphologic characteristics: *H. capsulatum* var. *capsulatum*, mainly described in the Americas; *H. capsulatum* var. *duboisii*, mainly described in Africa; and *H. capsulatum* var. *farciminosum*, described in equines from Africa and the Middle East [18].

The *H. capsulatum* species is not monophyletic and has been recently subdivided into geographically distinct phylogenetic lineages [19]. Based on concordance of multiple gene sequence genealogies, *Histoplasma* strains separate into at least eight clades: North American class 1 (Nam1), North American class 2 (Nam2), Latin American group A (LamA), Latin American group B (LamB), Australian, Netherlands (Indonesian), Eurasian, and African clades [20]. Seven of these eight clades comprise genetically and geographically distinct populations that can be regarded as phylogenetic species. The single exception, the Eurasian clade, originated from within the Latin American group A clade. *H. capsulatum* var. *farciminosum* was placed within the Eurasian clade. In addition to the seven phylogenetic species, another seven lineages represented by single isolates from Latin America were identified [18, 20].

Interestingly, clinical differences in histoplasmosis disease manifestation have been reported among the groups [19]. At this time, histoplasmosis due to *H. capsulatum* var. *duboisii* was considered a distinct entity, causing primarily cutaneous and subcutaneous lesions rather than pulmonary involvement. Unlike var. *capsulatum* and according to published data, var. *duboisii* is still not considered as an AIDS-defining illness [21]. Whether this manifestation is determined by genetic differences in *Histoplasma* strains is unclear, since the taxonomic placement of *H. capsulatum* var. *duboisii* has been called into question by the finding of one var. *capsulatum* isolate from South Africa (primarily causing pulmonary disease) that was placed in the African clade (var. *duboisii*-containing) [19, 20]. This extends the results of earlier studies that had shown that var. *duboisii* had mitochondrial DNA restriction patterns identical to those of var. *capsulatum* strains [18]. In the Americas, authors have reported mucocutaneous tropism differences between North and South strains in HIV-infected patients as linked with genetic differences in *Histoplasma* strains [22, 23]. These findings didn't reach consensus, since a marked decrease of mucocutaneous manifestation was observed by others during the HAART era, concomitant to the opening of a laboratory specialized in mycology [24, 25]. Other differences involving Nam1 and/or Nam2 and/or Latin American strains, concerning either the disease potential progression (acute or chronic) or the *Histoplasma* strain infection potential according to the immune status of the host (HIV-infected or not), have been described in the Americas with contradictory results [19].

Multiple typing methods have been developed to study the epidemiology of *H. capsulatum*. Nowadays, molecular typing methods are generally considered to have advantages over phenotypic methods in terms of stability of genomic markers and greater levels of type-ability. Several genotype-based methods, such as hybridization of target genes (probes), chromosomal DNA typing, restriction fragment length polymorphism (RFLP) analysis, random amplified polymorphic DNA (RAPD) analysis, and sequencing, have been described for *H. capsulatum* [26]. No single approach based on DNA assays has been the dominant method. Nevertheless, the analysis of concordance in the results obtained with different methods using the same set of isolates showed great convergence [26].

Thus, advances in the classification of *H. capsulatum* are sustained by the results of several genetic analyses that support the high diversity of this dimorphic fungus, suggesting diversity among strains in virulence, infectivity, pathogenesis and relationship to the immune status of the host [19]. But all these findings are based on relatively small sample size, and studies are required in order to explore the functional consequences of these variations on histoplasmosis pathogenesis in HIV-infected patients.

Epidemiology

H. capsulatum is a fungus that is distributed worldwide, endemic to geographically limited areas, according to local environmental conditions favorable to its development [27]. Since histoplasmosis is not classified among the notifiable diseases, hard data on the incidence and prevalence, as well as information on its morbidity and mortality, should be considered fragmentary or simply not available in many endemic areas [28, 29].

It is admitted that histoplasmosis occurs most commonly in the Americas, since most of the studies are published from patients residing in this area. But the organism exists in many diverse areas around the world and is described in the non-English literature [11, 28]. In North America, *H. capsulatum* is endemic in the Mississippi and Ohio River valleys and also in localized foci throughout the region [11, 30, 31]. In Central and South America and the Caribbean area, it has been described in almost all countries except Chile [4, 28, 32–34]. Other endemic areas include parts of Africa, Asia (India, China, Philippines, Thailand) and Australia [7, 35–38].

In endemic areas, histoplasmosis represents the first manifestation of AIDS in up to 50–75 % of patients and occurs in about 2–25 % in HIV-infected patients living in these areas [39]. Few incidence data using cohort studies are available. In one endemic area, before the HAART era, subclinical or symptomatic histoplasmosis occurred in 12/100 person-years at risk in a cohort of HIV-infected patients [40]. Nowadays, with the advent of effective antiretroviral therapy, North American authors state that the disease is less frequently seen in HIV-infected patients in USA [11]. Still, during the HAART era, in one South American endemic area, histoplasmosis is the first AIDS-defining event with an incidence of 15,4/1000 HIV-infected person-years [41]. In disease-endemic areas where research centers and/or reference centers for the diagnosis led studies and published papers, physicians are used to seeking and treating for histoplasmosis. Thus, at the AIDS stage, histoplasmosis-related mortality is mainly around 10 % during the HAART-era [24]. But, even in high-income countries, in settings far from the reference centers' influences and means, mortality increased up to 40 %. Apart from the host's medical history and socio-economic factors, this observation is probably due to the lack of disease recognition and knowledge by physicians who are unable to diagnose earlier and treat a treatable disease [42].

Histoplasmosis occurs infrequently in persons living outside of, and not travelling to, endemic areas. While precise incidence figures are unavailable, the low incidence can be inferred from case reports reviews on this subject [27]. In Europe, most of these cases are considered as "imported" with exposure decades before, when staying in or travelling to a known endemic region [8–10]. Also, as described in the soil of the Po River valley in Italy, microfoci contaminated with *H. capsulatum* can be found in regions considered as non-endemic. These may be the source of exposure causing autochtonous cases described in several European countries [10]. Historically, mortality in non-endemic areas was observed at around 50 to 60 % [8, 9]; but since the availability of HAART, a marked decrease to almost 20 % was observed in some places [8].

Pathogenesis and Risk Factors in HIV-Infected Patients

H. capsulatum is a thermally dimorphic ascomycete, displaying a filamentous mold form (saprophytic form) in the environment and in culture at temperatures below 35 °C, and a yeast phase (parasitic form) in tissue and body fluids at temperatures above 35 °C. The mold phase may contain macroconidia and microconidia. The latter, smooth walled with a diameter of 2 to 4 μm, are the infectious particles. The yeast phase develops as small oval budding cells with a diameter of 2 to 4 μm, mainly observed within the macrophages. In Africa, the yeast phase described is mostly thick walled and larger, 8 to 15 μm in diameter [18].

H. capsulatum is found in soils throughout the world. It grows best in soils with high nitrogen content, particularly those enriched with bird or bat guano. Birds do not become colonized or infected with *H. capsulatum* (since their body temperature is too high), and their droppings are primarily a nutrient source for the development of fungal pathogen. Soil samples from sites where birds have roosted have remained contaminated for at least 10 years after the roost has been cleared, even in urban areas [18, 43].

Upon disruption of soil containing the organism, infection with *H. capsulatum* mainly develops when microconidia or hyphal elements are inhaled and convert into yeasts in the lungs, or when organisms in old foci of infection reactivate during immunosuppression [44]. Once deposited in the lungs, *H. capsulatum* is internalized by resident and recruited phagocytes, including macrophages, dendritic cells, and neutrophils. The intracellular fate of the organism is divergent in these cellular populations. While neutrophils and dendritic cells have fungistatic and fungicidal activity, respectively, *H. capsulatum* survives in macrophages prior to cellular activation regulating the pH of the intraphagosomal environment. Phagocytes facilitate dissemination of *H. capsulatum* to several organs,

throughout the mononuclear phagocyte system, including the spleen, liver, bone marrow, and lymph nodes. Thus, to control intracellular growth of the organism, the host must mount a robust proinflammatory response, and cellular immunity plays an essential role in defense against *H. capsulatum* [45]. Therefore, with the development of HIV-infection and the progressive impairment of cellular immunity, patients are at a higher risk of disseminated and deadly infections with *H. capsulatum*.

In endemic areas, whether symptomatic diseases are caused by reactivation of old foci or by a recent exogenous exposure is unknown. Published data support reinfection in the context of outbreaks or reactivation when calcified lymph nodes are visualized on chest radiograms [39]. Recently, a time series attempted to estimate if new infection is prevailing over reactivation in endemic areas. Using climatic data, in one endemic area, 70 % of incident HIV-associated histoplasmosis cases could be predicted compared to observed cases [44]. Also, a clear seasonality pattern in incident cases of histoplasmosis was described [46]. These studies are in favor of new infection or reinfection rather than reactivation as the main pathogenesis mechanism to explain the occurrence of symptomatic histoplasmosis in endemic areas [44, 46].

Among Risk Factors Previously Studied in HIV-Infected Patients, Occupational or Environmental Factors and Host Factors Have Been Described

Environmental exposures to sources containing bat or bird guano are known to increase the risk of histoplasmosis in the general population, including histories of cave exploration, wood cutting, or exposure to bird roosts, excavation sites, farms or chicken coops [47]. In HIV-infected individuals experiencing histoplasmosis, prospectively studied and compared to control without histoplasmosis, a history of exposure to chicken coops was found to be significantly associated with histoplasmosis [47].

Current or prior occupations or activities with soil contaminated with bird or bat droppings have been described to be associated with an increased risk of histoplasmosis [40, 48].

Host factors previously found to be independently associated with histoplasmosis are: low CD4 count (<200/mm^3), low CD4 count at the NADIR (<50/mm^3), low CD8 count (<650/mm^3), absence of antiretroviral treatment or the first 6 months under antiretroviral therapy, a history of herpes simplex infection, absence of systemic antifungal therapy (fluconazole) and male gender [40, 49].

Out of *H. capsulatum* transmission to recipient by donor's solid organ transplant, no direct interhuman contamination has been previously described [50].

Clinical Findings

Although it is mostly asymptomatic and self-limiting in immunocompetent persons, histoplasmosis in immunocompromised HIV-infected patients presents as a symptomatic and disseminated infection in 95 % of the cases [11]. With the aggravation of CD4 Lymphocyte decline, the evolution is rapid and always fatal in the absence of treatment [2]. Thus, according to published series, there is up to 39 % death following diagnosis in endemic areas and 58 % death in non-endemic areas [9, 24].

During the HIV infection, the evolutive mode is very variable, from extreme latency to a fulminant form. For 10–20 % of patients, severe rapidly fatal forms are described. These forms present as a septic shock with intravascular disseminated coagulation, multiorgan failure (kidneys, liver, lungs), and rhabdomyolysis, all of which may be associated with a hemophagocytosis syndrome. These presentations of unclear pathogenesis seem to be a late manifestation of histoplasmosis [39]. Mortality of these severe cases is very high, with 50–70 % death [51].

Inflammatory reconstitution disease following antiretroviral treatment initiation has also been described [8, 52].

All organs and tissues may be clinically involved [11]. In HIV-infected patients histoplasmosis is disseminated in 95 % of the cases, and in 90 % of the cases, it concerns patients with CD4 counts below 200/mm3. A subacute presentation is the most frequent, with symptoms evolving for 1 or 2 months, during which the patient is seen by physicians. The clinical picture is misleading, the symptoms being mostly nonspecific. The general symptoms, particularly fever, fatigue and weight loss, are almost always found. Respiratory symptoms such as cough or dyspnea are observed in 50 % of the cases, and may be associated with hepatosplenomegaly (25 % of cases) and/or superficial lymph node enlargement (25 % of cases). Digestive, neurological or muco-cutaneous manifestations are less frequent (10–20 % of cases) and polymorphous. Isolated pulmonary manifestations have also been described in less immunocompromised patients (CD4>200). Pulmonary intersticial syndromes are often observed on chest radiograms or computed tomography (CT) scans. This implies that bronchoscopy must be performed to rule out the differential diagnosis of pneumocystosis [39].

Similarly to clinical symptoms, standard biology tests are nonspecific. However, they may give elements to suspect the diagnosis and lead to further investigations to confirm the diagnosis. The elevation of lactate dehydrogenase (LDH), liver enzymes (TGO, alkaline phosphatase), ferritin alone or isolated with pancytopenia and/or hemophagocytosis syndrome, are classically described [39].

Thus, a high suspicion index is required from clinicians because of the nonspecific nature of the clinical, biologic and radiologic spectrum of histoplasmosis. In disease-endemic areas, this nonspecific clinical picture makes tuberculosis the main differential diagnosis of histoplasmosis. Numerous publications report cases of histoplasmosis mimicking tuberculosis and state that making this differential diagnosis at the bed patient is difficult; mainly because of the absence of diagnostic

facilities or because a diagnosis of histoplasmosis was not considered. Nevertheless, tuberculosis and histoplasmosis could be easily distinguished, as reported recently. Numerous AIDS-related death caused by histoplasmosis and mistaken for multi-drug-resistant tuberculosis could be avoided [53].

Diagnosis

Direct examination of MGG-stained slides of all tissue or body fluid is a simple and rapid diagnostic method. In disseminated forms, smears obtained from tissue biopsies, bone marrow aspiration, or peripheral blood are fairly contributive. However, sensitivity varies according to the type of sample, the severity of the disease and the experience of the operator. With positive results for 50 to 75 % of patients, bone marrow aspiration is the most contributive [24, 39]. Special staining (PAS and Gomori Grocott) may be performed in pathology. These stainings allow one to rule out the main differential diagnoses, such as *C. glabrata*, *P. marneffei*, *Leishmania*, *Trypanosoma* and other staining artifacts [24].

Certain diagnosis rests on the culture and identification of *Histoplasma capsulatum* from any tissue or body fluid, obtained mainly by using invasive procedures. The isolation in culture is slow and may take several weeks (1–6 weeks). Sabouraud's dextrose agar media are thus incubated for several weeks between 25 °C and 30 °C. The observation of macronidia and micronidia are characteristic, but since they are highly infectious, this must be performed in a Biosafety Laboratory (BSL) level 2 or a BSL 3, according to the country's regulations. A definitive, specific and commercially available DNA probe assay should always be performed to confirm the diagnosis. The laborious task of converting the mold phase to the yeast phase is no longer required for the definitive identification of *H. capsulatum* [11].

Specificity is 100 %, but sensitivity varies between 85 and 90 % according to the fungal load and the laboratory experience [24, 54, 55]. Bone marrow aspirates yield the highest proportion of positive cultures (70–90 %). For peripheral blood culture, authors notably recommend the lysis-centrifugation technique, in order to increase sensitivity and to reduce the identification delay relative to other techniques [39]. For the diagnosis of isolated neuro-meningeal forms, the diagnosis must rely on other techniques, given the very low sensitivity of cerebrospinal fluid (CSF) culture [54].

Antibody detection by immunodiffusion or complement fixation is less sensitive in immunocompromised HIV-infected patients than in immunocompetent patients (90 %) [39, 54]. Serology is only positive in 50–70 % of immunocompromised HIV patients [39, 54]. The rise of antibody titers is observed 2–6 weeks after exposure, and the lack of discrimination between active and passive infection are not compatible with the management of acute cases. However, serology on CSF may be of interest for the diagnosis of neuromeningeal forms [56].

Immunodiffusion is more specific but less sensitive than complement fixation. Cross-reactions with other fungal pathogens, lymphoma, sarcoidosis and tuberculosis have been reported [57].

The detection of *Histoplasma capsulatum* var. *capsulatum* circulating antigen may be performed with several EIA methods.

The third generation polyclonal MVista *Histoplasma* antigen EIA allows the quantitative detection of *Histoplasma* polysaccharide circulating antigen with a sensitivity of 95–100 % in urine and 92–100 % in serum [55, 58]. The antigen levels detected are higher in the immunocompromised patients with a disseminated form. Moreover, the antigen level seems correlated with the severity of the clinical presentation (admission or not in ICU) [55]. Specificity is 99 %, and positive and negative predictive values are 91 % and 99.5 %, respectively (for a 10 % prevalence) [58]. In pulmonary forms of histoplasmosis, this test may be performed on bronchioloalveolar lavage fluid with a 93 % sensitivity [59]. Despite the evolution of the test, there are still cross–reactions with blastomycosis (90 %), paracoccidioidiomycosis or penicilliosis (80 %), coccidioidiomycosis (60 %), aspergillosis (10 %) and sporotrichosis [55, 60]. However, this method is currently the best validated method for the diagnosis of histoplasmosis in HIV-infected patients. Not commercialized, it is hardly used out of the USA [24, 61].

The IMMY ALPHA *Histoplasma* antigen EIA has been marketed since 2006. Sensitivity is 71 % and specificity is 98 % in urine. Using the same detection and antigen capture technique with polyclonal antigens, similar cross-reactions are observed with fungal pathogens (Paracoccidioidiomycosis, coccidioidiomycosis and blastomycosis) [62]. This first test seems less utilized, having generated debates in the literature [62–64]. However, recent modifications and the development of monoclonal antibodies seem very promising [65, 66].

The Mycotic Disease Branch of the Centers for Disease Control and Prevention (CDC) also developed an EIA *Histoplasma* antigen detection method with a sensitivity of 81 % and a specificity of 95 % in the urine of HIV patients in Guatemala. Cross-reactions were only observed for paracoccidioidiomycosis [67]. This test is routinely used in Colombia and is currently being evaluated on the Guiana Shield (ClinicalTrials.gov Registration number: NCT01884779).

Since MVista test can't be performed out of the MVista laboratory in Indianapolis, the IMMY and CDC EIA tests developments are of importance, because they are designed to improve methods for the detection of *Histoplasma capsulatum* in regions with limited resources where the organism is endemic and where delayed diagnosis of histoplasmosis results in high mortality rates.

False positives have been observed with the Platelia *Aspergillus* EIA (BioRad) in the serum of 50–70 % of patients with confirmed histoplamosis [68–71]. This cross-reaction may be of interest in hospitals that do not have access to *Histoplasma* EIAs, by taking into consideration the epidemiologic context and other potential causes of false positives in the Platelia *Aspergillus* test [71].

The molecular diagnosis by Polymerase Chain Reaction (PCR) gives rapid results with good sensitivity and specificity in tissues and body fluids of HIV-infected patients [72, 73]. However, its place in patient care is not clear, since it has not been externally validated and none of the methods have been commercialized [57, 74]. Recently, a loop-mediated isothermal amplification (LAMP) assay, a potential inexpensive point of care diagnostic tool, proved the concept that the assay can be used to detect *Histoplasma* DNA in urine. Still, further evaluation of this assay using body fluid samples from a larger patient population is warranted [75].

Treatment

The treatment of *Histoplasma capsulatum var. capsulatum* infection in HIV-infected patients has led to recommendations in the USA and in France [76, 77]. However, there are few recent data based on randomized controlled trials: the 2007 North American recommendations are still valid [78, 79].

While waiting for mycological confirmation of the diagnosis of a patient with a strong suspicion of histoplasmosis with or without severity symptoms, clinicians have two options for the treatment induction: intravenous (IV) amphotericin B or oral itraconazole. Although amphotericin B is fungicidal and has shown its efficacy in terms of survival, it is also nephrotoxic [80]. Itraconazole is fungistatic and is associated with drug interactions that complicate patient care in the context of profound immunosuppression. This, added to the diminished bioavailability of itraconazole in HIV patients, makes the recommended serum concentration level long and difficult to reach.

In a randomized clinical trial, IV liposomal amphotericin B was more effective than deoxycholate amphotericin B, with a quicker clinical response, a decreased toxicity, and a decreased mortality [80]. Thus, for the induction treatment of moderately severe and severe presentations of disseminated histoplasmosis, liposomal amphotericin B (3 mg/kg/day) is the recommended strategy for 2 weeks or until clinical improvement. A relay with oral itraconazole (200 mg three times a day for 3 days, then twice a day) must then be initiated for at least one year [78].

In order to avoid overlooking presentations with high short-term lethality, and to better guide treatment choice, studies were led to identify risk factors associated with severe histoplasmosis [40, 51]; these serve as the basis for the 2004 definition of histoplasmosis severity by the CDC [81]. Severe cases of histoplasmosis were defined as patients presenting one or more of the following criteria: temperature >39 °C, systolic blood pressure <90 mmHg, arterial oxygen pressure <70 mmHg, weight loss greater than 5 %, a Karnofsky score <70, haemoglobin concentration <10 g/dL, neutrophil counts $<1000/mm^3$, platelet count $<100\ 000/mm^3$, aspartate aminotransférase >2.5 times the normal threshold, serum bilirubine or creatinine exceeding twice the normal threshold, albumine concentration < 3.5 g/dL, the presence of a coagulopathy, the presence of another organ dysfunction or confirmed meningitis [81]. This definition seems scarcely used in routine, and no severity score is available to guide clinicians in their therapeutic choices.

In severe forms of histoplasmosis, renal failure is often described: either associated with multiorgan failure, or secondary to amphotericin B nephrotoxicity. Thus, clinicians tend to switch to itraconazole in order to not aggravate renal failure. However, the poor prognosis of these clinical presentations justifies the continuation of the most effective drug, amphotericin B, despite its nephrotoxicity. In most cases, renal function is restored *ad integrum,* despite the use of this nephrotoxic therapy [78].

For non-severe cases, itraconazole is the first-line treatment with the same protocol as above. The response is positive in 85 % of the cases [82].

The surveillance of itraconazole concentrations is recommended, notably when drug interactions are suspected (particularly with protease inhibitors, efavirenz or rifampicine). The surveillance of serum concentrations must take place 7–15 days following initiation. On a random blood sample, the serum concentration of itraconazole must be >1 μg/mL [78]. In terms of intestinal absorption, the itraconazole syrup formulation, prescribed in a fasting patient, seems preferable and better tolerated than capsules prescribed during a meal [76].

The evaluation of treatment efficacy with the regular monitoring of serum and urine *Histoplama* antigen concentrations using MVista *Histoplasma* antigen EIA is only available and recommended in the USA [76, 83]. For countries where this test is not available, under some conditions, notably the knowledge of the local epidemiology of cross-reacting fungal infections, certain authors consider the use of the Platelia *Aspergillus* EIA (BioRad) to monitor the therapeutic efficacy of antifungals [68, 71].

Acute non-disseminated pulmonary presentations in HIV-infected patients with CD4 counts $>300/mm^3$ are treated like immunocompetent individuals [78].

Confirmed neuro-meningeal HIV-associated histoplasmosis is initially treated with IV liposomal amphotericin B (5 mg/kg/day) for 4–6 weeks. A relay with oral itraconazole 200 mg two to three times per day is instated for at least one year, and until the normalization of the CSF abnormalities [78].

Oral posaconazole and voriconazole are considered second-line treatments for non-severe disseminated histoplasmosis.[76]. Fluconazole is less effective than itraconazole, but may be used as a second-line treatment in itraconazole-intolerant patients at a dose of 800 mg/day [84].

Antiretroviral treatment must be introduced rapidly following the initiation of the antifungal treatment. Immune reconstitution inflammatory syndromes have been described, but are mostly non-severe and are not considered sufficient to warrant delaying antiretrovirals to restore cellular immunity, the key defense against *Histoplasma capsulatum* infection [76, 78].

Prevention

Maintenance therapy (secondary prophylaxis) uses oral itraconazole 200 mg/day. It must be continued for life if the immunosuppression persists or if histoplasmosis relapses despite an appropriate treatment. Primary prophylaxis with itraconazole (200 mg daily) is recommended in HIV-infected patients with CD4 cell counts <150 cells/mm3 in specific areas of endemicity where the incidence of histoplasmosis is >10 cases per 100 patient-years [78]. The USA is the only endemic area recommending primary prophylaxis against histoplasmosis in immunosuppressed individuals, a strategy that has recently been shown to be cost-effective, particularly in low and middle income settings [85].

To our knowledge, the USA is also the only one to edit detailed recommendations regarding occupational or environmental exposure to protect workers at risk of histoplasmosis [86].

Recently, concerns about immunosuppressed travelers have been published. These patients represent an increasing group of travelers, for business or tourism. Those with severe cellular immunodeficiency, like advanced HIV infection, display the highest risk of fungal infections. Thus, a systematic visit in a travel clinic for immunocompromised patients traveling to the tropics ensures that the specific risks of acquiring fungal infections (and others) are understood. When immunocompromised hosts return to their area of residence, a nonbacteriologically documented, potentially severe, febrile pneumonia, with or without dissemination signs (skin lesions, cytopenia) should alert for travel-acquired fungal infection, even years after return. Localized subcutaneous nodule may be also ascribed to fungal infection. Finally, infectious disease physicians should be aware of major clinical patterns of travel-acquired fungal infection, as well as the fungi involved, and risk factors according to the geographical area visited [87].

Conclusion

Nowadays, the real burden of histoplasmosis is not known, it is only based on estimates and not on hard data. Histoplasmosis is still a public health problem during the HAART era, notably in HIV-infected individuals. The medical mycological iceberg statement, written 40 years ago, is still ongoing throughout the world [29]. Outside of research centers or reference centers for the diagnosis of histoplasmosis, in most of the countries where it is endemic, the disease remains either mistaken for tuberculosis or neglected. There are still research needs and accomplishments to target in order to better understand, diagnose and treat individuals infected with *H. capsulatum*. "*Know your epidemic, know your response*"; it is time to act against histoplasmosis [15, 17].

Acknowledgments "This work has benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-0025)."

Dr. Adenis and research team (Inserm CIC 1424) are leading a research project, in close collaboration with CDC MDB and the foundation for scientific research in Suriname, using the EIA CDC test for the detection of *Histoplasma* antigen in urine and serum (ClinicalTrials.gov Registration number: NCT01884779).

Compliance with Ethics Guidelines

Conflict of Interest Antoine A. Adenis, Christine Aznar, and Pierre Couppié declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Darling ST. A protozoon general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymphnodes. JAMA. 1906;46:1283–5.
2. Wheat LJ, Connolly-Stringfield PA, Baker RL, Curfman MF, Eads ME, Israel KS, et al. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and review of the literature. Medicine (Baltimore). 1990;69(6):361–74.
3. Gutierrez ME, Canton A, Sosa N, Puga E, Talavera L. Disseminated histoplasmosis in patients with AIDS in Panama: a review of 104 cases. Clin Infect Dis. 2005;40(8):1199–202.
4. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, Vaz T, et al. AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. AIDS. 2008;22(9):1047–53.
5. Arango M, Castaneda E, Agudelo CI, De Bedout C, Agudelo CA, Tobon A, et al. Histoplasmosis: results of the Colombian national survey, 1992-2008. Biomedica. 2011;31(3):344–56.
6. Pontes LB, Leitao Tdo M, Lima GG, Gerhard ES, Fernandes TA. Clinical and evolutionary characteristics of 134 patients with disseminated histoplasmosis associated with AIDS in the State of Ceara. Rev Soc Bras Med Trop. 2010;43(1):27–31.
7. Pan B, Chen M, Pan W, Liao W. Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases. Mycoses. 2013;56(3):212–21.

8. Peigne V, Dromer F, Elie C, Lidove O, Lortholary O. Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. Am J Trop Med Hyg. 2011;85(5):934–41.
9. Antinori S, Magni C, Nebuloni M, Parravicini C, Corbellino M, Sollima S, et al. Histoplasmosis among human immunodeficiency virus-infected people in Europe: report of 4 cases and review of the literature. Medicine (Baltimore). 2006;85(1):22–36.
10. Ashbee HR, Evans EG, Viviani MA, Dupont B, Chryssanthou E, Surmont I, et al. Histoplasmosis in Europe: report on an epidemiological survey from the European Confederation of Medical Mycology Working Group. Med Mycol. 2008;46(1):57–65.
11. Kauffman CA. Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev. 2007;20(1):115–32.
12. Jones PG, Cohen RL, Batts DH, Silva Jr J. Disseminated histoplasmosis, invasive pulmonary aspergillosis, and other opportunistic infections in a homosexual patient with acquired immune deficiency syndrome. Sex Transm Dis. 1983;10(4):202–4.
13. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Council of State and Territorial Epidemiologists; AIDS Program, Center for Infectious Diseases. MMWR Morb Mortal Wkly Rep. 1987;36 Suppl 1:1S-15S.
14. Homei A, Worboys M. Fungal Disease in Britain and the United States 1850-2000 Mycoses and Modernity. 2013/11/15 ed. Centre for the History of Science TaM, University of Manchester, England (http://chstm.manchester.ac.uk), editor. Manchester, England: Palgrave Macmillan; 2013.
15. 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. 2013.
16. Myint T, Al-Hasan MN, Ribes JA, Murphy BS, Greenberg RN. Temporal Trends, Clinical Characteristics, and Outcomes of Histoplasmosis in a Tertiary Care Center in Kentucky, 2000 to 2009. J Int Assoc Provid AIDS Care. 2013
17. Nacher M, Adenis A, Mc Donald S. Do Socorro Mendonca Gomes M, Singh S, Lopes Lima I, et al. Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage. PLoS Negl Trop Dis. 2013;7(11):e2319.
18. Brandt ME, Gómez BL, Warnock D. Histoplasma, Blastomyces, Coccidioides, and other dimorphic fungi causing systemic mycoses In: D VJaW, editor. Manual of Clinical Microbiology, 10th edition. Washington DC; 2011. p. 1902-18.
19. Edwards JA, Rappleye CA. Histoplasma mechanisms of pathogenesis–one portfolio doesn't fit all. FEMS Microbiol Lett. 2011;324(1):1–9.
20. Kasuga T, White TJ, Koenig G, McEwen J, Restrepo A, Castaneda E, et al. Phylogeography of the fungal pathogen Histoplasma capsulatum. Mol Ecol. 2003;12(12):3383–401.
21. Loulergue P, Bastides F, Baudouin V, Chandenier J, Mariani-Kurkdjian P, Dupont B, et al. Literature review and case histories of Histoplasma capsulatum var. duboisii infections in HIV-infected patients. Emerg Infect Dis. 2007;13(11):1647–52.
22. Karimi K, Wheat LJ, Connolly P, Cloud G, Hajjeh R, Wheat E, et al. Differences in histoplasmosis in patients with acquired immunodeficiency syndrome in the United States and Brazil. J Infect Dis. 2002;186(11):1655–60.
23. Goldani LZ, Aquino VR, Lunardi LW, Cunha VS, Santos RP. Two specific strains of Histoplasma capsulatum causing mucocutaneous manifestations of histoplasmosis: preliminary analysis of a frequent manifestation of histoplasmosis in southern Brazil. Mycopathologia. 2009;167(4):181–6.
24. Couppie P, Aznar C, Carme B, Nacher M. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis. 2006;19(5):443–9.
25. Smith JA, Jt R, Kauffman CA. Cutaneous manifestations of endemic mycoses. Curr Infect Dis Rep. 2013;15(5):440–9.
26. Muniz Mde M, Morais ESTP, Meyer W, Nosanchuk JD, Zancope-Oliveira RM. Comparison of different DNA-based methods for molecular typing of Histoplasma capsulatum. Appl Environ Microbiol. 2010;76(13):4438–47.
27. Wheat LJ. Histoplasmosis: a review for clinicians from non-endemic areas. Mycoses. 2006;49(4):274–82.
28. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol. 2011;49(8):785–98.
29. Ajello L. The medical mycological iceberg. HSMHA Health Rep. 1971;86(5):437–48.
30. Skillman D, Davis B, Harris JR, Nett RJ. Histoplasmosis in a state where it is not known to be endemic–Montana, 2012–2013. MMWR Morb Mortal Wkly Rep. 2013;62(42):834–7.
31. Allard A, Décarie D, Grenier JL, Lacombe MC, Levac F. Histoplasmosis outbreak associated with the renovation of an old house - Quebec, Canada, 2013. MMWR Morb Mortal Wkly Rep. 2014;62(51–52):1041–4.
32. Cruz R. Histoplasma capsulatum var. capsulatum Darling. Rev Chilena Infectol. 2013;30(3):311–2.
33. Garsaud P, Boisseau-Garsaud AM, Desbois N, Verneuil L, Cales-Quist D, Helenon R, et al. Epidemiology of histoplasmosis in the French West Indies (Martinique). Trans R Soc Trop Med Hyg. 1999;93(3):264–7.
34. Fernandez Andreu CM, Martinez Machin G. The sources of histoplasmosis infection on the Isla de la Juventud, Cuba. Rev Inst Med Trop Sao Paulo. 1992;34(5):441–6.
35. Gopalakrishnan R, Nambi PS, Ramasubramanian V, Abdul Ghafur K, Parameswaran A. Histoplasmosis in India: truly uncommon or uncommonly recognised? J Assoc Physicians India. 2012;60:25–8.
36. Bulmer AC, Bulmer GS. Incidence of histoplasmin hypersensitivity in the Philippines. Mycopathologia. 2001;149(2):69–71.
37. Chakrabarti A, Slavin MA. Endemic fungal infections in the Asia-Pacific region. Med Mycol. 2011;49(4):337–44.
38. McLeod DS, Mortimer RH, Perry-Keene DA, Allworth A, Woods ML, Perry-Keene J, et al. Histoplasmosis in Australia: report of 16 cases and literature review. Medicine (Baltimore). 2011;90(1):61–8.
39. Wheat J. Endemic mycoses in AIDS: a clinical review. Clin Microbiol Rev. 1995;8(1):146–59.
40. Hajjeh RA, Pappas PG, Henderson H, Lancaster D, Bamberger DM, Skahan KJ, et al. Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. Clin Infect Dis. 2001;32(8):1215–20.
41. 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(2):239–40.
42. Baddley JW, Sankara IR, Rodriquez JM, Pappas PG, Many Jr WJ. Histoplasmosis in HIV-infected patients in a southern regional medical center: poor prognosis in the era of highly active antiretroviral therapy. Diagn Microbiol Infect Dis. 2008;62(2):151–6.
43. Moquet O, Blanchet D, Simon S, Veron V, Michel M, Aznar C. Histoplasma capsulatum in Cayenne. Mycopathologia: French Guiana; 2012.
44. Hanf M, Adenis A, Couppie P, Carme B, Nacher M. HIV-associated histoplasmosis in French Guiana: recent infection or reactivation? AIDS. 2010;24(11):1777–8.
45. Kroetz DN, Deepe GS. The role of cytokines and chemokines in Histoplasma capsulatum infection. Cytokine. 2012;58(1):112–7.
46. Hanf M, Adenis A, Carme B, Couppie P, Nacher M. Disseminated Histoplasmosis Seasonal Incidence Variations: A Supplementary Argument for Recent Infection? J AIDS Clin Res. 2012;2012:3(8).
47. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, Brewer J, et al. Prospective study of histoplasmosis in patients

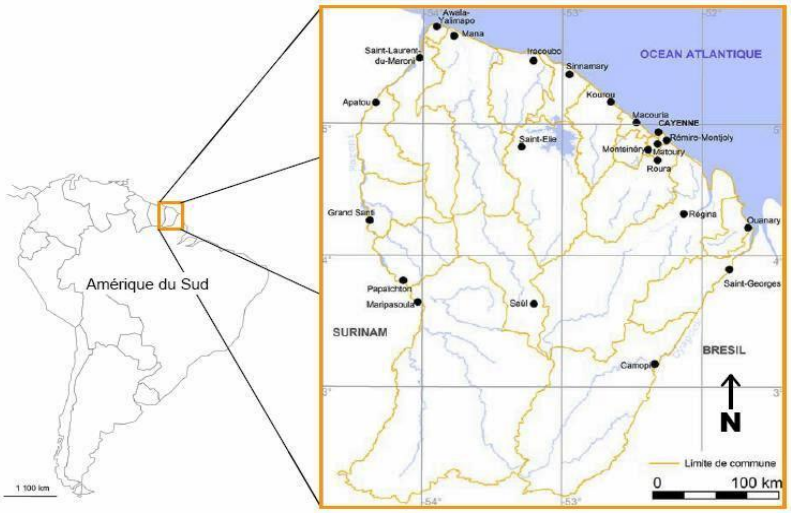
infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. Clin Infect Dis. 1997;24(6):1195–203.
48. Segura L, Rojas M, Pelaez N, Shor-Posner G, RosaRe D, Moreno J, et al. Disseminated histoplasmosis and human immunodeficiency virus type 1 infection: risk factors in Guatemala. Clin Infect Dis. 1997;25(2):343–4.
49. Nacher M, Adenis A, Blanchet D, Vantilcke V, Demar M, Basurko C, et al. Risk Factors for Disseminated Histoplasmosis in a Cohort of HIV-Infected Patients in French Guiana. PLoS Negl Trop Dis. 2014;8(1):e2638.
50. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, infectious diseases community of practice. Am J Transplant. 2012;12(9):2414–28.
51. Wheat LJ, Chetchotisakd P, Williams B, Connolly P, Shutt K, Hajjeh R. Factors associated with severe manifestations of histoplasmosis in AIDS. Clin Infect Dis. 2000;30(6):877–81.
52. Breton G, Adle-Biassette H, Therby A, Ramanoelina J, Choudat L, Bissuel F, et al. Immune reconstitution inflammatory syndrome in HIV-infected patients with disseminated histoplasmosis. AIDS. 2006;20(1):119–21.
53. Adenis A, Nacher M, Hanf M, Basurko C, Dufour J, Huber F, et al. Tuberculosis and Histoplasmosis among Human Immunodeficiency Virus-Infected Patients: A Comparative Study. Am J Trop Med Hyg. 2014.
54. Wheat LJ. Current diagnosis of histoplasmosis. Trends Microbiol. 2003;11(10):488–94.
55. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis. 2011;53(5):448–54.
56. Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. Clin Infect Dis. 2005;40(6): 844–52.
57. Kauffman CA. Histoplasmosis. Clin Chest Med. 2009;30(2):217–25.
58. Connolly PA, Durkin MM, Lemonte AM, Hackett EJ, Wheat LJ. Detection of histoplasma antigen by a quantitative enzyme immunoassay. Clin Vaccine Immunol. 2007;14(12):1587–91.
59. Hage CA, Davis TE, Fuller D, Egan L, Witt 3rd JR, Wheat LJ, et al. Diagnosis of histoplasmosis by antigen detection in BAL fluid. Chest. 2010;137(3):623–8.
60. Assi M, Lakkis IE, Wheat LJ. Cross-reactivity in the Histoplasma antigen enzyme immunoassay caused by sporotrichosis. Clin Vaccine Immunol. 2011;18(10):1781–2.
61. Gutierrez ME, Canton A, Connolly P, Zarnowski R, Wheat LJ. Detection of Histoplasma capsulatum antigen in Panamanian patients with disseminated histoplasmosis and AIDS. Clin Vaccine Immunol. 2008;15(4):681–3.
62. Cloud JL, Bauman SK, Neary BP, Ludwig KG, Ashwood ER. Performance characteristics of a polyclonal enzyme immunoassay for the quantitation of Histoplasma antigen in human urine samples. Am J Clin Pathol. 2007;128(1):18–22.
63. LeMonte A, Egan L, Connolly P, Durkin M, Wheat LJ. Evaluation of the IMMY ALPHA Histoplasma antigen enzyme immunoassay for diagnosis of histoplasmosis marked by antigenuria. Clin Vaccine Immunol. 2007;14(6):802–3.
64. Cloud JL, Bauman SK, Pelfrey JM, Ashwood ER. Biased report on the IMMY ALPHA Histoplasma antigen enzyme immunoassay for diagnosis of histoplasmosis. Clin Vaccine Immunol. 2007;14(10): 1389–90. *author reply 90-1*.
65. Theel ES, Jespersen DJ, Harring J, Mandrekar J, Binnicker MJ. Evaluation of an Enzyme Immunoassay for Detection of Histoplasma capsulatum Antigen from Urine Specimens. J Clin Microbiol. 2013.
66. Zhang X, Gibson B, Jr., Daly TM. Evaluation of commercially available reagents for the diagnosis of Histoplasmosis infection in immunocompromised patients. J Clin Microbiol. 2013.
67. Scheel CM, Samayoa B, Herrera A, Lindsley MD, Benjamin L, Reed Y, et al. Development and evaluation of an enzyme-linked immunosorbent assay to detect Histoplasma capsulatum antigenuria in immunocompromised patients. Clin Vaccine Immunol. 2009;16(6):852–8.
68. 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(12):941–3.
69. Wheat LJ, Hackett E, Durkin M, Connolly P, Petraitiene R, Walsh TJ, et al. Histoplasmosis-associated cross-reactivity in the BioRad Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2007;14(5):638–40.
70. Xavier MO, Pasqualotto AC, Cardoso IC, Severo LC. Cross-reactivity of Paracoccidioides brasiliensis, Histoplasma capsulatum, and Cryptococcus species in the commercial Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2009;16(1):132–3.
71. Riviere S, Denis B, Bougnoux ME, Lanternier F, Lecuit M, Lortholary O. Serum Aspergillus galactomannan for the management of disseminated histoplasmosis in AIDS. Am J Trop Med Hyg. 2012;87(2):303–5.
72. Simon S, Veron V, Boukhari R, Blanchet D, Aznar C. Detection of Histoplasma capsulatum DNA in human samples by real-time polymerase chain reaction. Diagn Microbiol Infect Dis. 2010;66(3):268–73.
73. Munoz C, Gomez BL, Tobon A, Arango K, Restrepo A, Correa MM, et al. Validation and clinical application of a molecular method for identification of Histoplasma capsulatum in human specimens in Colombia, South America. Clin Vaccine Immunol. 2010;17(1):62–7.
74. Kauffman CA. Diagnosis of histoplasmosis in immunosuppressed patients. Curr Opin Infect Dis. 2008;21(4):421–5.
75. Scheel CM, Zhou Y, Theodoro RC, Abrams B, Balajee SA, Litvintseva AP. Development of a Loop-Mediated Isothermal Amplification Method for Detection of Histoplasma capsulatum DNA in Clinical Samples. J Clin Microbiol. 2013;52(2):483–8.
76. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents:recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.; 2013. p. M11-7.
77. Yeni P. Prise en charge médicale des personnes infectées par le VIH : recommandations du groupe d'expert, rapport 2010. La documentation française ed. Paris: Ministère de la Santé et des Sports; 2010.
78. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, et al. 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):807–25.
79. Pappas PG. The role of azoles in the treatment of invasive mycoses: review of the Infectious Diseases Society of America guidelines. Curr Opin Infect Dis. 2011;24 suppl 2:S1–13.
80. Johnson PC, Wheat LJ, Cloud GA, Goldman M, Lancaster D, Bamberger DM, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. Ann Intern Med. 2002;137(2):105–9.
81. Benson CA, Kaplan JE, Masur H, Pau A, Holmes KK. Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/Infectious Diseases Society of America. MMWR Recomm Rep. 2004;53(RR-15):1–112.
82. Wheat J, Hafner R, Korzun AH, Limjoco MT, Spencer P, Larsen RA, et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clin Trial Group Am J Med. 1995;98(4):336–42.
83. Hage CA, Kirsch EJ, Stump TE, Kauffman CA, Goldman M, Connolly P, et al. Histoplasma antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. Clin Vaccine Immunol. 2011;18(4):661–6.

84. Wheat J, MaWhinney S, Hafner R, McKinsey D, Chen D, Korzun A, et al. Treatment of histoplasmosis with fluconazole in patients with acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Acquired Immunodeficiency Syndrome Clinical Trials Group and Mycoses Study Group. Am J Med. 1997;103(3):223–32.
85. Nacher M, Adenis A, Basurko C, Vantilcke V, Blanchet D, Aznar C, et al. Primary prophylaxis of disseminated histoplasmosis in HIV patients in French Guiana: arguments for cost effectiveness. Am J Trop Med Hyg. 2013;89(6):1195–8.
86. Lenhart SW, Schafer MP, Singal M, Hajjeh RA. Histoplasmosis protecting worker at risk revised edition. In: (DHHS-NIOSH) CfDCap, editor. Atlanta: CDC; 2004. p. 39.
87. Lortholary O, Charlier C, Lebeaux D, Lecuit M, Consigny PH. Fungal infections in immunocompromised travelers. Clin Infect Dis. 2013;56(6):861–9.

3. Contexte général d'étude

3.1. La Guyane française : un département français sur le plateau des Guyanes et en Amazonie

La Guyane française est un département français d'outre-mer localisé au Nord-Est de l'Amérique du Sud, proche de l'équateur entre 2° et 4° de latitude Nord, entouré par le Suriname à l'Ouest et le Brésil au Sud (Figure 7). Plus vaste région de France, elle s'étend sur une superficie de 84 000 km², équivalente à celle du Portugal ou de l'Angleterre. Son climat est de type équatorial, chaud et humide, avec plus de 90% du territoire occupé par la forêt tropicale. L'essentiel de la population totale, estimée par l'INSEE à 244 118 habitants au 1er janvier 2013, se concentre sur une plaine côtière de 40 km de large. Les principales agglomérations sont la région de Cayenne, appelée « Ile de Cayenne », les villes de Kourou et de Saint Laurent du Maroni.



(Source : Conseil Général de la Guyane)

Figure 7 : Situation géographique de la Guyane française

La Guyane française appartient au plateau des Guyanes ou bouclier guyanais : écosystème bien individualisé dont les contours sont discutés selon que l'on se place d'un point de vue géophysique ou biogéographique (46). En 2002, un consensus régional intergouvernemental a délimité un ensemble qualifié d' « écorégion » (Figure 8), visant notamment à mettre en œuvre une initiative à l'échelle du plateau des Guyanes pour la protection, la conservation et la valorisation des richesses naturelles et humaines (46).

De plus, la Guyane française et le plateau des Guyanes sont régulièrement inclus dans un vaste ensemble intitulé biome amazonien ou écorégion amazonienne (Figure 9) (47).

(Source : http://guianashield.org/index.php/maps)

Figure 8 : Situation géographique de la Guyane française au sein du plateau des Guyanes

(Source : FAO (48))

Figure 9 : Situation géographique du de la Guyane française au sein du biome amazonien

Le biome ou « macroécosystème » ou « zone de vie majeure » pouvant être définit comme un ensemble d'écosystèmes présentant des similarités importantes au niveau climatique et des espèces hébergées, il est nommé d'après la végétation qui y prédomine (49).
Ainsi, la Guyane française au sein du plateau des Guyanes appartient à un grand ensemble comprenant le bassin amazonien (vaste aire hydrographique de drainage des eaux dans le fleuve Amazone) étendu au Sud à la forêt sèche bolivienne de Chiquitanos et, au Nord, aux forêts tropicales du plateau des Guyanes (48). Si l'ensemble des eaux de ces régions ne se draine pas dans l'Amazone, on y retrouve des similarités d'habitats communément décrits comme appartenant à la forêt tropicale amazonienne (47, 50).

Dans sa position équatoriale au sein du vaste biome amazonien, reconnu comme une des principales zones de haute biodiversité à l'échelle mondiale, la Guyane française partage des caractéristiques biogéographiques et certains aspects de sa biodiversité avec le Brésil, la Bolivie, le Pérou, l'Equateur, la Colombie, le Venezuela, le Guyana et le Suriname.

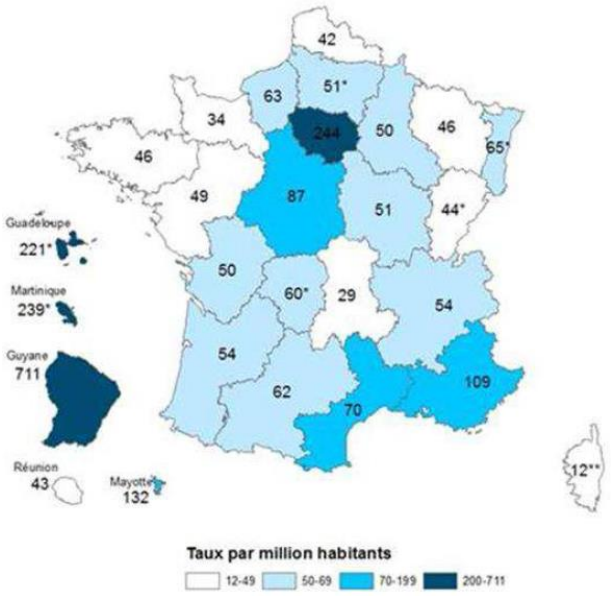
Cette proximité avec l'Equateur confère également à la Guyane française une grande biodiversité de pathogènes, responsables de maladies infectieuses et parasitaires chez l'Homme et l'Animal (51). Ces pathogènes évoluant au sein d'un même écosystème devraient être observés communément dans les différents pays de la région, avec de probables variations quantitatives et qualitatives, sous réserve que les conditions connues comme nécessaires au développement des maladies soient réunies et que chaque pays dispose d'un système de surveillance ou d'individus rapportant publiquement l'information. Ceci implique que la Guyane française partage des problématiques de santé publique, notamment au plan des maladies infectieuses humaines, avec les pays du plateau des Guyanes et plus largement du biome amazonien.

Pour mémoire, ce constat est l'hypothèse fondatrice à la dynamique de réflexions et de projets mis en œuvre dans le cadre de ce travail de thèse.
Hypothèse selon laquelle la problématique majeure de santé publique représentée par l'histoplasmose chez les patients infectés par le VIH, telle qu'elle est décrite en Guyane française, devrait être observée à minima dans les pays du plateau des Guyanes voire du biome amazonien (52). Si elle n'est pas ou peu observée dans les pays concernés, elle devrait être observable dans le cadre de programmes de recherche ou de collaborations.

3.2. Une épidémiologie particulière de l'infection par le VIH

Afin de cerner l'importance de la problématique du travail de thèse, une synthèse des principales données épidémiologiques déclarées à propos de l'infection par le VIH est présentée, sans avoir l'objectif de décrire toute la problématique de l'infection par le VIH en Guyane française.

La Guyane française est le département français le plus touché par l'épidémie de VIH/SIDA, bien que le nombre de découvertes de séropositivité et de cas incidents de SIDA aient diminués respectivement de 14% de 2011 à 2014 et de 90% de 2003 à 2013 (53).
Pour l'année 2014, on estime à 711 par million d'habitants (IC95% : [471-948]) le nombre de découvertes de sérologies positives à l'infection par le VIH, soit 7 fois plus que la moyenne nationale française estimée à 100 par million d'habitants [92-107] (FIGURE 10) (53). Pour l'année 2013, on retrouve une incidence de 67 diagnostics de SIDA par million d'habitants [IC95% : 46-88], soit pratiquement 5 fois plus que la moyenne nationale française (14/million d'habitants [IC95% : 12-16], hors Ile de France) (53).



Source : DO VIH France au 31/12/2014, corrigées pour les délais et la sous déclaration.

Figure 10 : Nombre de découvertes de séropositivité VIH par millions d'habitants, année 2014, France (53)

La prévalence de l'infection par le VIH en population générale, estimée sur la base des données exhaustives du Registre d'issue de Grossesse Informatisé de Guyane (RIGI), est régulièrement supérieure à 1% depuis le début des années 1990 (54). Le dernier chiffre disponible retrouve un niveau de prévalence à 1,1% chez les parturientes de Guyane en 2015 (55). Cette

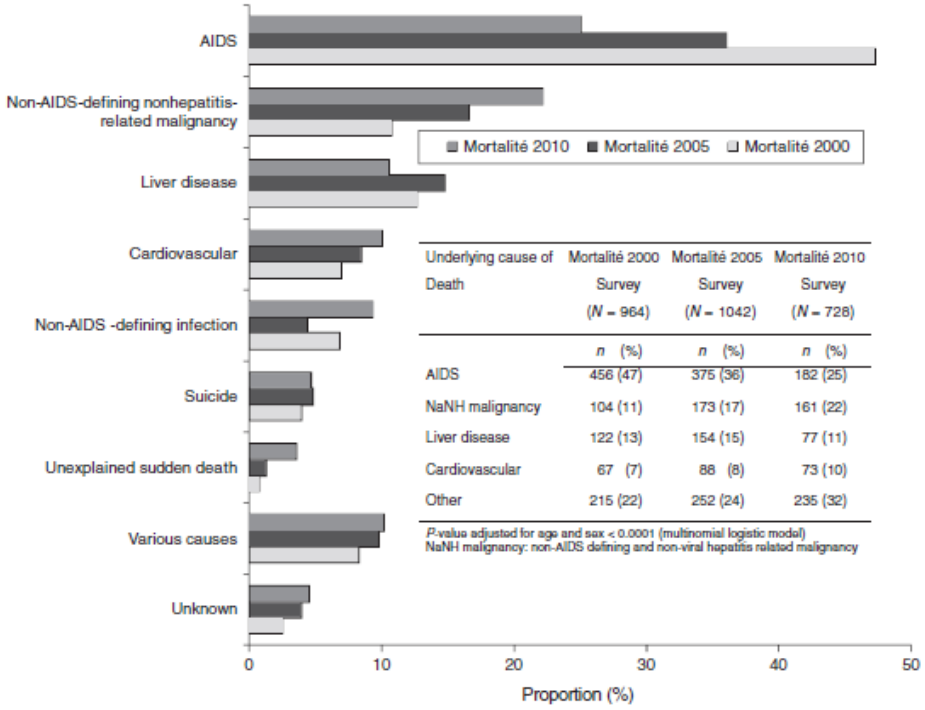
méthode d'estimation de la prévalence de l'infection par le VIH en population générale est habituellement considérée comme un bon indicateur de suivi de l'épidémie dans la mesure où le mode de contamination déclaré majoritaire est représenté par les rapports hétérosexuels (98% des nouveaux diagnostics d'infection par le VIH entre 2004 et 2013 en Guyane) (53).

Sur la période 2004-2013, au sein des patients nouvellement diagnostiqués on retrouve autant d'hommes que de femmes (sex ratio moyen H:F=1), majoritairement de la classe d'âge 25-49 ans (68%) et nés sur le continent américain hors Guyane (51%) (53).

Malgré des efforts importants pour le dépistage de l'infection par le VIH, illustrés par l'augmentation de 10% du nombre de sérologies VIH réalisées en Guyane entre 2011 et 2014, soit deux fois plus que la moyenne nationale française, la proportion de patients dépistés tardivement (CD4<200/mm3) reste stable autour de 30% des nouveaux diagnostics (53, 56).

Sur la période 2004-2013, les hommes (60%), âgés de 25 à 49 ans (72%) et nés hors de France (70%) représentent la majorité des cas de SIDA notifiés (53). Seulement 47% de ces nouveaux cas de SIDA avaient connaissance de leur séropositivité au VIH au moment du diagnostic (53). Les cinq pathologies opportunistes inaugurales du SIDA notifiées sur cette période sont : l'histoplasmose (19%), la tuberculose pulmonaire (11%), la candidose œsophagienne (11%), la toxoplasmose cérébrale (10%) et la pneumocystose (8%) (53).

Peu de données récentes sont disponibles concernant la mortalité brute et les causes de décès des patients infectés par le VIH en Guyane française. D'après les données de déclarations obligatoires SIDA seuls 67 décès sont rapportés sans autre précision pour la période 2005-2012 (57). Les enquêtes « Mortalité 2000 », « Mortalité 2005 » et « Mortalité 2010 », menées auprès d'un grand nombre d'investigateurs français, retrouvent une tendance à la baisse de la mortalité brute, de 1,5% en 2000 à 0,9% en 2010 (58). Si les pathologies opportunistes survenant au stade sida ou « causes SIDA » restent la première cause de décès sur la période, on observe une tendance à la baisse s'accompagnant d'une augmentation de la part des décès pour des « causes non-SIDA » (Figure 11) (58). Seuls les résultats de l'enquête « Mortalité 2000 » sont déclinés à l'échelle des Antilles et de la Guyane française. Les « causes SIDA » de décès représentent la plupart des décès chez les patients infectés par le VIH dans ces territoires (n=67/81, 83%). Parmi ces « causes SIDA », l'histoplasmose est la première cause de décès en Guyane et à l'échelle des départements français d'Amérique (DFA) au début des années 2000 (Figure 12) (59).



Underlying cause of Death	Mortalité 2000 Survey (*N* = 964)	Mortalité 2005 Survey (*N* = 1042)	Mortalité 2010 Survey (*N* = 728)
	n (%)	*n* (%)	*n* (%)
AIDS	456 (47)	375 (36)	182 (25)
NaNH malignancy	104 (11)	173 (17)	161 (22)
Liver disease	122 (13)	154 (15)	77 (11)
Cardiovascular	67 (7)	88 (8)	73 (10)
Other	215 (22)	252 (24)	235 (32)

P-value adjusted for age and sex < 0.0001 (multinomial logistic model)
NaNH malignancy: non-AIDS defining and non-viral hepatitis related malignancy

Figure 11 : Distribution des causes de décès chez les patients infectés par le VIH, Enquête nationale 2000 (n=964), 2005 (n=1042) et 2010 (n=728), France (58)

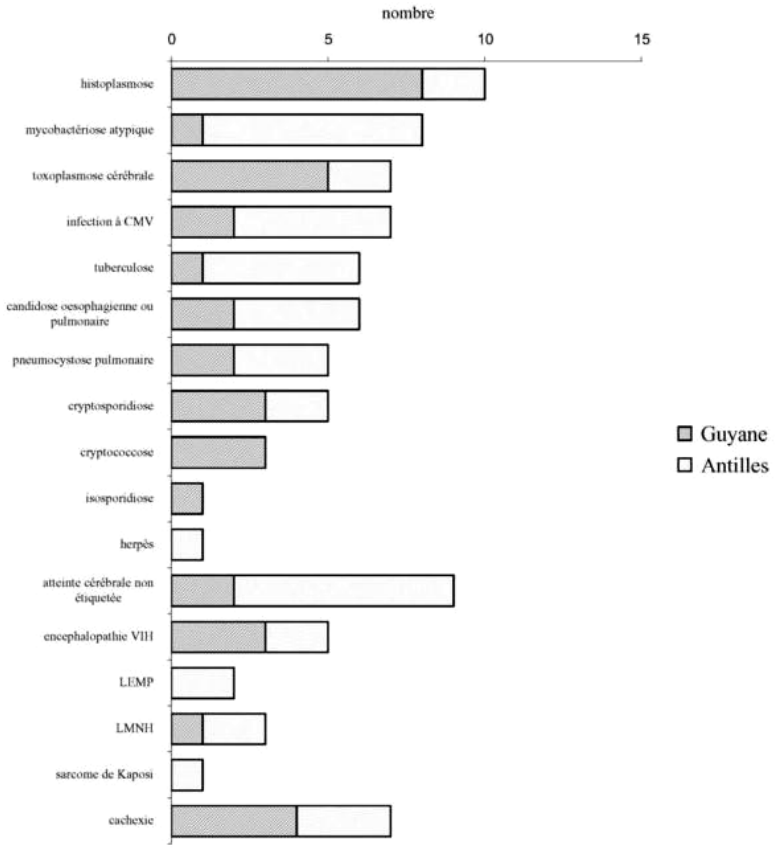


Figure 12 : Pathologies classant sida déclarées chez les personnes décédées de « causes sida » dans les départements français d'Amérique (DFA) en 2000 (Guyane *n*=26, Antilles *n*=28) (59)

Ces données sur les nouvelles infections à VIH, les cas incidents de SIDA et la mortalité des patients infectés par le VIH sont à prendre avec précautions car elles sont soit considérées comme brutes et non corrigées pour la sous déclaration, soit issues d'enquêtes sur des échantillons de patients infectés par le VIH suivis par des investigateurs référents localement pour cette prise en charge. Néanmoins, elles renseignent sur les grandes tendances observables et sont cohérentes avec les données localement renseignées dans la cohorte de patients infectés par le VIH de Guyane française.

Au moment de commencer ce travail de thèse, les données de l'épidémiologie de l'infection par le VIH observée en Guyane française rendent compte de différences importantes par rapport à la France hexagonale.

La fréquence de survenue et la mortalité du stade SIDA de l'infection par le VIH reste un problème important chez les patients infectés par le VIH en Guyane française alors que ce poids tend à diminuer en France hexagonale.

L'ordre de fréquence des infections opportunistes classant SIDA et la mortalité imputable à celles-ci retrouvent l'histoplasmose comme principale cause d'infection opportuniste classant SIDA (accompagnée de la tuberculose) et première cause de mortalité au stade SIDA en Guyane française. A la même période, en France hexagonale, c'est la pneumocystose pulmonaire qui est la plus fréquente, accompagnée de la tuberculose, avec une tendance à la baisse de la mortalité liée au SIDA qui se confirme (60, 61). L'histoplasmose y est, à juste titre, reléguée au rang d'infection opportuniste rarement observée.

Ces données confirment que le positionnement biogéographique de la Guyane française influe de manière significative sur l'épidémiologie locale de l'infection par le VIH, avec notamment l'histoplasmose, élevée au rang de priorité de santé publique quand elle considérée comme une curiosité de médecine des voyages dans un contexte européen.

3.3. L'histoplasmose : un sujet d'étude au long cours en Guyane française

3.3.1. Etats des connaissances à l'initiation du travail de thèse

Historiquement, les premières descriptions publiées à propos de l'histoplasmose en Guyane française datent des années 1950 avec l'identification d'*H. capsulatum* à partir d'échantillons de sol puis une campagne d'IDR à l'histoplasmine en population générale retrouvant 32,5% de positivité (62). Ce niveau de prévalence est confirmé par une étude similaire menée en 1966 sur une population de soldats ayant séjourné en Guyane française pendant un an et rapportant 30% de tests cutanés positifs à l'histoplasmine (63).

Les premières descriptions de formes disséminées d'histoplasmose rapidement fatales chez des patients présentant un syndrome d'immunodéficience acquise (SIDA) datent du début des années 1980 (64). Si le premier cas d'histoplasmose disséminée en provenance de Guyane française est identifié en France hexagonale chez un jeune patient haïtien en 1979, officiellement, le premier cas d'histoplasmose chez un patient infecté par le VIH a été confirmé rétrospectivement à l'aide d'un sérum conservé datant de 1982 (64).

Par la suite, la majorité des diagnostics et les principales descriptions publiées sont réalisées par les dermatologues de Guyane à propos de formes cutanées et/ou muqueuses d'histoplasmose chez des patients infectés par le VIH (65). Car, en consultation de Dermatologie-Vénérologie au Centre Hospitalier de Cayenne, les médecins disposent de microscope optique et lisent quotidiennement des frottis de prélèvement cutanés réalisés par leurs soins. Initialement, la pratique de l'examen direct avec une coloration « Giemsa rapide » (RAL 555) des prélèvements cutanéo-muqueux était réalisée à la recherche des formes amastigotes intracellulaires de *Leishmania* spp.. Mais, dans le cytoplasme des cellules ou en dehors, ils retrouvent la forme levure d'*H. capsulatum*, dont la forme et la taille se rapprochent de *Leishmania* spp., sans toutefois retrouvé le kinétoplaste caractéristique de ce dernier. Ainsi, en l'absence de mycologue au laboratoire de l'hôpital de Cayenne, les dermatologues font de plus en plus de diagnostic d'histoplasmose cutanéo-muqueuse ou disséminée chez des patients infectés par le VIH (66-68).

A la fin des années 1990, la fréquence du diagnostic de formes cutanéo-muqueuses d'histoplamose associée au développement progressif d'une expertise en mycologie dans les laboratoires hospitaliers de Guyane française, plus particulièrement sur le site de Cayenne, est à l'origine d'une augmentation croissante du diagnostic d'autres formes d'histoplasmose disséminée, pour l'essentiel chez des patients infectés par le VIH.

Sur la période 1982 à 2007, une étude dans les trois centres hospitaliers de Guyane rapporte 200 cas d'histoplasmose associés à l'infection par le VIH (69). Pour 77,5% des cas, l'histoplasmose était l'événement inaugural du stade SIDA et 92% des patients ne prenaient pas de trithérapie antirétrovirale au diagnostic. Le taux de CD4 était inférieur à 100/mm³ pour 80% des individus et 37% présentaient une infection opportuniste concomitante (notamment la tuberculose pour 8% des cas). La plupart des cas présentaient une fièvre, des adénopathies, des signes pulmonaires ou digestifs, un taux de lacticodeshydrogénase (LDH) supérieur à 4 fois la normale et un taux d'aspartate aminotransférase (TGO) supérieur au taux d'alanine aminotransférase (TGP). Le myélogramme était jugé utile pour le diagnostic, les cultures de tissus ganglionnaire ou hépatique étaient les plus contributives. Un traitement antifongique présomptif était démarré pour 14% des individus, la confirmation diagnostique survenant en moyenne après 29,6 jours de traitement. Le pronostic était marqué par 19,5% de décès dans le mois suivant l'admission.

Une étude sur les seuls cas d'histoplasmose chez des patients infectés par le VIH du Centre Hospitalier de l'Ouest Guyanais, rapporte 82 cas sur la période 2002-2012. Si le profil de patient est similaire, les formes avec atteinte du tractus digestif sont au premier plan et la mortalité à un mois est de 13% (70). A la frontière avec le Suriname, ce sont 41% et 86% des patients des patients infectés par le VIH arrivés fébriles en hospitalisation, avec respectivement un taux de lymphocytes T CD4+<200/mm^3 ou <50/mm^3, pour lesquels l'histoplasmose est le diagnostic principal de sortie (ARTICLE 3).

Avec des définitions diverses dans la littérature, des formes sévères d'histoplasmose sont décrites. Une étude sur la période 1994-2002 rapporte 82 cas d'histoplasmose chez des patients infectés par le VIH, dont 15 cas (18%) à 18 cas (22%) de formes sévères selon la définition employée. Après ajustement, la dyspnée, un taux de plaquettes < 100 000 :mm3 et un taux de LDH > 2 fois la normale étaient associés à la survenue d'un décès dans les trente jours suivant la mise en route d'un traitement antifongique (71).

Une surincidence de cas d'histoplasmose est décrite après la mise en route d'une trithérapie antirétrovirale chez les patients infectés par le VIH suivis en Guyane française. Ces cas correspondent à des syndromes inflammatoires de reconstitutiion immunitaire infectieux ou paradoxaux à *H. capsulatum* (72).

Peu de données sont publiées à propos de l'histoplasmose chez des individus immunocomptétents de Guyane française. Suite aux descriptions princeps de Floch dans les années 1950, les cas décrits concernent essentiellement la prise en charge de nodules pulmonaires isolés chez des militaires non immunodéprimés de retour de Guyane (73-76).

Toutefois, de façon sporadique mais régulière (tous les 12 à 24 mois), des formes sévères disséminées et rapidement fatales d'histoplasmose sont décrites chez des individus qualifiés « d'immunocompétents » (77). La plupart font l'objet d'investigations immunogénétiques à la recherche de déficits immunitaires rares ou isolés, non connus antemortem.

ARTICLE 3 : Fever in hospitalized HIV-infected patients in Western French Guiana: first think histoplasmosis

Author: Vantilcke, V., Boukhari, R., Jolivet, A., Vautrin, C., Misslin, C., **Adenis, A**. and Nacher, M.

Year: 2014 **Journal:** Int J STD AIDS

ISSN: 1758-1052 (Electronic) **DOI:** 10.1177/0956462413516299

Abstract: In Western French Guiana, there was a dramatic increase in HIV prevalence between 1990 and 2000. The present study describes the causes of fever among HIV patients hospitalized in the medical ward of the only hospital in the western part of French Guiana. A retrospective descriptive study was conducted between 1 January 2008 and 30 June 2010 in the department of medicine of Saint Laurent du Maroni Hospital. The main characteristics of 67 patients having presented with fever in the first 48 hours of hospitalization were described. Among patients with CD4 <200/mm3 the main febrile opportunistic infection was disseminated histoplasmosis (41.1%). Among patients with CD4 counts <50/mm3 and fever without focal points 85.7% had disseminated histoplasmosis. Three patients died and all had disseminated histoplasmosis. Disseminated histoplasmosis is the most common febrile opportunistic infection in western French Guiana. Primary prophylaxis with itraconazole among immunocompromised patients seems warranted.

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24598976

Original research article

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
0(0) 1–7

DOI: 10.1177/0956462413516299
std.sagepub.com
SAGE

Fever in hospitalized HIV-infected patients in Western French Guiana: first think histoplasmosis

Vincent Vantilcke[1], Rachida Boukhari[2], Anne Jolivet[1], Cyrille Vautrin[1], Caroline Misslin[1], Antoine Adenis[3,4] and Mathieu Nacher[3,4]

Summary

In Western French Guiana, there was a dramatic increase in HIV prevalence between 1990 and 2000. The present study describes the causes of fever among HIV patients hospitalized in the medical ward of the only hospital in the western part of French Guiana. A retrospective descriptive study was conducted between 1 January 2008 and 30 June 2010 in the department of medicine of Saint Laurent du Maroni Hospital. The main characteristics of 67 patients having presented with fever in the first 48 hours of hospitalization were described. Among patients with CD4 $<200/mm^3$ the main febrile opportunistic infection was disseminated histoplasmosis (41.1%). Among patients with CD4 counts $<50/mm^3$ and fever without focal points 85.7% had disseminated histoplasmosis. Three patients died and all had disseminated histoplasmosis. Disseminated histoplasmosis is the most common febrile opportunistic infection in western French Guiana. Primary prophylaxis with itraconazole among immunocompromised patients seems warranted.

Keywords

HIV, French Guiana, fever, histoplasmosis, primary prophylaxis

Date received: 29 July 2013; accepted: 20 October 2013

Introduction

For the past 20 years, French Guiana has been the French territory with the highest HIV prevalence in mainland and overseas French territories.[1] The western part of French Guiana, which is separated from Suriname by the Maroni river, is very different from the rest of coastal French Guiana. The population consisting mostly of maroons (runaway slaves who formed independent settlements together) often lives in rural areas along the Maroni river and is highly mobile between the French and Surinamese sides of the border. In the early 1990s there were no known HIV infections along the Maroni, but within a decade HIV prevalence exceeded 1% and has remained above 1% since. Boatmen transporting cargo along the river are thought to have been a bridging group between sex workers and the general maroon population.[2]

In the department of medicine of the Hospital of Saint Laurent du Maroni, the only hospital for western French Guiana, over 1 in 10 patients are admitted for an opportunistic infection linked to HIV. Outpatients receive free antiretrovirals and have access to the latest molecules, regular viral load monitoring, and viral genotyping. Clinicians and laboratories are very aware of histoplasmosis, therefore they have locally implemented standardized and proactive diagnostic and therapeutic procedures. If an immunocompromised patient comes with fever and no clear focal sign i.e. pyelonephritis, bacterial pneumonia, a bone marrow sample is rapidly taken, and direct examination followed by fungal culture are systematic. Physicians are prompt to initiate

[1]Centre hospitalier de l'ouest Guyanais, service de médecine, Saint Laurent du Maroni
[2]Centre hospitalier de l'ouest Guyanais, Laboratoire Polyvalent, Saint Laurent du Maroni
[3]Centre d'investigation clinique épidémiologie clinique Antilles Guyane, CIE INSERM 802, Centre hospitalier de Cayenne
[4]Epidemiologie des parasitoses et mycoses tropicales, EA 3593, Université des Antilles et de la Guyane

Corresponding author:
Mathieu Nacher, Centre Hospitalier de Cayenne , Rue des Flamboyants BP 6006 Cayenne Cedex Cayenne, 97306, French Guiana.
Email: mathieu.nacher@ch-cayenne.fr

antifungal treatment when they suspect histoplasmosis may be causing fever (immunosuppression, fever and weight loss, liver enzyme abnormalities, cytopenia...). Presumptive treatment may consist of itraconazole in the absence of severity criteria, or liposomal amphotericin B in the presence of severity signs.[3]

Given the general epidemiological and environmental particularities of French Guiana,[4] and the specific conditions of western French Guiana (rural setting, populations living along the Maroni river, on the Surinamese border, a malaria endemic area, lower access to care), a retrospective study of medical records of HIV patients hospitalized for fever between 1 January 2008 and 30 June 2010 was conducted in this department.

The principal objective of the study was to identify the main febrile opportunistic infections associated with HIV in western French Guiana in order to refine diagnostic and therapeutic decisions in the light of the local epidemiology.

Methods

Study design

The study was retrospective and descriptive. It concerned patients admitted between 1 January 2008 and 30 June 2010.

The three inclusions criteria were: HIV infection, age>15 years and hyperthermia (>38°C). The HIV positive serostatus was either previously known or had been discovered at the time of admission.

Data was extracted from medical files identified by the hospital medical information system. The files of patients with HIV infection hospitalized in the department of medicine were then checked for the other two inclusion criteria. The analysis performed locally on anonymized data extracted from medical records from a single department in a single hospital, performed in order to improve patient care, falls within the authorization granted by the commission nationale informatique et libertés.

When the patients fulfilled the three inclusion criteria other variables were collected such as age, gender, nationality, date of HIV diagnosis, date of hospitalization, immunovirological data, treatment data, clinical and biological data, the final diagnoses, survival or death at the end of hospitalization. Mycologic diagnosis was performed in Saint Laurent du Maroni hospital either by direct examination of fluid or tissue samples or culture, the gold standard of mycological examination. According to EORTC/MSG criteria for fungal diseases,[5] proven histoplasmosis was considered when the fungus was recovered in culture from a specimen or when the microorganism was observed using histopathology or direct microscopy. Cases were considered probable on clinical arguments (hyperthermia, elevated ferritin, elevated LDH, elevated liver enzymes, pancytopenia) and evolution on presumptive antifungal treatment, and a positive *H. capsulatum* P.C.R.[6] In French Guiana, to avoid deaths due to treatment delays, cases with a compatible clinical presentation (immunosuppression, hyperthermia, elevated ferritin, elevated LDH, elevated liver enzymes, pancytopenia) are treated. Here, we considered that patients with a favourable evolution on antifungals had histoplasmosis as reported in the conclusion of the hospitalisation in the patient records.

The diagnosis of bacterial pneumonia relied on the clinical and radiological presentation, positive blood culture, and/or pneumococcal antigen results and evolution after antibiotic treatment.

Patients upon admission get a standard biological screening, immunovirological screening, blood and urine cultures, chest X ray, serologies for toxoplasmosis, hepatitis, CMV, tuberculin test, gastric tubages, often they get PCR for CMV, they are screened for *Cryptococcus* (latex). If there are symptoms orienting towards specific diagnoses or organs ultrasounds, CT scanner, or MRI (in Cayenne) of the pertinent regions are available, endoscopy, bronchiolo-alveolar lavage, *Cryptosporidium*, *Microsporidium*, biopsies of liver or adenopathies are performed when indicated.

Data analysis

Data were entered in an excel file and then analyzed using STATA 8.2® (College Station, TX). The data analysis was descriptive. Univariate analysis was followed by bivariate analysis. Measures of association were obtained. Comparison of binary or qualitative variables was performed using the Chi square test or Fischer's exact test when appropriate. Liver enzymes were considered to be elevated when they exceeded the laboratory threshold. Quantitative variables were compared using Mann Whitney's test or Student's *t* test when appropriate. The sample size was insufficient to perform multivariate analysis.

Results

Population characteristics

There were 67 patients hospitalized during the study period for a total of 81 hospitalizations, hence 1.21 hospitalizations per patient.

The gender-ratio was 1.48 men per woman. The mean age was 40.6 years (range 27–59) in men and 38.97 years (range 16–74) in women without any significant difference between genders ($p = 0.44$); 61% of

XML Template (2013) [30.11.2013–2:32pm] [1–8]
//blnas3/cenpro/ApplicationFiles/Journals/SAGE/3B2/STDJ/Vol00000/130170/APPFile/SG-STDJ130170.3d (STD) [PREPRINTER stage]

the patients were Surinamese and 22% were French; 67.9% of hospitalizations concerned patients who already knew their HIV status at the time of hospitalization (mean 3.8 years, SD 4.33 years).

Diagnosis

There were 24 diagnoses of histoplasmosis (14 certain, 4 probable, and 6 suspected) and 69 other diagnoses. Table 1 shows the Top 10 most common diagnoses; 52% of patients with CD4 counts >200 had bacterial pneumonia.

Among patients with CD4<200/mm^3, histoplasmosis was the main opportunistic infection. Among patients with CD4 <100/mm^3 and isolated fever, 68.4% had histoplasmosis. In those presenting a very profound immunosuppression (CD4<50/mm^3) and fever without infectious focal signs, 85.7% had histoplasmosis.

In 33% of cases, histoplasmosis was associated with another infection: *Mycobacterium avium* complex,[2] cytomegalovirus[2] bacterial pneumonia, salmonellosis, shigellosis.

The diagnosis of histoplasmosis was mostly obtained by the culture of bone marrow aspirates (9/24) or colonic biopsy (4/24). The low sample size did not allow us to do statistics for other diagnoses.

Table 1. Top 10 febrile causes of admission in Saint Laurent du Maroni hospital over a 30-month period.

Final diagnosis	CD4 <200 (n = 56)	CD4 >199 (n = 25)
Histoplasmosis (proven)	15	0
Histoplasmosis (probable)	3	0
Histoplasmosis (suspected)	5	1
Bacterial pneumonia	7	13
Pyelonephritis	2	6
Tuberculosis	4	0
Cerebral toxoplasmosis	3	0
Pneumocystosis	3	0
Cytomegalovirus	3	0
Mycobacteria avium	2	0
Herpes zoster	0	1
Cryptococcosis	1	0

Immune status at the time of hospitalization

Forty eight percent of hospitalized men vs 32% of women had less than 50 CD4 lymphocytes, and 73% of men vs 61% of women had less than 200 CD4/mm^3 but again the difference failed to reach statistical significance ($p = 0.2$ et 0.17).

Clinical presentation of patients

The mean duration of fever was 26.9 days (range 1–180).

The main clinical signs for severely immunocompromised patients (CD4 <50/mm^3) relative to those with CD4 ≥50/mm^3 were weight loss (89%) and diarrhoea (58%) ($p < 0.001$ and $p = 0.004$, respectively).

Table 2 compares the demographics of patients with histoplasmosis and patients with other diagnoses. Figure 1 shows the main clinical symptoms during histoplasmosis and other diagnoses. For histoplasmosis,

Table 2. Comparison of the demographic and immunovirologic characteristics of proven histoplasmosis and other diagnoses.

	Histoplasmosis (proven or probable) N = 18	Other diagnoses (excluding suspected histoplasmosis) N = 69	*p*
Mean age (SD)	38.5 (12.8)	41.4 (10.9)	0.3
Gender ratio M/F	1.5	1.1	0.8
Nationality (%)			0.4
French	27	19	
Surinamese	60	58	
Brazilian	13	5	
Haitian	0	4	
Guyanese	0	14	
Median CD4 (interquartile range)	17 (31)	175 (283)	<0.001
Median viral load (interquartile range)	270 000 (490 000)	147 000 (349 000)	0.4
Prior antiretroviral treatment prescription (%)	7	18	0.27

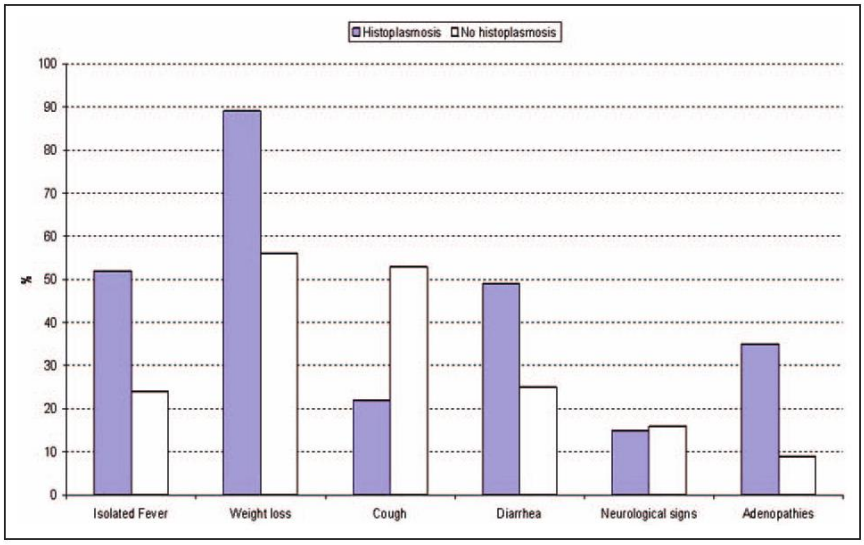


Figure 1. There were 24 histoplasmosis cases and 69 other diagnoses. There was a significant association of histoplasmosis with weight loss ($p < 0.001$), diarrhoea ($p = 0.01$) and isolated fever ($p = 0.02$).

60% of patients were aware of their HIV status. Histoplasmosis was significantly associated with weight loss ($p < 0.001$), diarrhoea ($p = 0.01$), and isolated fever ($p = 0.02$) (Figure 1). Patients with histoplasmosis had a longer median fever duration (30 days interquartile range (IQR) 53 days) than those without histoplasmosis (6 days IQR = 12 days), $p < 0.001$.

Biological presentation

Severely immunocompromised patients had leukopenia and neutropenia, elevated ferritin, liver test abnormalities. CRP was significantly lower in immunocompromised patients (CD4 count <200) than patients with greater CD4 counts (84.7 mg/l vs 195.4 mg/l, respectively, $p < 0.001$).

Of the 24 histoplasmosis cases, 14 were confirmed by culture of peripheral blood, bone marrow, liver biopsy, intestinal biopsy, or lymph node biopsy. Four were considered probable because of the presence of clinical arguments and a positive *H. capsulatum* P.C.R. Six patients with clinical arguments (hyperthermia, elevated ferritin, elevated LDH, elevated liver enzymes, pancytopenia) and a favourable evolution on presumptive treatment (favourable evolution on liposomal amphotericin B or itraconazole) were considered histoplasmoses.

The biological signs associated with the diagnosis of histoplasmosis were pancytopenia ($p < 0.001$), hyperferritinemia ($p = 0.001$), and hepatic cytolysis ($p < 0.001$) (Figure 2).

Treatment and outcome

Overall, only one patient was still on antiretrovirals on admission. Treatment of histoplasmosis consisted of intravenous liposomal amphotericin B 3 mg/kg/day for severe patients until clinical improvement followed by itraconazole 400 mg/day orally (with a 600 mg/day loading dose for 3 days). For bacterial pneumonia, the treatment was amoxicillin + clavulanic acid 3 g/day or, in severe cases, ceftriaxone 2 g/24 h combined with fluoroquinolones. Overall, there were three deaths out of 81 hospitalizations, thus a 3.7% mortality: all three deaths had disseminated histoplasmosis which amounts to a mortality of 12.5% for histoplasmosis.

Discussion

This study emphasizes that histoplasmosis was, by far, the most frequent opportunistic infection on the border between French Guiana and Surinam, notably in the most profound cases of immunodepression (CD4 $<50/mm^3$). Indeed, a significant proportion of the

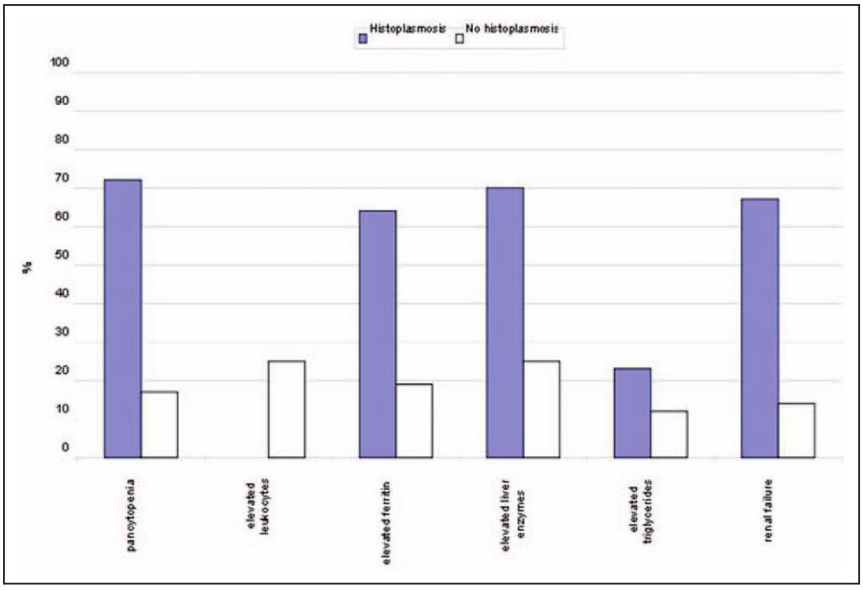


Figure 2. Comparison between the proportion of parents with different biological abnormalities between histoplasmosis and other diagnoses admitted in Saint Laurent du Maroni hospital 2008–2010.

hospitalized patients were severely immunocompromised. This particularly concerned older men, as observed elsewhere,[7–9] a group that remains hard to reach for the HIV testing system.

The observed clinical and biological signs of histoplasmosis did not differ from the literature.[10–12] Throughout French Guiana, histoplasmosis fluctuates depending on the year between 20 to 30% of AIDS defining illnesses. In the present study, it concerned 23/56 (41.1%) patients hospitalized for fever with less than 200 CD4/mm^3 and up to 85.6% of patients hospitalized for isolated fever with less than 50 CD4/mm^3. The mortality of histoplasmosis over the study period was 12.5% whereas historically it was around 40%. The aggressive stance of local clinicians in investigating and treating suspected cases supported by a laboratory trained in fungal diagnosis probably explains this decrease in mortality. This emphasizes the crucial importance of awareness of this disease and prompt diagnosis, and when in doubt presumptive treatment.

Histoplasmosis was more frequent than pneumocystosis and toxoplasmosis which require primary prophylaxis as soon as CD4 counts drop below 200/mm^3. This high incidence of histoplasmosis in western French Guiana suggests primary prophylaxis should be implemented in immunocompromised patients. The American society of infectious diseases recommends itraconazole prophylaxis as long as CD4 counts are <150/mm^3 in endemic areas where the incidence of histoplasmosis is greater than 10 cases for 100 person-years.[13] In French Guiana the incidence of histoplasmosis is 15 cases per 100 person-years for patients with CD4 counts <100/mm^3. If this prophylaxis had been initiated in our patients, 15 histoplasmoses out of 24 could have been avoided since these patients knew their HIV status before being hospitalized.

Histoplasmosis has been documented in French Guiana and Brazil.[14] There are fewer data in other countries of the Guiana shield, from which a significant number of patients are followed in Saint Laurent du Maroni. Given the fact that imported cases from Suriname have been reported in the netherlands[15–17] and that diagnosis is difficult, the most likely hypothesis is that it is underdiagnosed on the Guiana Shield, emphasizing the need for simple reliable diagnostic tools in resource-limited countries.

In our study, tuberculosis was rather infrequent with only two documented cases. This is surprising because the incidence of tuberculosis is high representing the 4th opportunistic infection. The living conditions in

Western French Guiana are more rural than in Cayenne, which may explain the lower incidence of tuberculosis.

The low frequency of cryptococcosis is somewhat surprising since in some Brazilian series over 50% of systemic mycotic infections were cryptococcosis.[18] Environmental and weather conditions could influence the incidence of histoplasmosis,[4] which again suggests acute infections are significant and points towards prophylaxis..

Although the sample size was small, the present study gives a snapshot of the importance of histoplasmosis in this amazonian region. The risk of death should incite clinicians to suspect it first in front of isolated fever in an immunocompromised patient. Histoplasmosis is the most commonly found opportunistic infection associated with AIDS in this region and primary prophylaxis in patients with CD4 counts $<150\,mm^3$ could be indicated.[19] Although it has shown no significant impact on survival in the USA, it has shown significant incidence reduction.[20] It could also be cost-effective in limited resource contexts. Simple diagnostic tools are needed to facilitate diagnosis in resource-limited countries and increase awareness of this problem in South America.

Author contributions

Conceived the study: VV; designed the study: VV, RB, CV, CM, MN; analysed the data: VV, MN; interpreted the data: VV, RB, CV, AJ, CM, AA, MN; drafted the manuscript: MN; critically revised the manuscript for intellectual content: VV, RB, CV, AJ, CM, AA, MN; read and approved the final version: VV, RB, CV, AJ, CM, AA, MN. Guarantor of the paper MN.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Not required.

References

1. Nacher M, Vantilcke V, Parriault MC, et al. What is driving the HIV epidemic in French Guiana? *Int J STD AIDS* 2010; 21: 359–361.
2. Gaubert Maréchal Emilie JA, Van melle Astrid, Parriault Marie Claire, et al. Knowledge, attitudes, beliefs, practices on HIV/AIDS among boatmen on the Maroni river: a neglected bridging group? *J AIDS Clin Res* 2012.
3. Couppie P, Sobesky M, Aznar C, et al. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. *Clin Infect Dis* 2004; 38: 134–138.
4. Hanf M, Adenis A, Couppie P, et al. HIV-associated histoplasmosis in French Guiana: recent infection or reactivation? *AIDS* 24: 1777–1778.
5. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; 46: 1813–1821.
6. Simon S, Veron V, Boukhari R, et al. Detection of Histoplasma capsulatum DNA in human samples by real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 66: 268–273.
7. Nacher M, El Guedj M, Vaz T, et al. Risk factors for late HIV diagnosis in French Guiana. *AIDS* 2005; 19: 727–729.
8. Mugavero MJ, Castellano C, Edelman D, et al. Late diagnosis of HIV infection: the role of age and sex. *Am J Med* 2007; 120: 370–373.
9. Delpierre C, Cuzin L, Lauwers-Cances V, et al. High-risk groups for late diagnosis of HIV infection: a need for rethinking testing policy in the general population. *AIDS Patient Care STDS* 2006; 20: 838–847.
10. Pontes LB, Leitao Tdo M, Lima GG, et al. [Clinical and evolutionary characteristics of 134 patients with disseminated histoplasmosis associated with AIDS in the State of Ceara]. *Rev Soc Bras Med Trop* 43: 27–31.
11. Couppie P, Aznar C, Carme B, et al. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. *Curr Opin Infect Dis* 2006; 19: 443–449.
12. Huber F, Nacher M, Aznar C, et al. AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. *AIDS* 2008; 22: 1047–1053.
13. Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007; 45: 807–825.
14. Nacher M, Aznar C, Blanchet D, et al. AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? *AIDS* 2006; 20: 951–952.
15. Van der Schee AC, Dinkla BA and Festen JJ. Gonarthritis as only manifestation of chronic disseminated histoplasmosis. *Clin Rheumatol* 1990; 9: 92–94.
16. Peters EJ, Kauffmann RH and Blok P. Fever and high lactate dehydrogenase in HIV-positive patients from the Antilles and Surinam: histoplasmosis? *Neth J Med* 2006; 64: 302–306.
17. Joore JC, Marcelis JH, Sie-Go DM, et al. [Histoplasma capsulatum infection, a manifestation of AIDS unusual for The Netherlands]. *Ned Tijdschr Geneeskd* 1990; 134: 1908–1910.
18. Ribeiro LC, Hahn RC, Favalessa OC, et al. [Systemic mycosis: factors associated with death among patients

infected with the human immunodeficiency virus, Cuiaba, State of Mato Grosso, Brazil, 2005–2008]. *Rev Soc Bras Med Trop* 2009; 42: 698–705.
19. Nacher M, Adenis A, Basurka C, et al. Primary prophylaxis of disseminated histoplasmosis in HIV patients in French Guiana: a cost effectiveness study. *Am J Trop Med Hyg* 2013.
20. McKinsey DS. Histoplasmosis in AIDS: advances in management. *AIDS Patient Care STDS* 1998; 12: 775–781.

3.3.2. Bases de données pour l'étude de l'histoplasmose en Guyane française

La prise de conscience du problème représenté par l'histoplasmose en Guyane française et le développement du diagnostic ont été accompagnés par la mise en œuvre de bases de données. En dehors des données issues du Programme de Médicalisation des Systèmes d'Information (PMSI) (principalement exploitable à compter de 2007) ou des dossiers médicaux de patients ou des laboratoires hospitaliers pratiquant le diagnostic des infections fongiques profondes dans les trois centres hospitaliers publics de Guyane française, nous avons principalement utilisés deux bases de données dans le cadre de ce travail de thèse.

- **La cohorte guyanaise de patients infectés par le VIH**

Depuis 1997, l'ensemble des patients infectés par le VIH suivis dans un des trois centres hospitaliers de Guyane (Cayenne, Kourou, Saint Laurent du Maroni) ont été progressivement inclus dans la base de données du DMI-2/DOMEVIH, administrée par l'ancien Centre d'Information et de Suivi de l'Immunodéficience Humaine (CISIH), désormais remplacé par la COordination RÉgionale de lutte contre le VIH Guyane (COREVIH Guyane) et basée au Centre Hospitalier de Cayenne. Cette base de données appartient à la FHDH (French Hospital Database on HIV), cohorte nationale de patients infectés par le VIH dont les données socio-démographiques, cliniques, biologiques, radiologiques et thérapeutiques, sont inclues prospectivement par des techniciens(nes) d'études cliniques depuis le 01/01/1992.
Cette base de données a reçu un avis favorable de la Commission Nationale Informatique et Libertés (CNIL) le 27/11/1991. L'ensemble des patients inclus reçoivent une information et signent un consentement éclairé (78). Par ailleurs, depuis 2006-2007, les données sont également inclues dans la base de données Dat'Aids via le dossier médical en ligne eNADIS, basé au CHU de Nice.

La file active de patients, définie par l'ensemble des patients vus au moins une fois dans l'année dans un des trois hôpitaux de Guyane française est de 2174 patients fin 2015 (79). Si cette base de données ne comprend pas l'ensemble des patients infectés par le VIH vivant en Guyane française (patient infectés non dépistés ou, patients suivis en libéral ou dans des centres ayant des difficultés de connexion internet), elle autorise l'analyse des grandes tendances épidémiologiques.

Elle permet la prise en compte du temps dans l'analyse statistique de problématique telle que l'histoplasmose chez le patient infecté par le VIH.

- **La base de données Histoplasmose et VIH Guyane**

A partir de 1992, dans le cadre de l'objectif principal de la FHDH concernant la description et l'étude de la morbi-mortalité des patients infectés par le VIH au stade Sida, le Pr Couppié a initié la mise en œuvre d'une base de données anonymisées (administrée par le Dr F. Huber de 2006 à 2008 puis par moi-même depuis 2009).

L'objectif principal est la description de l'épidémiologie clinique spécifique aux cas incidents d'histoplasmose chez les patients infectés par le VIH. Tout patient adulte et infecté par le VIH présentant un cas incident confirmé d'histoplasmose dans un des trois centres hospitaliers publics de Guyane française est inclus. Si le diagnostic d'histoplasmose est confirmé sur la base des critères du groupe de consensus EORTC-MSG (80) (examen direct mycologique ou anatomopathologique et/ou culture mycologique de tous types de fluides ou tissus humains identifiant la forme levure ou mycélienne d'*H. capsulatum*), un recueil rétrospectif de données socio-démographiques, cliniques, biologiques, radiologiques et thérapeutiques est réalisé à l'aide d'une fiche standardisée sur la base du dossier médical d'hospitalisation.

Entre 1992 et 2011, suivant l'évolution des aspects législatifs de la recherche médicale française, cette base de données a fait l'objet d'une demande d'autorisation CNIL (n° JZU0048856X, le 16/07/2010). Cette dernière était précédée des avis favorables du Comité de Qualification Institutionnel (CQI) de l'Institut National de la Santé et de la Recherche Médicale (INSERM) (IRB0000388, FWA00005831 le 18/05/2010), puis du Comité Consultatif pour le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS) (N°10.175bis, le 10/06/2010).

En comparaison aux données de la cohorte de patients infectés par le VIH, cette base de données spécifique aux cas incidents s'envisage comme une étude transversale au long cours. Elle autorise une description plus fine des caractéristiques cliniques et de prise en charge médicale. Si elle n'autorise pas la prise en compte du temps dans les analyses statistiques, elle renseigne sur la survie à 1 mois des patients.

4. Plan et objectifs de travail

4.1. Première partie : Revue des travaux scientifiques réalisés

Préambule structurant à la présentation des travaux et activités menés dans le cadre de mon travail de thèse, un état des lieux (ARTICLE 2) permettait de fixer le cadre scientifique. L'objectif principal était de guider le développement et la mise en place de travaux ou autres programmes de recherche adaptés à la situation observée.

Au cours du travail, l'insertion de la problématique dans le cadre plus large des infections fongiques invasives et de l'infection par le VIH s'est avérée intéressante à présenter (ARTICLE 1).

De la même manière, si l'objectif initial de ce travail de thèse était restreint à la Guyane française, très rapidement, le besoin de s'intéresser à la problématique dans sa dimension régionale amazonienne, voire internationale, s'est avéré nécessaire et pertinent.

Depuis la fin des années 1990, les cliniciens de Guyane en charge de l'infection par le VIH rencontraient régulièrement leurs homologues des pays du plateau des Guyanes et plus largement de la Caraïbe. Dès le début des années 2000, ces cliniciens sont frappés par l'absence totale d'informations liées à l'histoplasmose chez les patients infectés par le VIH dans les pays voisins, d'où sont originaires nombre de leurs patients. En l'absence de publications rapportant des cas dans ces pays, tout se passait comme si cette maladie n'existait pas chez les patients infectés par le VIH. Sans compter les rares publications de cas chez des patients originaires de ces pays et ayant émigrés en Europe, ou les séries autopsiques de patients brésiliens infectés par le VIH, touchant une faible audience, mais rapportant la présence d'*H. capsulatum* dans les tissus comme pouvant être à l'origine du décès. Constat jugé alors péjoratif pour le pronostic vital des patients infectés par le VIH originaires de ces pays, notamment ceux pris en charge pour une suspicion de tuberculose, principal diagnostic différentiel de l'histoplasmose, alors qu'ils présentent d'authentiques formes d'histoplasmose non identifiées sur place.

Deux publications guyanaises au cours de l'année 2006 posent les bases des réflexions et initiatives toujours à l'œuvre en 2017.

Nacher et al. posent la question de la fréquence de survenue de l'histoplasmose et de sa description épidémiologique au cours de l'infection par le VIH dans les pays de la grande région Caraïbe élargie au nord de l'Amérique du Sud, sachant qu'elle est une des principales causes de SIDA et de mortalité au stade SIDA en Guyane française (52). Question légitime car la région Caraïbe est la deuxième région OMS la plus touchée par l'infection par le VIH et des études historiques rapportent la

présence d'*H. capsulatum* dans l'environnement ou au sein de la population générale de la plupart des pays concernés (52).
Couppié et al. envisagent la problématique de l'histoplasmose dans les « pays en développement » avec une revue de la littérature décrivant les différences entre Amérique du Sud et Amérique du Nord au plan de la présentation clinique, des pratiques et des options disponibles pour le diagnostic et la prise en charge médicale des patients coinfectés par l'histoplasmose et le VIH (81). Dans un contexte où les chiffres de mortalité observés semblent plus important au Sud, il évoque notamment les besoins de critères pour distinguer les formes sévères des formes non sévères d'histoplasmose ainsi que pour le diagnostic différentiel entre les cas de tuberculose et d'histoplasmose. Il envisage également la possible primauté du traitement antifongique sur le traitement antituberculeux lors de l'initiation empirique d'un traitement dans l'attente des résultats des explorations microbiologiques.

Ainsi, la première partie de ce travail de thèse déclinera les travaux réalisés selon un plan similaire à la revue de la littérature initiale (ARTICLE 2) avec pour objectif de répondre à certaines des questions identifiées localement ou dans la littérature comme restant en suspend à propos: des tendances épidémiologiques et du poids de la maladie en santé publique, de la physiopahtologie et des facteurs de risque de la maladie, des aspects diagnostiques (cliniques et paracliniques) et de prise en charge médicale des patients coinfectés par le VIH et l'histoplasmose.

4.2.Deuxième partie : coopération régionale et projets mis en œuvre

Au sein de cette deuxième partie, nous envisagerons la description et les résultats préliminaires d'un projet de recherche développé autour de la problématique de la coinfection histoplasmose et VIH en Guyane française et dans la région.
Puis, nous décrirons le cadre plus général de la coopération régionale engendrée par ces projets, base à l'activité de paidoyer et de mise en réseau des partenaires menée depuis 2011.

4.3. Troisième partie : développement d'une initiative internationale

Suite logique aux travaux scientifiques et autres programmes de coopération régionale, inscrits d'emblée dans une perspective de santé publique visant à la réduction de la morbi-mortalité, s'impose la difficile mais nécessaire activité de plaidoyer autour de la problématique de l'histoplasmose au cours de l'infection par le VIH.

Dans le contexte du début des années 2000, qui a façonné les ojectifs de ce travail de thèse, si des ajustements pour la prise en charge de la coinfection histoplasmoe et VIH étaient nécessaires en Guyane française, notamment sur l'évolution de l'arsenal diagnostic de l'histoplasmose, les patients de la plupart des pays d'Amérique Latine ne bénéficiaient pas de la même qualité de prise en charge du VIH et encore moins de l'histoplasmose.

La valorisation des résultats de nos travaux publiés dans des revues spécialisées de langue anglaise devait toucher non seulement la communauté de spécialistes de la prise en charge de l'infection par le VIH à majorité hispanophone et lusophone en Amérique Latine, mais aussi les gestionnaires de la mise en œuvre des politiques de santé publique aux plans national et international dans la région.

Tout était à faire au début de ce travail de thèse et beaucoup reste encore à faire au moment de rendre ce manuscrit. Cette troisième partie présente, sous la forme d'un cheminement chronologique, les principales réalisations dans notre tentative d'initier une dynamique sur la problématique de la coinfection histoplasmose et VIH.

PREMIERE PARTIE : REVUE DES TRAVAUX SCIENTIFIQUES

Les travaux et publications sont présentés selon un plan similaire à celui de la revue de la littérature présentée à l'ARTICLE 2.

1. Taxonomie et épidémiologie moléculaire

Aucun travail de recherche fondamentale n'a été mené. Ce travail de thèse consistait en des réalisations en épidémiologie clinique et santé publique.

Toutefois, dans le cadre des projets et collaborations engendrés autour de ce travail de thèse, nous avons sanctuarisé des collaborations scientifiques visant à tester l'hypothèse de la variabilité génétique d'*H. capsulatum* sur le plateau des Guyanes. Cette étude n'avait pas été réalisée dans notre contexte et la variabilité génétique des souches pourraient avoir des implications dans le développement ou l'utilisation de méthodes diagnostiques de biologie moléculaire. De plus, l'hypothèse cladistique développée par Kasuga & al. en 2003 ne comprenait qu'une seule souche en provenance d'Amazonie, plus particulièrement issue d'un patient surinamais (82). Dans cet objectif, nos projets ont participé de la collection déclarée de souches d'*H. capsulatum* en provenance du Brésil amazonien, du Suriname, du Guyana. Ces souches sont utilisées dans le cadre d'une thèse d'université en cours à l'Université de Guyane.

Par ailleurs, dans le débat sur le potentiel dermatotropisme des souches sud américaines d'*H. capsulatum* en comparaison aux souches nord américaines d'*H. capsulatum*, une étude des tendances temporelles d'incidence et de mortalité observées dans les formes cutanéo-muqueuses d'histoplasmose chez le patients infectés par le VIH de Guyane a été menée dans le cadre du travail de thèse de Médecine de Mme S. Moroté (soutenance le 3/10/2017, directeur de thèse A. Adenis). Selon notre expérience, il n'y a probablement pas de dermatotropisme proprement dit en Amérique du Sud mais un diagnostic plus tardif de la maladie histoplasmose en Amérique du Sud par rapport à l'Amérique du Nord. Pour la première fois sur une période aussi importante, il semblerait que les tendances observées dans le cadre de ce travail viennent nourrir l'idée d'absence de dermatotropisme. Ces résultats devraient faire l'objet d'une publication scientifique prochainement.

2. Epidémiologie descriptive

Comme nous l'avons vu précédemment, les données issues des déclarations obligatoires et des enquêtes mortalité de l'Inserm décrivaient une tendance épidémiologique particulière de

l'infection par le VIH au stade SIDA en Guyane française. L'histoplasmose était parmi les premières causes d'infections opportunistes déclarées et parmi les premières causes de décès observées.

Les données de la cohorte de patients infectés par le VIH suivis dans un des trois hôpitaux de Guyane française permettaient une analyse plus fine. Elle autorisait la comparaison du taux d'incidence des diagnostics classants SIDA chez les patients infectés par le VIH au cours de deux périodes de temps, avant et après 1997. Pour mémoire, cette date est classiquement utilisée dans la littérature car, elle marque le début des trithérapies antirétrovirales hautement actives, avec notamment la mise à dispostion d'une classe médicamenteuse appelée inhibiteurs de protéases. Ces molécules, utilisées dans cette stratégie, ont eu un impact majeur sur la survie des patients infectés par le VIH, modifiant définitivement la prise en charge de l'infection par le VIH.

Ces comparaisons d'incidences au cours du temps, inédites en Guyane française, devaient être réalisées. Elles constituaient des éléments simples et utiles pour faire un constat de la situation locale et justifier de la pertinence de développer des travaux autour de la problématique de l'histoplasmose chez le patient infecté par le VIH en Guyane française et probablement au-delà en Amazonie (ARTICLE 4).

De plus, les données d'incidence publiées étaient majoritairement issues de séries de cas dans le cadre d'études transversales. Seule une publication nord américaine rapportait un taux d'incidence stricto sinsu de l'histoplasmose à partir d'une cohorte de patients infectés par le VIH (83).

ARTICLE 4 : What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis.

Author: Nacher, M., **Adenis, A**., Adriouch, L., Dufour, J., Papot, E., Hanf, M., Vantilcke, V., Calvez, M., Aznar, C., Carme, B. and Couppie, P.

Year: 2011 **Journal:** Am J Trop Med Hyg

ISSN: 1476-1645 (Electronic) **DOI:** 10.4269/ajtmh.2011.10-0251

Abstract: The pathogen ecology of Amazonian regions may lead to specific differences in the most frequent clinical presentations of acquired immunodeficiency syndrome (AIDS). A retrospective cohort study was thus conducted to describe the main AIDS-defining events in French Guiana. Disseminated histoplasmosis was the most frequent opportunistic infection (15.4/1000 person years).

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21292891

Am. J. Trop. Med. Hyg., 84(2), 2011, pp. 239–240
doi:10.4269/ajtmh.2011.10-0251

Short Report: What Is AIDS in the Amazon and the Guianas? Establishing the Burden of Disseminated Histoplasmosis

Mathieu Nacher,* Antoine Adenis, Leila Adriouch, Julie Dufour, Emmanuelle Papot, Matthieu Hanf, Vincent Vantilcke, Mélanie Calvez, Christine Aznar, Bernard Carme, and Pierre Couppié

Centre d'Investigation Clinique, Epidémiologie Clinique Antilles Guyane (CIC EC CIE802), Centre Hospitalier de Cayenne; COREVIH Guyane, CH de Cayenne; Service de Médecine, Centre Hospitalier de l'Ouest Guyanais; Hôpital de Jour, CH de Cayenne; Service Dermatologie, CH de Cayenne; Equipe EA 3593 Epidemiologie des Parasitoses et mycoses tropicales, Université Antilles Guyane, Campus Saint Denis, French Guiana

Abstract. The pathogen ecology of Amazonian regions may lead to specific differences in the most frequent clinical presentations of acquired immunodeficiency syndrome (AIDS). A retrospective cohort study was thus conducted to describe the main AIDS-defining events in French Guiana. Disseminated histoplasmosis was the most frequent opportunistic infection (15.4/1000 person years).

The AIDS-defining illnesses are numerous. The prevalence of different opportunistic pathogens may vary between regions. There is an increase of pathogens as one moves toward the equator.[1] The positive predictive value of a symptom or a diagnostic test increases with the prevalence of the considered diagnosis.[2] On the contrary, the negative predictive value decreases when prevalence of the considered diagnosis increases. Knowledge of the local epidemiology is therefore important for clinicians making diagnostic and therapeutic decisions. In this perspective, we aimed to describe the major AIDS-defining illnesses in the context of French Guiana, a French territory of South America.

The standards of healthcare in French Guiana are close to those of metropolitan France. All human immunodeficiency virus (HIV) patients receive free antiretroviral treatments (including the most recent drugs) regardless of their origin or socio-economic level. Radiology, viral loads, CD4 counts and genotyping, and antiretroviral concentration measurements are available for routine care. There is a reference university laboratory specialized in parasitology-mycology since 1997 in Cayenne Hospital, with a mycologist performing fungal culture and a Pasteur institute for the diagnosis of tuberculosis (Pasteur Institute of Guadeloupe and Pasteur Institute of French Guiana). The diagnosis of histoplasmosis relied on the identification of histoplasma on direct examination of samples, culture, or histopathology. The manifestations of histoplasmosis are described elsewhere.[3]

The HIV-positive patients followed in Cayenne, Kourou, and Saint Laurent du Maroni hospitals between January 1, 1992 and October 31, 2008 were enrolled in the French Hospital Database for HIV (FHDH) and right censoring occurred after the last visit. The database includes > 80% of the patients followed in French Guiana. Trained technicians entered demographic data, weight, diagnoses, therapeutic data, medical events, viral loads, CD4 and CD8 counts. Diagnoses are coded according to the 10th International Classification of Diseases.[4] Incidence rates were obtained. Patients included in the FHDH give informed consent to the use of their data. Their identity is encrypted before the data is sent to the Ministry of Health and the Institut National de la Recherche Médicale (INSERM), which centralize data from Regional Coordination for the fight against HIV (COREVIH) throughout France. This data collection is approved by the Commission Nationale Informatique et Libertés (CNIL). The data were analyzed with STATA version 9.0 (College Station, TX).

A total of 2,320 subjects were included for a total of 40,404 records. There were 9,606 patient-years of follow-up. Before the HAART era 553 patients were followed for a total of 773 years at risk and after HAART became available, 2,048 were followed for a total of 8,829 years at risk. Figure 1 shows the most common AIDS-defining illnesses by period. Results show that, in the HAART era, disseminated histoplasmosis is the most frequent opportunist infection, followed by esophageal candidiasis, cerebral toxoplasmosis, and tuberculosis.

Here, we give an overview of what AIDS is in French Guiana, where diagnostic means are that of a rich country. This description may be of use for surrounding countries in the Guianas and in the Amazon region that would be expected to share the same pathogen ecology.[5] Contrary to most opportunistic infections, histoplasmosis incidence did not decrease with the availability of HAART. This is because of the arrival of a parasitology and mycology team in Cayenne Hospital in 1997. Presumably, before this upgrade of mycological facilities, a number of cases were not diagnosed before 1997, and classified as HIV wasting syndrome. When the clinical symptoms involve respiratory symptoms, histoplasmosis, tuberculosis, and pneumocystosis are the usual suspects. Similarly, when encountering adenopathies, histoplasmosis and tuberculosis are also alternative diagnoses. The diagnosis procedures required to differentiate tuberculosis and histoplasmosis are often invasive.[3] The risk is that diagnostic hesitation and delays in diagnostic procedures, notably in the case of histoplasmosis, may rapidly lead to death. Thus, it is important to improve the description of the presentation of these pathologies and to develop better diagnostic tools to aid the therapeutic decisions of the clinicians.[6] In the meantime, a better knowledge of what causes AIDS in the region is precious because we strongly suspect histoplasmosis is a major but unrecognized cause of numerous deaths in and around the Amazon.

*Address correspondence to Mathieu Nacher, 25/28 rue du Dr. Roux, Pavillon Biotop, Paris, France 75015. E-mails: m_nacher@lycos.com or Mathieu.nacher@ch-cayenne.fr

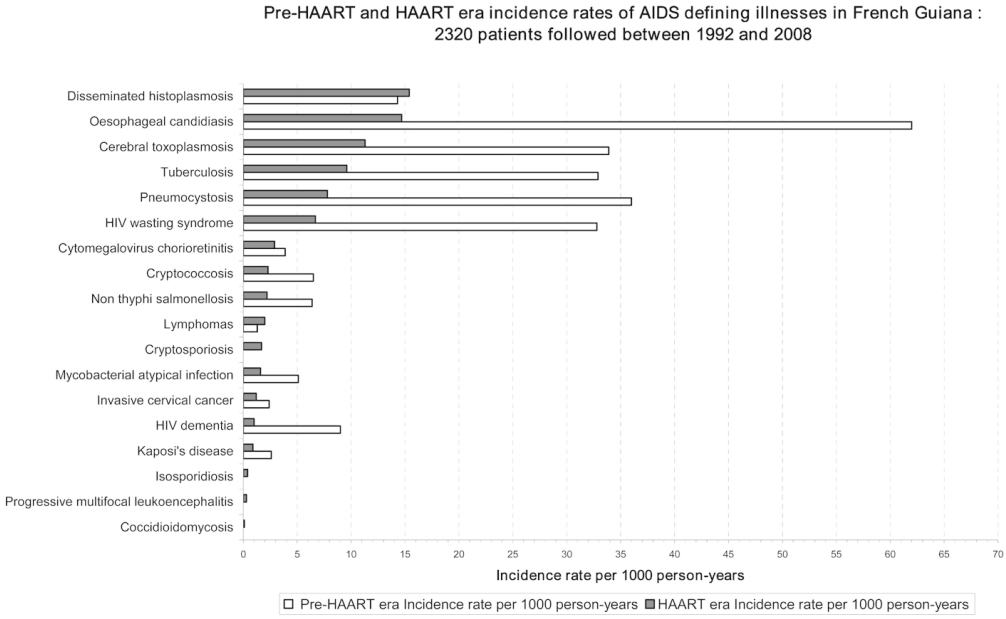


FIGURE 1. Main opportunistic infections before and after the availability of HAART in French Guiana.

Received May 3, 2010. Accepted for publication August 11, 2010.

Authors' addresses: Mathieu Nacher, Antoine Adenis, Matthieu Hanf, and Bernard Carme, Centre d'Investigation Clinique, Epidémiologie Clinique Antilles Guyane (CIC EC INSERM CIE802), Centre Hospitalier de Cayenne, 97300 Cayenne, French Guiana. Leila Adriouch, COREVIH Guyane, CH de Cayenne, 97300 Cayenne, French Guiana. Julie Dufour, Emmanuelle Papot, and Pierre Couppié, Service Dermatologie, CH de Cayenne, 97300 Cayenne, French Guiana. Vincent Vantilcke, Service de Médecine, Centre Hospitalier de l'Ouest Guyanais 97310, Saint Laurent du Maroni, French Guiana. Mélanie Calvez, Hôpital de Jour, CH de Cayenne, 97300 Cayenne, French Guiana. Christine Aznar, Equipe EA 3593 Epidemiologie des Parasitoses et Mycoses Tropicales, Université Antilles Guyane, Campus Saint Denis, French Guiana.

REFERENCES

1. Guernier V, Hochberg ME, Guégan J-F, 2004. Ecology drives the worldwide distribution of human diseases. *PLoS Biol 2:* e141.
2. Bernard PM, Lapointe C, 1995. *Mesures Statistiques en Epidemiologie*. Quebec: Presses de l'Université du Québec, 182.
3. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, Vaz T, Vantileke V, Mahamat A, Magnien C, Chauvet E, Carme B, Couppié P, 2008. AIDS-related *Histoplasma capsulatum* var. *capsulatum* infection: 25 years experience of French Guiana. *AIDS 22:* 1047–1053.
4. World Health Organization, 1995. *CIM-10: Classification Statistique Internationale des Maladies et des Problèmes de Santé Connexes, Dixième Révision, Volume 2, Manuel d'Utilisation*. Geneva: WHO.
5. Nacher M, Aznar C, Blanchet D, Demar M, El Guedj M, Vaz T, Clyti E, Carme B, Couppié P, 2006. AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? *AIDS 20:* 951–952.
6. Couppié P, Aznar C, Carme B, Nacher M, 2006. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. *Curr Opin Infect Dis 19:* 443–449.

En Guyane française, depuis le début de l'épidémie d'infection par le VIH, l'histoplasmose était considérée comme une des principales causes de décès au stade SIDA. Au moment d'initier ce travail de thèse, la tendance observée en routine clinique était à une réduction significative des décès depuis le développement d'un laboratoire de mycologie médicale et la mise à disposition des trithérapies antirétrovirales dans le département. Décrire les tendances temporelles d'incidence et de mortalité liées à l'histoplasmose permettait de discuter des déterminants des évolutions observées. Autant de leçons épidémiologiques qui pourraient être utiles au développement de programmes de lutte contre cette pathologie en Guyane française et sur le plateau des Guyanes (ARTICLE 5).

ARTICLE 5 : HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority.

Author: Adenis, A., Nacher, M., Hanf, M., Vantilcke, V., Boukhari, R., Blachet, D., Demar, M., Aznar, C., Carme, B. and Couppie, P.

Year: 2014 **Journal:** PLoS Negl Trop Dis

ISSN: 1935-2735 (Electronic) **DOI:** 10.1371/journal.pntd.0003100

Abstract: BACKGROUND: Histoplasmosis is an endemic fungal infection in French Guiana. It is the most common AIDS-defining illness and the leading cause of AIDS-related deaths. Diagnosis is difficult, but in the past 2 decades, it has improved in this French overseas territory which offers an interesting model of Amazonian pathogen ecology. The objectives of the present study were to describe the temporal trends of incidence and mortality indicators for HIV-associated histoplasmosis in French Guiana. METHODS: A retrospective study was conducted to describe early mortality rates observed in persons diagnosed with incident cases of HIV-associated Histoplasma capsulatum var. capsulatum histoplasmosis admitted in one of the three main hospitals in French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using standard statistical methods. RESULTS: There were 124 deaths (45.3%) and 46 early deaths (16.8%) among 274 patients. Three time periods of particular interest were identified: 1992-1997, 1998-2004 and 2005-2011. The two main temporal trends were: the proportion of early deaths among annual incident histoplasmosis cases significantly declined four fold (chi2, $p<0.0001$) and the number of annual incident histoplasmosis cases increased three fold between 1992-1997 and 1998-2004, and subsequently stabilized. CONCLUSION: From an occasional exotic diagnosis, AIDS-related histoplasmosis became the top AIDS-defining event in French Guiana. This was accompanied by a spectacular decrease of early mortality related to histoplasmosis, consistent with North American reference center mortality rates. The present example testifies that rapid progress could be at reach if awareness increases and leads to clinical and laboratory capacity building in order to diagnose and treat this curable disease.

URL: http://www.ncbi.nlm.nih.gov/pubmed/25144374

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

HIV-Associated Histoplasmosis Early Mortality and Incidence Trends: From Neglect to Priority

Antoine Adenis[1,2]*, Mathieu Nacher[1,2], Matthieu Hanf[1,2], Vincent Vantilcke[3], Rachida Boukhari[4], Denis Blachet[2,5], Magalie Demar[2,5,6], Christine Aznar[2,5], Bernard Carme[1,2,5], Pierre Couppie[2,7]

1 Inserm CIC 1424, Centre d'Investigation Clinique Antilles Guyane, Centre Hospitalier de Cayenne, Cayenne, France, **2** Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, France, **3** Service de Médecine Interne, Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, France, **4** Laboratoire de Biologie Médicale, Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, France, **5** Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier de Cayenne, Cayenne, France, **6** Unité des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne, Cayenne, France, **7** Service de Dermatologie Vénérologie, Centre Hospitalier de Cayenne, Cayenne, France

Abstract

Background: Histoplasmosis is an endemic fungal infection in French Guiana. It is the most common AIDS-defining illness and the leading cause of AIDS-related deaths. Diagnosis is difficult, but in the past 2 decades, it has improved in this French overseas territory which offers an interesting model of Amazonian pathogen ecology. The objectives of the present study were to describe the temporal trends of incidence and mortality indicators for HIV-associated histoplasmosis in French Guiana.

Methods: A retrospective study was conducted to describe early mortality rates observed in persons diagnosed with incident cases of HIV-associated *Histoplasma capsulatum* var. *capsulatum* histoplasmosis admitted in one of the three main hospitals in French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using standard statistical methods.

Results: There were 124 deaths (45.3%) and 46 early deaths (16.8%) among 274 patients. Three time periods of particular interest were identified: 1992–1997, 1998–2004 and 2005–2011. The two main temporal trends were: the proportion of early deaths among annual incident histoplasmosis cases significantly declined four fold ($\chi 2$, $p<0.0001$) and the number of annual incident histoplasmosis cases increased three fold between 1992–1997 and 1998–2004, and subsequently stabilized.

Conclusion: From an occasional exotic diagnosis, AIDS-related histoplasmosis became the top AIDS-defining event in French Guiana. This was accompanied by a spectacular decrease of early mortality related to histoplasmosis, consistent with North American reference center mortality rates. The present example testifies that rapid progress could be at reach if awareness increases and leads to clinical and laboratory capacity building in order to diagnose and treat this curable disease.

Citation: Adenis A, Nacher M, Hanf M, Vantilcke V, Boukhari R, et al. (2014) HIV-Associated Histoplasmosis Early Mortality and Incidence Trends: From Neglect to Priority. PLoS Negl Trop Dis 8(8): e3100. doi:10.1371/journal.pntd.0003100

Editor: Richard James Hamill, Baylor College of Medicine, United States of America

Received September 18, 2013; **Accepted** July 7, 2014; **Published** August 21, 2014

Funding: This work has benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-0025). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: antoine.adenis@gmail.com

Introduction

French Guiana is a French overseas territory, located in the North-Eastern part of South America. The Human Immunodeficiency Virus (HIV) epidemic there is the most preoccupying among French territories [1]. During the Highly Active AntiRetroviral Therapy (HAART) era, disseminated histoplasmosis has remained the most common Acquired Immunodeficiency Syndrome (AIDS) defining illness with an incidence of 15.4/1000 person-years in HIV-infected patients [2].

In immunocompetent patients, *Histoplasma capsulatum* var. *capsulatum* infection is typically asymptomatic or pauci-symptomatic and spontaneous resolution is the rule in the great majority of cases [3]. On the contrary, in HIV-infected patients it presents mostly as a disseminated infection. With the worsening of the immunosuppression, the disease progression is often rapid and always fatal in the absence of treatment [4].

Thus, different studies have observed up to 39% of deaths following diagnosis in endemic areas, where it is supposedly well known, and 58% in non endemic areas, where it is perhaps less known [5,6]. In endemic areas, although there are different outcome measures and inclusion criteria, the death rates observed in AIDS-associated histoplasmosis differ between the USA (12–23%) and South America (19–39%) [6]. Hypotheses advanced to explain these differences are a delayed recognition due to the lack of awareness of physicians, a delayed diagnosis due to the lack of diagnostic facilities and the late presentation of HIV-infected patients in resource limited settings [6,7,8]. Delayed treatment due

Author Summary

Histoplasmosis is an endemic fungal infection in French Guiana. It is the most common AIDS-defining illness and the leading cause of AIDS-related deaths. Diagnosis is difficult, but in the past 2 decades, it has improved. The objectives of the present study were to describe the temporal trends of incidence and mortality indicators for HIV-associated histoplasmosis in French Guiana. A retrospective study was conducted to describe early mortality rates observed in persons diagnosed with incident cases of HIV-associated histoplasmosis admitted in one of the three main hospitals of French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using standard statistical methods. Among 274 patients there were 46 early deaths (16.8%). The two main temporal trends were: the proportion of early deaths significantly divided four fold and the number of annual incident histoplasmosis cases increased three fold. The present example testifies that rapid progress could be at reach if awareness increases and leads to clinical and laboratory capacity building in order to diagnose and treat this curable disease.

to the unavailability of the most effective therapy in severe cases, the impossibility of monitoring drug concentrations and/or drug-drug interactions with antituberculosis treatments are other possible explanations [6].

In French Guiana, disseminated histoplasmosis has also been the leading cause of death among HIV-infected patients [9]. Despite HIV care and treatment standards close to those in Mainland France, the mortality rate of AIDS-associated histoplasmosis remains high in the HAART era (30.7% at 6 months and 17.5 at 1 month), whereas in Mainland France, a non-endemic area, this mortality rate was divided by two [10,11].

The objective of this study was to describe the temporal trends of incidence and mortality indicators for AIDS-associated histoplasmosis in French Guiana. This knowledge is important to guide and improve AIDS-associated histoplasmosis diagnosis, care and treatment, and to illustrate that awareness and standard practices in mycology can dramatically change prognosis.

Materials and Methods

Ethics Statement

Since 1992, an anonymized database compiles retrospectively and continuously *Histoplasma capsulatum var. capsulatum* histoplasmosis confirmed incident cases diagnosed in HIV-infected patients according to the case definition of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group [12]. The revised EORTC/MSG criteria defining a proven case of histoplasmosis are: recovery in culture from a specimen obtained from the affected site or from blood; and/or histopathologic or direct microscopic demonstration of appropriate morphologic forms with a truly distinctive appearance characteristic such as intracellular yeasts forms in a phagocyte in a peripheral blood smear or in tissue macrophages. By contrast, molecular methods of detecting fungi in clinical specimens, such as Polymerase Chain Reaction (PCR), were not included in the classifications of "proven," "probable," and "possible" invasive fungal disease (IFD) definitions because there is as yet no standard, and none of the techniques has been clinically validated.

All HIV-infected patients hospitalized or seen in the outpatient department before admission, suspicious for histoplasmosis and receiving antifungal therapy in one of the three main hospitals of French Guiana (the Centre Hospitalier de Cayenne (CHC), the Centre Hospitalier Médico-Chirurgical de Kourou (CMCK) and the Centre Hospitalier de l'Ouest Guyanais in Saint Laurent du Maroni (CHOG), were identified and checked for a confirmed diagnosis of histoplasmosis in all laboratories where biological samples were sent. Then, they were finally enrolled according to the following inclusion criteria: age >18 years, admission in one of the three hospitals (the inclusion date corresponding to the date of antifungal treatment initiation), confirmed HIV infection (by Western blot), confirmed incident histoplasmosis infection (EORTC/MSG criteria), and baseline blood screening within 7 days prior to antifungal therapy initiation. Non inclusion criteria were: histoplasmosis relapse or diagnosis of histoplasmosis relying only on Histoplasma Polymerase Chain Reaction (PCR). Data were collected on a standardized form and included socio-demographic, clinical, biologic, radiologic, therapeutic and survival information. These data were then entered in an anonymized database. Ethical approval was obtained for the database and related studies (IRB0000388, FWA00005831). A descriptive study of the patients included in this database until April 2007 was published elsewhere [10].

Methods

An observational, retrospective and multicentric study was conducted from 01/01/1992 to 09/30/2011, using the French Guiana HIV-Histoplasmosis database described above.

In this study, the primary endpoint was the vital status on day 30 following antifungal therapy initiation. Patients lost to follow up within 30 days following antifungal therapy initiation, or deceased with an unknown date of death, or presenting a relapse of histoplasmosis were excluded from the analysis.

This early death criterion appeared as a good compromise to attribute mortality to the histoplasmosis infectious episode under consideration, in a context of severe immunosuppression favouring multiple opportunistic pathogens, ensuring simplicity and reproducibility of the study.

The statistical analysis was performed using STATA 10.0 (College Station, Texas, USA) (38). Descriptive analysis used proportions, medians and trend χ^2 test.

Results

There were 278 patients with AIDS-associated histoplasmosis. Four cases were excluded before the analysis (3 because they were lost to follow up and one because of an unknown date of death). Their socio-demographic characteristics and median CD4 count did not differ from the 274 patients finally selected in this study (data not shown).

Among the 274 patients selected for whom the vital status at 30 days after antifungal therapy initiation was known, there were 124 deaths (45.3%). The median time to death was 110 days (Interquartile Range [IQR] = 13–481) and the median age at the time of death was 39 years (IQR = 33–47). Early death occurred in 46 patients (16.8%) with a median survival time of 7 days (IQR = 3–16) after antifungal treatment initiation. The median age at the time of early death was 37 years (IQR = 32–47).

Figure 1 shows that the proportion of deaths occurring the same year as the diagnosis of incident histoplasmosis cases remained stable around 5 deaths per year until 2005/2006 and then

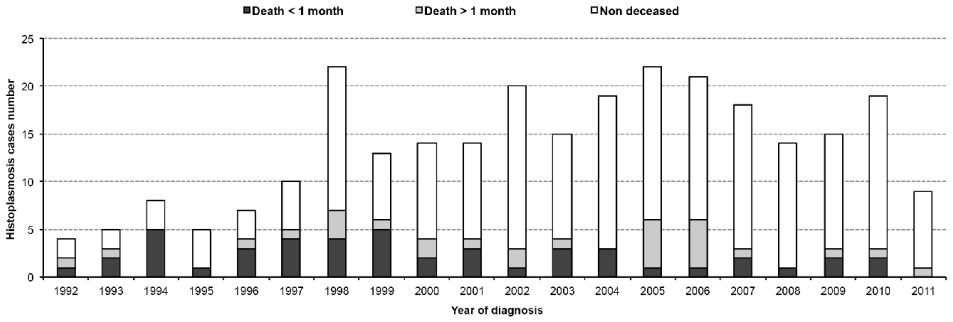


Figure 1. Number of deaths and early deaths observed among annual incident histoplasmosis cases diagnosed in the three main hospitals of French Guiana between 01/01/1992 and 09/30/2011.
doi:10.1371/journal.pntd.0003100.g001

stabilized around 3 deaths per year. Among these deaths cases, almost half were early deaths until 2004. From 2005 onwards there was a notable decline of early deaths along with the overall decline of mortality. In addition, starting in 1998, the number of histoplasmosis cases diagnoses increased, and subsequently the number of incident cases oscillated between 14 and 22 cases per year. Data were incomplete for 2011, the study considering cases only until 09/30/2011.

Thus, three time periods of particular interest have been identified: 1992–1997, 1998–2004 and 2005–2011. Figure 2 summarizes the two main temporal trends observed in Figure 1. First, the proportion of early deaths among annual incident histoplasmosis cases was significantly divided four fold (χ^2, $p<$ 0.0001). Second, the number of annual incident histoplasmosis cases increased three fold between 1992–1997 and 1998–2004, and subsequently stabilized at the same level.

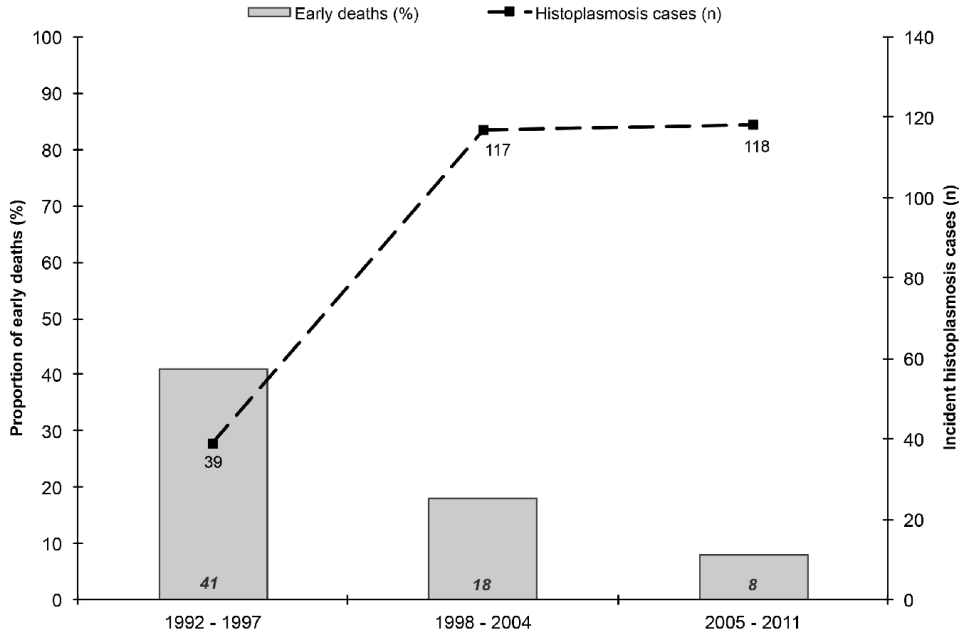


Figure 2. Incident histoplasmosis cases (n) and proportion of early deaths (%) observed in the three main hospitals of French Guiana between 01/01/1992 and 09/30/2011.
doi:10.1371/journal.pntd.0003100.g002

Table 1. Description of baseline HIV infection and histoplasmosis infection characteristics and treatments in patients with AIDS-related histoplasmosis incident cases early death, in French Guiana, between 01/01/1992 and 09/30/2011.

	Study time period			
	1992–1997	1998–2004	2005–2011	Overall
	n = 16	n = 21	n = 9	n = 46
Demographics and HIV characteristics on admission				
Sex male, *n*(%)	11 (69)	14 (67)	5 (56)	30 (65)
Mean age +/− SD, years	38 (8)	37 (13)	44 (7)	39 (11)
HIV diagnosis <1 year, *n*/N(%)	1/4 (25)	4/7 (57)	6/9 (67)	11/20 (55)
Histoplasmosis as the first AIDS-defining illness, *n*(%)	11 (69)	15 (71)	8 (89)	34 (74)
Concomitant opportunistic infection, *n*(%)	7 (44)	8 (38)	3 (33)	18 (19)
Patient on HAART, *n*(%)	0 (0)	2 (10)	1 (11)	3 (7)
Median CD4 count (IQR 25–75%),/mm3*	15 (5–30)	43 (8–54)	33 (15–52)	24 (7–50)
Histoplasmosis infection disease Classification				
Progressive disseminated histoplasmosis, *n*(%)	14 (87)	20 (95)	9 (100)	43 (93)
Pulmonary histoplasmosis, *n*(%)	2 (13)	1 (5)	0 (0)	3 (7)
Histoplasmsosis infection diagnostic methods†				
Fungal culture, *n*/N (%)	8/15 (53)	19/20 (95)	8/9 (89)	35/44 (80)
Direct examination (MGG), *n*/N (%)	13/16 (81)	16/21 (76)	6/9 (67)	35/46 (76)
Pathology (PAS and silver staining), n/N (%)	7/10 (70)	4/6 (67)	1/1 (100)	12/17 (71)
RT-PCR, *n*/N (%)	0/0 (0)	0/0 (0)	4/4 (100)	4/4 (100)
Serology (Immunodiffusion), *n*/N (%)	0/0 (0)	0/1 (0)	0/1 (0)	0/2 (0)
First-line antifungal regimen for histoplasmosis‡				
Deoxycholate amphotericin B (IV), *n*(%)	10 (63)	9 (43)	0 (0)	19 (41)
Itraconazole (oral), *n*(%)	4 (25)	9 (43)	5 (56)	18 (39)
Liposomal amphotericin B (IV), *n*(%)	0 (0)	4 (19)	5 (56)	9 (20)
Fluconazole (oral or IV), *n*(%)	2 (12)	0 (0)	1 (11)	3 (7)

* One CD4 count missing value during the 1992–1997 period.
†Good practices for fungal culture and serology were implemented in 1997–1998 and RT-PCR (Polymerase Chain Reaction using a Real-Time detection method) was implemented in 2006 in Cayenne General Hospital.
‡3 patients received amphotericin B (liposomal or deoxycholate) and itraconazole or fluconazole simultaneously.
SD: Standard Deviation, IQR 25–75%: Interquartile range 25%–75%, HIV: Human Immunodeficiency Virus, HAART: Highly Active Antiretroviral Therapy, MGG: May Grünwald Giemsa, IV: Intravenously.
doi:10.1371/journal.pntd.0003100.t001

Table 1 showed that early deaths associated with histoplasmosis occurred mainly in men, late presenters with HIV infection (CD4 count <50/mm3) among whom 10% were on HAART on admission. The incident histoplasmosis cases were mainly disseminated and often recognized as the first AIDS-defining illness in the course of HIV infection. Fungal culture and direct examination were the main methods used for the diagnosis of histoplasmosis cases. The Real Time Polymerase Chain Reaction (RT-PCR) detection method for *Histoplasma* only became available during the 2005–2011 period. Amphotericin B and itraconazole were the first line antifungal regimen used to treat these patients. During the study period, liposomal amphotericin B and itraconazole became the standard antifungal regimen over deoxycholate amphotericin B and fluconazole, respectively.

Discussion

This study described 19 years of experience in French Guiana. Three periods of interest and two main trends could be observed from 1998 onwards: the spectacular decrease of early deaths among incident histoplasmosis cases, and a simultaneous marked increase of the annual incidence of histoplasmosis cases. Whereas, during the same period, HIV prevalence in pregnant women was quite stable >1% since the 1990's: 0.8%–1.4% between 1992–1997, 1.2%–1.4% between 1998–2004 and 1.0%–1.2% between 2005–2011 [1,13].

The increased number of annual histoplasmosis cases can be attributed to the development of medical mycology skills in hospitals laboratories, notably a reference university laboratory specialized in parasitology-mycology established since 1997 in Cayenne Hospital. By the same time, highly active antiretroviral therapy was introduced, which could have led to more patent cases of histoplasmosis due to the immune reconstitution inflammatory syndrome [14]. In addition, a PCR diagnostic method became available for histoplasmosis in 2006 [15]. Unfortunately, urinary antigen detection for histoplasmosis is still unavailable in French Guiana.

The sharp decline of the proportion of early deaths can be attributed to the improvement of the diagnostic capacity along with the improvement of the clinical management of HIV-infected patients following French recommendations [16]. Thus, French Guiana reached HIV-virological suppression levels comparable to those in Mainland France by 2004. In addition, this trend can also be attributed to the improvement of the clinical management of

AIDS-related disseminated histoplasmosis cases. The accurate recognition of severe cases and the supply of liposomal amphotericin B since 1998, an effective and less nephrotoxic treatment recommended for severe disseminated histoplasmosis cases, were two important factors behind the progress.

This study had limitations. Data were collected retrospectively, which might have led to selection biases. Determining retrospectively if death was related to AIDS-associated histoplasmosis incident cases under study is challenging, considering the high percentage of concomitant opportunistic infections. Thus, we chose early death as the primary outcome because we thought that retrospectively it was the simplest and most reproducible indicator of histoplasmosis AIDS-related deaths.

Despite its limitations, this study showed that capacity building both in laboratory and clinical practice, effective drug availability both for HIV and histoplasmosis infections, and an effective bench to bed collaboration between actors progressively helped in reducing the burden of overall deaths and early deaths. Mortality indicators are now consistent with those described in North America, where the most effective and non invasive histoplasmosis diagnostic method is available. To further reduce early mortality, reducing diagnostic delays and antifungal therapy initiation is still a major objective. To reach it, a diagnostic method that meets the World Healh Organization's A.S.S.U.R.E.D. (Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free and Delivered) should be developed.

Although our results may seem parochial, they illustrate the rapid progress that took place within a decade. The increased awareness of clinicians, who became more aggressive in their investigations, and the increased laboratory capacity led to find and treat a disease that was present but probably not identified and not treated in time. Thus, histoplasmosis, previously known as a mild disease in immunocompetent individuals, became a public health problem in HIV-infected patients, known by almost all health practitioners in French Guiana. By dealing with the mycology diagnostic tool box limitations and starting prompt presumptive antifungal treatment in HIV-infected patients it was possible to reduce early deaths considerably.

The historical 40% of early deaths observed in French Guiana, where histoplasmosis was known, plausibly reflects a low estimate of what happens in the Amazon region and probably beyond, where histoplasmosis is endemic but probably still widely misdiagnosed for tuberculosis and/or neglected [17]. Although cost effective strategies to prevent the disease and very effective diagnostic methods have been developed and are well known by scattered medical teams in Latin America [18], this knowledge does not percolate to too many HIV care units and hospital laboratories [19].

The present example testifies that rapid progress could be at reach if awareness increased and led to implement clinical and laboratory capacity building in order to diagnose and treat this curable disease before it is too late.

Author Contributions

Conceived and designed the experiments: AA MN PC. Performed the experiments: AA MN MH VV RB DB MD CA BC PC. Analyzed the data: AA MN MH PC. Contributed reagents/materials/analysis tools: RB DB MD CA BC. Wrote the paper: AA MN MH VV RB DB MD CA BC PC.

References

1. Nacher M, Vantilcke V, Parriault MC, Van Melle A, Hanf M, et al. (2010) What is driving the HIV epidemic in French Guiana? Int J STD AIDS 21: 359–361.
2. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, et al. (2011) What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. Am J Trop Med Hyg 84: 239–240.
3. Kauffman CA (2009) Histoplasmosis. Clin Chest Med 30: 217–225, v.
4. Wheat LJ, Connolly-Stringfield PA, Baker RL, Curfman MF, Eads ME, et al. (1990) Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and review of the literature. Medicine (Baltimore) 69: 361–374.
5. Antinori S, Magni C, Nebuloni M, Parravicini C, Corbellino M, et al. (2006) Histoplasmosis among human immunodeficiency virus-infected people in Europe: report of 4 cases and review of the literature. Medicine (Baltimore) 85: 22–36.
6. Couppie P, Aznar C, Carme B, Nacher M (2006) American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis 19: 443–449.
7. Karimi K, Wheat LJ, Connolly P, Cloud G, Hajjeh R, et al. (2002) Differences in histoplasmosis in patients with acquired immunodeficiency syndrome in the United States and Brazil. J Infect Dis 186: 1655–1660.
8. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M (2011) Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol 49: 785–798.
9. Lewden C, Sobesky M, Cabie A, Couppie P, Boulard F, et al. (2004) Causes of death among HIV infected adults in French Guyana and the French West Indies in the era of highly active antiretroviral therapy (HAART). Med Mal Infect 34: 286–292.
10. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, et al. (2008) AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. AIDS 22: 1047–1053.
11. Peigne V, Dromer F, Elie C, Lidove O, Lortholary O (2011) Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. Am J Trop Med Hyg 85: 934–941.
12. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, et al. (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 46: 1813–1821.
13. Nacher M (2013) Rapport d'activités 2012 du Comité de Coordination Régionale de la Lutte contre l'Infection par le VIH de Guyane. Cayenne: Centre Hospitalier de Cayenne. 1–48 p.
14. Nacher M, Sarazin F, El Guedj M, Vaz T, Alvarez F, et al. (2006) Increased incidence of disseminated histoplasmosis following highly active antiretroviral therapy initiation. J Acquir Immune Defic Syndr 41: 468–470.
15. Simon S, Veron V, Boukhari R, Blanchet D, Aznar C (2010) Detection of Histoplasma capsulatum DNA in human samples by real-time polymerase chain reaction. Diagn Microbiol Infect Dis 66: 268–273.
16. Yeni P (2010) Prise en charge médicale des personnes infectées par le VIH : recommandations du groupe d'expert, rapport 2010. Paris: Ministère de la Santé et des Sports. 417 p.
17. Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, et al. (2013) Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage. PLoS Negl Trop Dis 7: e2319.
18. Nacher M, Adenis A, Basurko C, Vantilcke V, Blanchet D, et al. (2013) Primary prophylaxis of disseminated histoplasmosis in HIV patients in French Guiana: arguments for cost effectiveness. Am J Trop Med Hyg 89: 1195–1198.
19. Nacher M, Adenis A, Aznar C, Blanchet D, Vantilcke V, et al. (2013) How many have died from undiagnosed human immunodeficiency virus-associated histoplasmosis, a treatable disease? Time to act. Am J Trop Med Hyg 90: 193–194.

3. Physiopathologie et facteurs de risque

Hormis les rares cas de transmission verticale et la faible proportion de cas consécutifs à une transplantation d'organes, l'inhalation de spores dans l'environnement constitue le principal mécanisme de contamination par *H. capsulatum*.

Dans les années 1950, H. Floch a fait les descriptions princeps de la présence d' *H. capsulatum* dans le sol et des premiers cas humains d'histoplasmose chez des individus résidants en Guyane française. Au début des années 2000, *H. capsulatum* est toujours présent dans le sol de Guyane française, retrouvé notamment dans le centre-ville de Cayenne (Figure 13) (84).

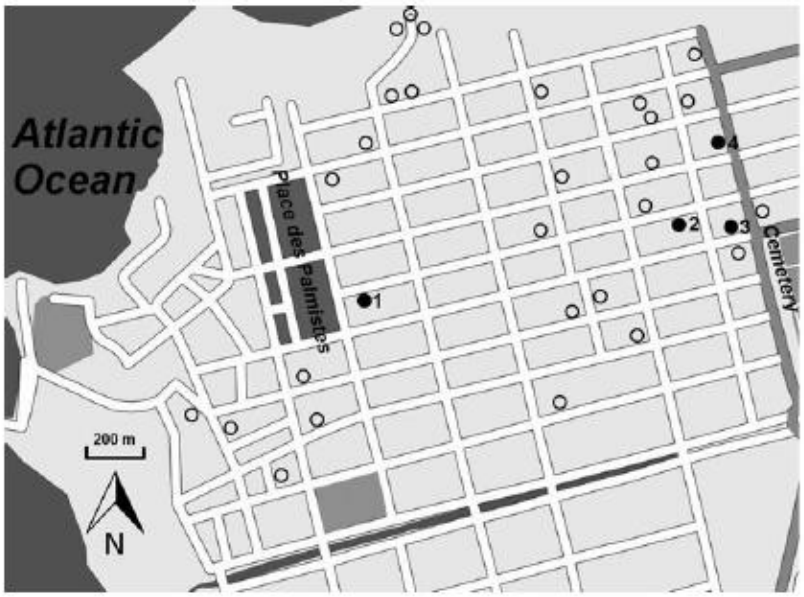


Figure 13 : Carte d'échantillonnage des prélèvements environnementaux à la recherche d'*H. capsulatum* dans le centre-ville de Cayenne, Guyane française, 2008 (cercles noirs : recherche positive) (84)

Considérant le réservoir de pathogènes dans l'environnement et la franche saisonnalité du climat tropical humide de Guyane française, il était pertinent de tester l'hypothèse d'une saisonnalité de l'incidence observée des cas symptomatiques d'histoplasmose chez les patients infectés par le VIH (ARTICLE 6). Par la suite, si cette saisonnalité existait, il fallait décrire quels paramètres climatiques pouvaient l'influencer et dans quelle mesure (ARTICLE 7). Si l'ARTCILE 7 a été publié avant l'ARTICLE 6, la réflexion et les travaux ont été menés dans l'ordre présenté ci-dessus.

L'idée sous-jacente à ces travaux était de venir nourrir un débat dans la littérature à propos du principal mécanisme physiopathologique menant à l'expression clinique de l'histoplasmose chez les patients infectés par le VIH vivants en zone d'endémie pour *H. capsulatum* : nouvelle infection ou réactivation ?

ARTICLE 6 : Disseminated Histoplasmosis Seasonal Incidence Variations: A Supplementary Argument for Recent Infection?

Author: Hanf, Matthieu, **Adenis, Antoine**, Carme, Bernard, Couppié, Pierre and Nacher, Mathieu

Year: 2012 **Journal:** J AIDS Clinic Res

ISSN: 2155-6113 **DOI:** 10.4172/2155-6113.1000175

Abstract: Background: In French Guiana, a recent study has shown that a major part of the histoplasmosis incidence temporal fluctuations could be explained by climatic factors and thus postulated that disseminated histoplasmosis cases could be in a large proportion due to new infections. The description of the seasonal pattern of histoplasmosis could potentially help to test this new hypothesis. Patients and methods: A study using prospective data from the French Hospital Database for HIV was conducted in order to determine seasonal variations of the incidence of first cases of disseminated histoplasmosis in HIV persons in Cayenne, French Guiana. Single failure Cox proportional hazards models were used. Results: After adjusting for CD4 counts and antiretroviral treatment, the incidence of disseminated histoplasmosis was significantly higher during the Short Wet Season-Long Dry Season than during the Short Dry Season-Long Wet Season (Adjusted Hazard ratio 1.7 (1.1-2.5), P= 0.01). Conclusion: This result gives both valuable epidemiologic information to clinicians and a supplementary argument in favour of the hypothesis that an important proportion of cases were due to recent exposure. Therefore, the use of a primary prophylaxis must be discussed in French Guiana.

URL: http://www.hal.inserm.fr/inserm-00915009

AIDS & Clinical Research

Hanf et al., J AIDS Clinic Res 2012, 3:8
http://dx.doi.org/10.4172/2155-6113.1000175

Rapid communication Open Access

Disseminated Histoplasmosis Seasonal Incidence Variations: A Supplementary Argument for Recent Infection?

Matthieu Hanf[1]*, Antoine Adenis[1], Bernard Carme[1,3], Pierre Couppie[2,3] and Mathieu Nacher[1,4,3]

[1]*Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane CIC-EC INSERM CIE 802, Cayenne General Hospital, Cayenne, French Guiana*
[2]*Department of Dermatology, Cayenne General Hospital, Cayenne, French Guiana*
[3]*Department of Parasitology and Mycology – Team EA 3593, Université des Antilles et de la Guyane, Cayenne General Hospital, Cayenne, French Guiana*
[4]*COREVIH, Cayenne General Hospital, Cayenne, French Guiana*

Abstract

Background: In French Guiana, a recent study has shown that a major part of the histoplasmosis incidence temporal fluctuations could be explained by climatic factors and thus postulated that disseminated histoplasmosis cases could be in a large proportion due to new infections. The description of the seasonal pattern of histoplasmosis could potentially help to test this new hypothesis.

Patients and methods: A study using prospective data from the French Hospital Database for HIV was conducted in order to determine seasonal variations of the incidence of first cases of disseminated histoplasmosis in HIV persons in Cayenne, French Guiana. Single failure Cox proportional hazards models were used.

Results: After adjusting for CD4 counts and antiretroviral treatment, the incidence of disseminated histoplasmosis was significantly higher during the Short Wet Season–Long Dry Season than during the Short Dry Season–Long Wet Season (Adjusted Hazard ratio 1.7 (1.1-2.5), P= 0.01).

Conclusion: This result gives both valuable epidemiologic information to clinicians and a supplementary argument in favour of the hypothesis that an important proportion of cases were due to recent exposure. Therefore, the use of a primary prophylaxis must be discussed in French Guiana.

Keywords: Disseminated histoplasmosis; HIV; French Guiana; Environment; Climate; Seasonality

Introduction

French Guiana is the French overseas territory where the HIV epidemic is most preoccupying. HIV prevalence among pregnant women is 1.5%. AIDS incidence is 10 times greater than in metropolitan France. Disseminated histoplasmosis is one of the most frequent opportunistic infections due to HIV in French Guiana, and is the first AIDS defining illness with an incidence rate of 1.5 per 100 person-years [1]. Furthermore, disseminated histoplasmosis is the first causes of AIDS-related death in French Guiana [2].

It generally occurs when a patient's CD4+ T lymphocyte count is less than 100 cells/mm^3. Without treatment, this disseminated disease has a rapidly fatal course.

In French Guiana, clinicians in the region usually assumed that disseminated histoplasmosis was a relapse of a past infection consecutive to the decline of cellular immunity [3]. However, a recent study has shown that a major part of the histoplasmosis incidence temporal fluctuations could be explained by climatic factors and thus postulated that disseminated histoplasmosis cases could be in a large proportion due to new infections [4]. The description of the seasonal pattern of histoplasmosis could potentially give both valuable information for clinicians and help to test the new contamination hypothesis in this area. Furthermore, the seasonality of histoplasmosis was never studied in French Guiana. In this perspective, we aimed in the present study to determine the relation between first disseminated histoplasmosis events and seasonality in a cohort of HIV-infected patients in French Guiana.

Patients and Methods

French Guiana is a large French overseas territory with a surface of 83,534 km^2. Its latitude ranges from 2°C to 5°C and its longitude from 51°C to 54°C. The hygrometry rate is about 90%. The mean temperature is 27°C and the precipitation is heavy in particular during the rainy season. There are four main seasons: The Long dry season (LDS) from July to November, the Short wet season (SWS) from December to February, the Short dry season (SDS) in March and the long wet season (LWS) from April to June.

Monthly first cases of disseminated histoplasmosis in HIV-infected patients followed at the Cayenne General Hospital between 1st January 1992 and 31th December 2007 were obtained from the French Hospital Database for HIV (FHDH). The FHDH is an ongoing prospective observational nationwide, hospital based cohort to which patients have been continuously recruited in 62 hospitals since 1992. The only FHDH inclusion criteria are HIV type 1 or 2 infection and written informed consent. Data are collected prospectively by trained research assistants using standardized forms. Clinical events are coded using the International Statistical Classification of Diseases, 10th Revision. Demographic data are recorded at inclusion. A follow-up form is completed at least every 6 months or at each visit or hospital admission

***Corresponding author:** Matthieu Hanf, Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane CIC-EC CIE 802, Cayenne General Hospital, EHPAD, Avenue des Flamboyants, BP 6006 97300 Cayenne, French Guiana, France, E-mail: matthieu@hanf.fr

Received August 30, 2012; **Accepted** October 10, 2012; **Published** October 15, 2012

Citation: Hanf M, Adenis A, Carme B, Couppie P, Nacher M (2012) Disseminated Histoplasmosis Seasonal Incidence Variations: A Supplementary Argument for Recent Infection? J AIDS Clinic Res 3:175. doi:10.4172/2155-6113.1000175

during which a new illness is diagnosed, a new treatment is prescribed, or a noteworthy change in biological markers is noted. Patient identity is encrypted before the data are sent to the Ministry of Health and the Institut National de la Recherche Médicale, which centralizes data from COREVIHs (Regional Coordination of the fight against HIV) throughout France. This data collection is approved by the Commission Nationale Informatique ET Libertés.

A confirmed case of disseminated histoplasmosis was defined by an identification of *Histoplasma capsulatum* var. *capsulatum* in tissues or fluids (direct either microscopic examination of May–Grunwald–Giemsa-stained smears or fungal culture or histo-pathological examination).

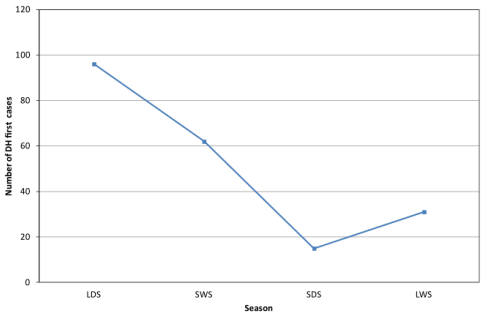
For each season, cumulated number of monthly new cases was computed. Single failure Cox proportional hazards models were used to evaluate the crude and CD4 and antiretroviral treatment adjusted relationship between first disseminated histoplasmosis failure and the two main periods of cases occurrence (LDS – SWS versus SDS – LWS). The 9.0 version of the STATA software was used to conduct all statistical analyses.

Results

A total of 2275 subjects were followed for a total of 9202 years. The median follow up time was 2.8 years. There were 204 single failure events. The incidence rate was 1.07 per 100 person years during the SDS – LWS and 1.77 per 100 person-years during the LDS – SWS. The seasonal distribution of cases is shown in figure 1. First disseminated histoplasmosis event survival curves in HIV-infected patients for LDS–SWS and SDS–LWS are shown in figure 2. After adjusting for CD4 counts and antiretroviral treatment, the incidence of disseminated histoplasmosis was still significantly higher during the SWS – LDS than during the SDS – LWS (Adjusted Hazard ratio 1.7 (1.1-2.5), $P = 0.01$).

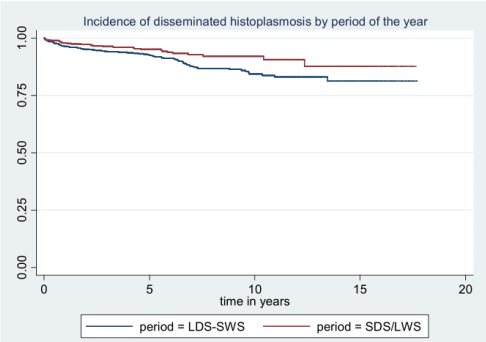
Conclusions

This is the first study to describe the seasonal pattern of histoplasmosis in French Guiana. We here show evidence that histoplasmosis incidence in HIV patients was strongly related to seasonal cycles with a significantly higher incidence during the long dry and short wet seasons than during the long wet and short dry seasons.



LDS: Long Dry Season From July To November; SWS: Short Wet Season From December To February; SDS: Short Dry Season In March; LWS: Long Wet Season From April To June

Figure 1: Seasonal monthly cumulated disseminated histoplasmosis first cases in HIV-infected patients in French Guiana 1992-2007.



LDS: Long Dry Season From July To November; SWS: Short Wet Season From December To February; SDS: Short Dry Season In March; LWS: Long Wet Season From April To June

Figure 2: Kaplan Meier Survival curves comparing the incidence of disseminated histoplasmosis between 2 climatic sequences: Long Dry Season-Short Wet Season and Short Dry Season-Long Wet Season.

In HIV patients, during the SWS and LDS, there is a greater risk of disseminated histoplasmosis than during the SDS and LWS.

In a previous study describing the correlation between climatic factors and histoplasmosis incidence [4], one of the hypotheses raised to explain this correlation was that the climate influences the level of immunodepression and so produces reactivation in particular conditions. Thus, many recent studies have emphasized the role of vitamin D, mainly obtained from sun exposure, in the susceptibility to respiratory infections and immune system regulation [5]. However, here, this hypothesis seems unlikely because of the contamination peak occurred during the dry season (the sunniest one). The role of ultraviolet-mediated immunosuppression is another theoretical possibility but the seasonal pattern was not affected by adjustment on CD4 count.

The second, and, in our view, most plausible hypothesis was that cases were largely due to new infections instead of reactivation. In this situation, climatic factors could favour ideal conditions for the development of mold and its subsequent dispersion, thereby increasing the risk of exogenous exposure. In French Guiana, most human activities are practiced during the dry season and therefore are conducive to greater exposition to histoplasmosis molds. Furthermore, the role of the dry season as an activator of the development/aerial dispersion of mold is plausible [6–9].

Our results have several limitations. First, a bias consecutive to the fact that seasonality was assessed by date of diagnosis rather than date of onset of symptoms could not be excluded in this analysis. Furthermore, patients who were first diagnosed with HIV infection when they presented with histoplasmosis could also not be included in our time-dependent statistical analyses.

However, the observed well marked seasonality is in favour of the hypothesis that in endemic areas as French Guiana, HIV-associated disseminated histoplasmosis is mainly due to recent infections rather than reactivations as postulated elsewhere [4]. As recommended by the current American guidelines [10], this is therefore a supplementary argument for using primary prophylaxis in French Guiana.

Citation: Hanf M, Adenis A, Carme B, Couppie P, Nacher M (2012) Disseminated Histoplasmosis Seasonal Incidence Variations: A Supplementary Argument for Recent Infection? J AIDS Clinic Res 3:175. doi:10.4172/2155-6113.1000175

References

1. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, et al. (2011) What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. Am J Trop Med Hyg 84: 239-240.
2. Couppié P, Sobesky M, Aznar C, Bichat S, Clyti E, et al. (2004) Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin Infect Dis 38: 134-138.
3. Wheat LJ (2006) Histoplasmosis: a review for clinicians from non-endemic areas. Mycoses 49: 274-282.
4. Hanf M, Adenis A, Couppie P, Carme B, Nacher M (2010) HIV-associated histoplasmosis in French Guiana: recent infection or reactivation? AIDS 24: 1777-1778.
5. Norman AW (2008) From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 88: 491S-499S.
6. FURCOLOW ML (1965) Environmental Aspects Of Histoplasmosis. Arch Environ Health 10: 4-10.
7. Hajjeh RA, Pappas PG, Henderson H, Lancaster D, Bamberger DM, et al. (2001) Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. Clin Infect Dis 32: 1215-1220.
8. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, et al. (1997) Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. Clin Infect Dis 24: 1195-1203.
9. Silverman FN, Schwarz J, Lahey ME, Carson RP (1955) Histoplasmosis. Am J Med 19: 410-459.
10. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, et al. (2007) Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. Clin Infect Dis 45: 807-825.

ARTICLE 7 : HIV-associated histoplasmosis in French Guiana: recent infection or reactivation?

Author: Hanf, M., **Adenis, A**., Couppie, P., Carme, B. and Nacher, M.

Year: 2010 **Journal:** AIDS

ISSN: 1473-5571 (Electronic) **DOI:** 10.1097/QAD.0b013e32833999c9

Abstract: In order to determine whether HIV-associated disseminated histoplasmosis was a recent infection or a reactivation, time series of first episodes of disseminated histoplasmosis were analyzed. Climatic variables were associated with histoplasmosis incidence. This suggested an important proportion of cases were due to recent exposure, and therefore primary prophylaxis may be warranted in French Guiana.

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20453632

Research Letters

AIDS 2010, **24**:1777–1785

HIV-associated histoplasmosis in French Guiana: recent infection or reactivation?

Matthieu Hanf[a], Antoine Adenis[a], Pierre Couppie[b,c], Bernard Carme[a,c] and Mathieu Nacher[a,c,d]

In order to determine whether HIV-associated disseminated histoplasmosis was a recent infection or a reactivation, time series of first episodes of disseminated histoplasmosis were analyzed. Climatic variables were associated with histoplasmosis incidence. This suggested an important proportion of cases were due to recent exposure, and therefore primary prophylaxis may be warranted in French Guiana.

French Guiana is the French overseas territory where the HIV epidemic is most preoccupying. HIV prevalence among pregnant women is 1.5%. AIDS incidence is 10 times greater than in metropolitan France. *Histoplasma capsulatum* var. *capsulatum* infection seems to be a major AIDS-defining illness in endemic areas of South and Central America. It is the most frequent opportunistic infection due to HIV in French Guiana, along with tuberculosis, and the first cause of AIDS-related death [1]. It usually occurs in patients with severe immunosuppression and therefore is presumed to be mostly a recurrence of a past infection in nonendemic areas [2]. For patients residing in areas of endemicity, it is not possible to ascertain whether disseminated histoplasmosis is due to a new infection or to the reactivation of an old infection [3,4]. In this perspective, the main objective was to quantify the relation between environmental factors and disseminated histoplasmosis using an ARIMA model with exogenous variables [5]. Time series data with monthly totals of first episodes of disseminated histoplasmosis were modeled with climatic variables in order to identify and quantify an eventual relation between the two.

Monthly histoplasmosis incidences in HIV patients followed at the Cayenne General Hospital between 1 January 1999 and 31 December 2007 were obtained from the French Hospital Database for HIV (FHDH). Clinical and therapeutic data are entered by trained technicians. The FHDH is approved by the commission nationale informatique et libertés. Climatic data including monthly rainfall, minimum temperature, maximum temperature, minimum humidity, maximum humidity, relative humidity and mean daily insolation were obtained from the Cayenne station of the French meteorological institute.

ARIMA models are the most general class of statistical models for analyzing and forecasting a time series. Multiplicative seasonal ARIMA models were used to evaluate the relationship between climatic factors and monthly histoplasmosis incidence rates. Modeling with seasonal ARIMA involves the estimation of a series of parameters to account for the inherent dynamics in the time series, including the trend, and seasonal and nonseasonal autoregressive and moving average processes. The series was natural log-transformed to stabilize variance. The smallest values of Akaike's information criterion (AIC) were set as the standard to identify the best-fit model.

In the model, each of the climatic input series, at lags of 0–12 months, respectively, was then fitted into the ARIMA model to screen for potential climatic predictors of histoplasmosis incidence. Those input series significantly associated with histoplasmosis incidence, with a P value of less than 0.05, were singled out to fit the best multivariate ARIMA model. A manual descendent variable selection was used to eliminate the nonsignificant variables in the final multivariate model. The Ljung-Box Q-test was applied to ascertain whether the residual series was white noise.

Of all the models tested, the ARIMA (0, 0, 0) model fitted the data best according to AIC, showing that no particular pattern explained histoplasmosis incidence.

In the best fitting model with climatic data, histoplasmosis incidence was positively associated with rainfall at a lag of 7 months ($\beta = 0.001$, $P = 0.001$), minimum temperature at a lag of 5 months ($\beta = 0.091$, $P = 0.026$), minimum humidity at a lag of 9 months ($\beta = 0.011$, $P = 0.004$) and monthly mean daily insolation ($\beta = 0.001$, $P = 0.009$). Negatively associated climatic variables with histoplasmosis incidence were minimum temperature of the month ($\beta = -0.114$, $P = 0.008$) and maximum humidity at a lag of 6 months ($\beta = -0.107$, $P < 0.001$).

The incorporation of climatic data in the ARIMA model reduced the AIC by 70%. The monthly observed and predicted histoplasmosis incidences are shown in Fig. 1.

Although clinicians in the region usually assume that disseminated histoplasmosis is a relapse of a past infection consecutive to the decline of cellular immunity, we here show evidence that a significant proportion of cases is in fact influenced by environmental factors.

This suggested that climatic factors favored the development and the dispersion of molds, thereby increasing the

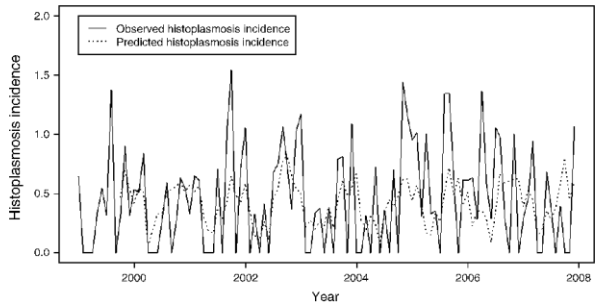


Fig. 1. Observed versus predicted histoplasmosis incidence in HIV patients, Cayenne General Hospital, 1999–2007. Predicted histoplasmosis incidences were estimated using a multiplicative seasonal ARIMA with exogenous variables.

risk of exogenous exposure and explaining the fluctuations of histoplasmosis incidence (70% in terms of AIC). An alternative hypothesis, however, could be the influence of climate on the level of immunodepression.

This study leads to important conclusions. First, further studies have to be done to confirm and explain this trend of environmental exposure. Second, primary prophylaxis should be discussed in French Guiana. Given that different communities do not have the same risk of disseminated histoplasmosis, the indication for prophylaxis could be adapted to the patients' environment as suggested by some authors [6].

[a]*Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane CIC-EC CIE 802,* [b]*Department of Dermatology,* [c]*Department of Parasitology and Mycology – Team EA 3593, Université des Antilles et de la Guyane, and* [d]*COREVIH, Cayenne General Hospital, Cayenne, French Guiana, France.*

Correspondence to Matthieu Hanf, Cayenne General Hospital, EHPAD, Avenue des Flamboyants, BP 6006 97300 Cayenne, French Guiana.
E-mail: matthieu@hanf.fr

Received: 27 January 2010; revised: 1 March 2010; accepted: 10 March 2010.

References

1. Couppie P, Sobesky M, Aznar C, Bichat S, Clyti E, Bissuel F, *et al.* **Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors.** *Clin Infect Dis* 2004; **38**:134–138.
2. Wheat LJ. **Histoplasmosis: a review for clinicians from non-endemic areas.** *Mycoses* 2006; **49**:274–282.
3. Kauffman CA. **Histoplasmosis: a clinical and laboratory update.** *Clin Microbiol Rev* 2007; **20**:115–132.
4. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, Brewer J, *et al.* **Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology.** *Clin Infect Dis* 1997; **24**:1195–1203.
5. Box G, Jenkins G. *Time series analysis: forecasting and control.* San Francisco: Holden-Day; 1970.
6. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, Kauffman CA. **Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America.** *Clin Infect Dis* 2007; **45**:807–825.

DOI:10.1097/QAD.0b013e32833999c9

Hormonal contraception and HIV acquisition: reanalysis using marginal structural modeling

Charles S. Morrison[a], Pai-Lien Chen[b], Cynthia Kwok[b], Barbra A. Richardson[c], Tsungai Chipato[d], Roy Mugerwa[e], Josaphat Byamugisha[f], Nancy Padian[g], David D. Celentano[h] and Robert A. Salata[i]

Hormonal contraceptives are used widely worldwide; their effect on HIV acquisition remains unresolved. We reanalyzed data from the Hormonal Contraception and HIV Study using marginal structural modeling to reduce selection bias due to time-dependent confounding. Replicating our original analysis closely, we found that depo-medroxyprogesterone acetate (DMPA) but not combined oral contraceptive (COC) was associated with increased HIV acquisition. Also, young (18–24 years) but not older women who used DMPA and COCs were at increased HIV risk.

In 2007, we published the results of a large multicenter cohort study designed specifically to investigate whether hormonal contraceptive use increased HIV acquisition [1]. We found no significant increased overall risk of HIV acquisition for either depo-medroxyprogesterone acetate (DMPA) or combined oral contraceptives (COCs) [1]. In a prespecified subgroup analysis, we found that whereas herpes simplex virus (HSV)-2-positive women using hormonal contraception had no increased HIV risk,

Parmi les facteurs de risque associés à la survenue de l'histoplasmose chez les patients infectés par le VIH, des facteurs environnementaux et professionnels ont été décrits.
En Guyane française, ce type d'étude n'a jamais été mené. Ces données pourraient guider les conseils de prévention d'exposition donnés par les cliniciens aux patients infectés par le VIH suivis localement ou simplement de passage dans le département.
Pour mémoire, dans cet objectif, un questionnaire d'exposition environnementale et professionnelle a été développé et administré à l'ensemble des participants au programme de recherche ANRS 12260 EDIRAPHIS qui sera détaillé par la suite.

Une dernière catégorie de facteurs de risque associés à la survenue de l'histoplasmose chez les patients infectés par le VIH, était liée aux caractéristiques de l'hôte. A notre connaissance, seules deux études retrouvaient après ajustement le taux de CD4 <150/mm^3, les antériorités de maladies chroniques ou d'infection par le virus de l'Herpès comme associée à la survenue de l'histoplasmose au cours de l'infection par le VIH (83, 85).
A l'aide des données disponibles dans la cohorte de patients infectés par le VIH, il semblait pertinent de décrire les facteurs individuels associés à la survenue des cas d'histoplasmose. Par la suite, l'analyse a été étendue à la description des caractéristiques associées à la survenue de la mortalité chez ces mêmes individus (ARTICLE 8).

ARTICLE 8 : Risk Factors for Disseminated Histoplasmosis in a Cohort of HIV-Infected Patients in French Guiana.

Author: Nacher, M., **Adenis, A.**, Blanchet, D., Vantilcke, V., Demar, M., Basurko, C., Gaubert-Marechal, E., Dufour, J., Aznar, C., Carme, B. and Couppie, P.

Year: 2014 **Journal:** PLoS Negl Trop Dis

ISSN: 1935-2735 (Electronic) **DOI:** 10.1371/journal.pntd.0002638

Abstract: Disseminated histoplasmosis is the first AIDS-defining infection in French Guiana. A retrospective cohort study studied predictive factors of disseminated histoplasmosis in HIV-infected patients between 1996 and 2008. Cox proportional hazards models were used. The variables studied were age, sex, last CD4/CD8 count, CD4 nadir, herpes or pneumocystosis, cotrimoxazole and fluconazole use, antiretroviral treatment and the notion of recent initiation of HAART. A total of 1404 patients were followed for 6833 person-years. The variables independently associated with increased incidence of disseminated histoplasmosis were CD4 count<50 per mm3, CD4 count between 50 and 200 per mm3, a CD4 nadir <50 per mm3, CD8 count in the lowest quartile, herpes infection, and recent antiretroviral treatment initiation (less than 6 months). The variables associated with decreased incidence of histoplasmosis were antiretroviral treatment for more than 6 months, fluconazole treatment, and pneumocystosis. There were 13.5% of deaths at 1 month, 17.5% at 3 months, and 22.5% at 6 months after the date of diagnosis of histoplasmosis. The most important predictive factors for death within 6 months of diagnosis were CD4 counts and antiretroviral treatment. The present study did not study environmental/occupational factors but provides predictive factors for disseminated histoplasmosis and its outcome in HIV patients in an Amazonian environment during the HAART era.

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24498446

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Risk Factors for Disseminated Histoplasmosis in a Cohort of HIV-Infected Patients in French Guiana

Mathieu Nacher[1,2,3]*, Antoine Adenis[1], Denis Blanchet[3,4], Vincent Vantilcke[5], Magalie Demar[3,6], Célia Basurko[1], Emilie Gaubert-Maréchal[2], Julie Dufour[7], Christine Aznar[3,4], Bernard Carme[3,4], Pierre Couppié[3,7]

1 Centre d'Investigation Clinique, Epidémiologie Clinique Antilles Guyane (CIC EC CIE802), Centre Hospitalier de Cayenne, Cayenne, French Guiana, **2** COREVIH Guyane, CH de Cayenne, Cayenne, French Guiana, **3** Equipe EA 3593 Epidemiologie des Parasitoses et Mycoses Tropicales, Université Antilles Guyane, Campus Saint Denis, Cayenne, French Guiana, **4** Laboratoire Hospitalo Universitaire de Parasitologie Mycologie, CH de Cayenne, Cayenne, French Guiana, **5** Service de Médecine, Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, French Guiana, **6** Unité des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne, Cayenne, French Guiana, **7** Service Dermatologie, CH de Cayenne, Cayenne, French Guiana

Abstract

Disseminated histoplasmosis is the first AIDS-defining infection in French Guiana. A retrospective cohort study studied predictive factors of disseminated histoplasmosis in HIV-infected patients between 1996 and 2008. Cox proportional hazards models were used. The variables studied were age, sex, last CD4/CD8 count, CD4 nadir, herpes or pneumocystosis, cotrimoxazole and fluconazole use, antiretroviral treatment and the notion of recent initiation of HAART. A total of 1404 patients were followed for 6833 person-years. The variables independently associated with increased incidence of disseminated histoplasmosis were CD4 count<50 per mm3, CD4 count between 50 and 200 per mm3, a CD4 nadir <50 per mm3, CD8 count in the lowest quartile, herpes infection, and recent antiretroviral treatment initiation (less than 6 months). The variables associated with decreased incidence of histoplasmosis were antiretroviral treatment for more than 6 months, fluconazole treatment, and pneumocystosis. There were 13.5% of deaths at 1 month, 17.5% at 3 months, and 22.5% at 6 months after the date of diagnosis of histoplasmosis. The most important predictive factors for death within 6 months of diagnosis were CD4 counts and antiretroviral treatment. The present study did not study environmental/occupational factors but provides predictive factors for disseminated histoplasmosis and its outcome in HIV patients in an Amazonian environment during the HAART era.

Citation: Nacher M, Adenis A, Blanchet D, Vantilcke V, Demar M, et al. (2014) Risk Factors for Disseminated Histoplasmosis in a Cohort of HIV-Infected Patients in French Guiana. PLoS Negl Trop Dis 8(1): e2638. doi:10.1371/journal.pntd.0002638

Editor: Bodo Wanke, Fundação Oswaldo Cruz, Brazil, United States of America

Received June 24, 2013; **Accepted** November 27, 2013; **Published** January 30, 2014

Funding: This work has benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mathieu.nacher@ch-cayenne.fr

Introduction

Histoplasma capsulatum var. capsulatum (HC) is found throughout the world, but there are great differences in the levels of endemicity [1]. On the South American continent, histoplasmin sensitivity studies showed proportions of the population with positive tests ranging from 7% to nearly 90%. On the Guiana Shield, the proportion of persons with positive tests is around 30%. Microconidia and mycelial forms of HC are present in the soil and aerial dispersion exposes persons to inhale these infective forms. In immunosupressed persons, HC yeasts then disseminate to various organs through phagocytes, notably macrophages where they can survive for lack of cellular activation by a robust proinflammatory immune response. Disseminated histoplasmosis has been an AIDS defining infection of HIV-infected patients since 1987 [2]. The disease may follow the resurgence of a previous infection due to immunodepression, or it may be newly acquired [3]. It often affects the most severely immunosupressed patients, and when untreated, usually leads to death. Approximately 10% of patients present with a septic shock-like syndrome [4] with high mortality. Even in the absence of initial shock, a significant proportion of cases of disseminated histoplasmosis (ranging from 22% to 47%) are severe and have a poor prognosis [5].

There have been few prospective studies on the predictive factors of disseminated histoplasmosis in HIV patients, mostly in the United States of America [3,6]. Environmental and occupational aspects, and the patient characteristics that were associated with increased risk have been studied in the 1990's. In the Amazonian area and in the Guianas, some data about histoplasmosis suggest that this is a major –but dramatically underdiagnosed- AIDS defining illness [7,8]. In the absence of diagnosis, the problem remains invisible, and therefore, in some endemic countries, standard drugs such as itraconazole are not available. There is thus a need to describe its epidemiology and to raise the awareness of clinicians and decision makers about this disease. The objective of the present study was thus to describe the predictive factors of disseminated histoplasmosis in a cohort of HIV-infected patients followed in French Guiana and to determine predictors of death.

Methods

Patients

HIV positive patients followed in Cayenne, Kourou, and Saint Laurent du Maroni Hospitals between January 1st 1996 and October 31st 2008 were enrolled in the French Hospital Database

Author Summary

Disseminated histoplasmosis is the first AIDS-related disease in French Guiana, and probably in the Amazonian area. In order to determine the factors that are associated with histoplasmosis, a retrospective looked at a cohort of HIV-infected patients between 1996 and 2008. Multiple models were used to study the relation of age, sex, last CD4/CD8 count, CD4 nadir, herpes or pneumocystosis, cotrimoxazole and fluconazole use, antiretroviral treatment and the notion of recent initiation of antiretroviral treatment with the occurrence of disseminated histoplasmosis. A total of 1404 patients were followed for 6833 person-years. The variables independently associated with the incidence of disseminated histoplasmosis were low CD4 counts, the lowest CD4 counts were most at risk; Patients with the lowest CD8 counts were also at increased risk; Antiretroviral treatment was generally associated with lower histoplasmosis incidence, but for the first 6 months following antiretroviral treatment initiation there was a transient period of increased risk of diagnosing histoplasmosis; Herpes was also associated with more histoplasmosis; Pneumocystosis and Fluconazole treatment were negatively associated with histoplasmosis. Of 156 patients with histoplasmosis, there were 13.5% of deaths at 1 month, 17.5% at 3 months, and 22.5% at 6 months after the date of diagnosis of histoplasmosis. The most important predictive factors for death within 6 months of diagnosis were low CD4 counts and no antiretroviral treatment. The present study did not study environmental/occupational factors but provides predictive factors for disseminated histoplasmosis and its outcome in HIV patients in an Amazonian environment during the HAART era. These results are useful to guide clinicians working in an area where this diagnosis is often overlooked.

for HIV (FHDH). The data is entered in the FHDH database by trained technicians from the medical records. Diagnoses were coded according to the 10th international classification of diseases. Occupational and environmental data were not available in the FHDH. The variables usually used as prognostic factors [5] (LDH, haemoglobin, platelet counts, ferritine, liver enzymes, creatinine, albumine, symptoms) were not available in the database which was created to follow broader trends.

Diagnosis of histoplasmosis

The diagnosis of histoplasmosis was performed by direct examination using May Grünvald Giemsa staining and culture of tissue and fluid samples for up to 3 months. Primary prophylaxis for disseminated histoplasmosis is not given.

HIV care in French Guiana

All HIV patients in French Guiana can receive free antiretroviral treatments (including the most recent drugs) regardless of their origin or socio-economic level. Imagery, Viral loads, CD4 counts and genotyping and antiretroviral concentration measurements are available for routine care.

Study design and statistical analysis

In this retrospective cohort study, incidence rates were obtained. Kaplan Meier curves were used to visualize the differences in the incidence of histoplasmosis between CD4 strata and between different CD4 and CD8 strata. Single failure multiple Cox proportional hazards models were used to evaluate the adjusted relationship between failure and a set of explanatory variables. Right censoring occurred after the last visit. For the first model, including 1404 patients, the failure event was a first episode of disseminated histoplasmosis. The main explanatory variables were for the time independent variables: sex, age, and a nadir of CD4 count$<$50/mm3, a prior history of herpes or pneumocystosis [6]; for the time dependent variables: last available CD4 cell count at the time of the visit (categorized 0–50, 51–200, 201–350, 350–500, and $>$500 cells per mm^3), last available CD8 cell count at the time of the visit (dichotomous variable corresponding to CD8 values within the lowest quartile or not), cotrimoxazole and fluconazole use, the presence or absence of HAART and the notion of recent initiation of HAART ($<$6 months) [9]. A variable reflecting the annual frequency of visits was also added to the models. First the crude hazard ratios were obtained for each predictor, afterwards a multiple model with the relevant variables was constructed. Confounding was considered when the difference between crude and adjusted hazard ratios exceeded 20%. Different interaction terms were created between explanatory variables and added in succession to the full model and removed when non significant. Overall, none of the interaction terms was retained in the final model. The proportionality of the hazard functions was determined using Schoenfeld and scaled Schoenfeld residuals and the global proportional hazards test.

A second model was constructed in a subgroup of 156 patients with disseminated histoplasmosis with death within 6 months of diagnosis as a failure event and CD4 count, CD8 counts, age, sex and antiretroviral treatment as explanatory variables. Other treatments, such as fluconazole and cotrimoxazole were also explored.

The significance level was 0.05. The Data were analyzed with STATA 12.0 (College Station, Texas, USA).

Ethics statement

Patients included in the FHDH gave written informed consent to the use of their data for the study. Their identity was encrypted before the data was sent to the Ministry of Health and the Institut National de la Recherche Médicale (INSERM) which centralize data from Regional Coordination for the fight against HIV (COREVIH) throughout France. This cohort is approved by the Commission Nationale Informatique et Libertés (CNIL) since Nov 27th 1991 and has led to numerous international publications.

Results

Follow up

A total of 1404 patients were included. This amounted to 30838 records and 6833 years at risk. There were 141 first episodes of disseminated histoplasmosis observed. The average time at risk was 4.04 years. The general characteristics of the patients at inclusion are shown in table 1.

Incidence

The overall incidence rate for a first episode of disseminated histoplasmosis was 1.41 per 100 person years.

Figure 1 shows the Kaplan Meier curves for different CD4 strata, with a marked increase of the incidence of histoplasmosis in patients with CD4 counts $<$50 per mm^3. Patients with both CD4 counts $<$50 per mm3 and CD8 counts under 643 had the highest risk of histoplasmosis (Fig. 2).

Table 2 shows the variables associated with disseminated histoplasmosis in the HIV cohort of French Guiana.

The incidence rate increased proportionally to the level of CD4 decline. Table 1 also shows that patients that had a CD4 nadir $<$50 had a greater risk of disseminated histoplasmosis. Patients in

Table 1. General characteristics of the patients included in the cohort.

Age group (years)	Female N (%)	Male N (%)	French nationality N (%)	CDC Stage C* (%)
<20	91 (86.7)	14 (13.3)	18 (26.8)	9.5
[20–30[	387 (68.7)	176 (31.2)	71 (15.6)	12.9
[30–40[	407 (51.4)	385 (48.6)	148 (24)	24.4
[40–50[	211 (43)	280 (57)	85 (21.7)	27.7
[50–60[	92 (37.4)	154 (62.6)	66 (33.3)	28.5
>60	46 (36.5)	80 (63.5)	43 (45.7)	27.8

*1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* **41** (RR-17): 1–19. December 1992.
doi:10.1371/journal.pntd.0002638.t001

the lowest CD8 quartile had an increased incidence of disseminated histoplasmosis. There were important differences between the crude and adjusted hazard ratios suggesting confounding, notably by the CD4 nadir.

Antiretroviral treatment was associated with protection from histoplasmosis (models with different antiretroviral classes did not show any difference between classes, data not shown). However, the first 6 months following antiretroviral treatment initiation were a period of increased risk of diagnosing histoplasmosis (table 2).

After adjustments in Cox multiple models, cotrimoxazole was not associated with any protection from disseminated histoplasmosis while herpes was associated with an increased risk of disseminated histoplasmosis (table 2). When looking at the temporal relation between herpes and histoplasmosis 11 herpes cases (50%) were simultaneous with disseminated histoplasmosis, and 4 cases (18%) occured within six months before the diagnosis of disseminated histoplasmosis. There was a notable difference between the crude and adjusted hazards reflecting confounding.

On the contrary, after controlling for CD4 count, and cotrimoxazole use, a history of pneumocystosis (but not toxoplasmosis) was independently associated with a decreased risk of disseminated histoplasmosis. However, there were 3 simultaneous cases of pneumocystosis and disseminated histoplasmosis. Adjustments for the annual frequency of visits did not change the observed association between pneumocystosis and protection from histoplasmosis and thus, for the sake of parsimony, this variable was excluded from the final model. No other opportunistic infection was associated with histoplasmosis in the multiple single failure model. It is of note that tuberculosis was associated with disseminated histoplasmosis in an analysis with a single covariable and in a multiple failure model (but not in the single failure model) multiple model with the same covariables (Adjusted Hazard

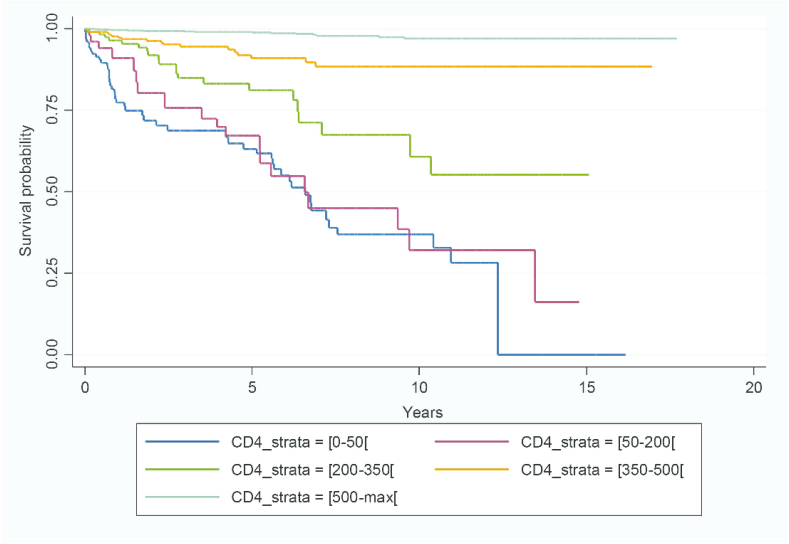


Figure 1. Incidence of a first episode of histoplasmosis stratified by CD4 count. The Y axis represents the percentage of persons that have never had histoplasmosis.
doi:10.1371/journal.pntd.0002638.g001

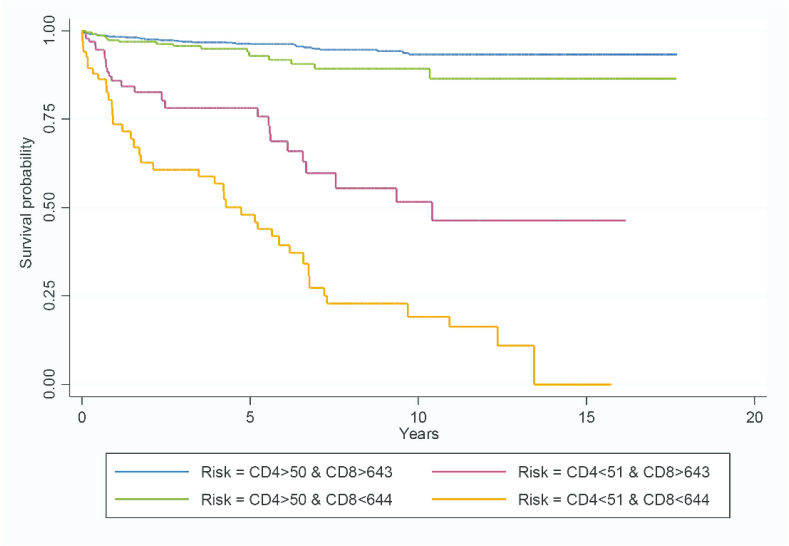


Figure 2. Incidence of a first episode of histoplasmosis stratified by different combinations of CD4 and CD8 counts: The Y axis represents the percentage of persons that have never had histoplasmosis.
doi:10.1371/journal.pntd.0002638.g002

Ratio = 2.3 (95%CI = 1.1–4.6, $P = 0.016$). Altogether, 12% of first histoplasmosis cases had a concomitant opportunistic infection.

After adjustments in Cox multiple models, fluconazole treatment was associated with a reduction of the incidence of histoplasmosis. The crude incidence rate seemed higher in those receiving fluconazole (Table 3), but this reflected the underlying immunosuppression. When looking at the incidence in the <50 CD4 per mm3 strata, those having received fluconazole had a lower incidence of histoplasmosis than those who did not receive it (6.5 per 100 person-years vs 12.9 per 100 person-years, respectively). Unsurprisingly, curative treatments such as itraconazole, amphotericine B, or liposomal amphotericine were positively associated with histoplasmosis because they were initiated when the diagnosis of histoplasmosis was made, before that they were exceptionally prescribed. They were thus not included in the predictive models. Interaction terms between CD8 and CD4 counts were created but were not significant and thus removed from the Cox model.

Mortality

Of 156 patients with disseminated histoplasmosis, there were 13.5% of deaths at 1 month, 17.5% at 3 months, and 22.5% at 6 months after the date of diagnosis of histoplasmosis. The factors associated with death are shown in table 4. Among the available variables, the most important predictive factors were CD4 counts and antiretroviral treatment. A history of oral fluconazole or cotrimoxazole treatment prior to disseminated histoplasmosis was not associated with any significant differences in mortality.

Discussion

The present results show a markedly lower incidence of histoplasmosis in HIV infected patients in French Guiana (1.41 per 100 person years) than in the USA (4.7% per year) [3]. First, while the American study took place before highly active antiretroviral therapy (HAART) was available, the present study covered a period where over 80% of patients received HAART, which may have globally increased the level of immunity and reduced the incidence of disseminated histoplasmosis. In addition, histoplasmin skin test positivity studies suggest that histoplasma is much more Frequent in the middle west (60–90%) [10] than in French Guiana where, on the basis of local studies [11] and studies in neighbouring countries [8,12], it is estimated to be around 30%.

HIV-positive men had a higher risk of histoplasmosis, and of death within 6 months if they had histoplasmosis, both of which had not been observed in the studies in the USA [3,6]. However, histoplasmin skin test studies have shown a slight male bias [10], which presumably reflects the gender differences regarding their environmental and occupational niches. Previous studies have shown that males had a higher AIDS mortality in French Guiana [13] and elsewhere [14]; The present observation may also result from the same contextual determinants. The patients with the lowest CD4 counts were both at increased risk of histoplasmosis and death within six months for patients with histoplasmosis. In addition to CD4 counts around the time of diagnosis, the CD4 nadir was also an independent predictor of disseminated histoplasmosis as was demonstrated for other indicators of HIV disease progression [15].

A less straightforward finding was the observation that CD8 counts in the lowest quartile were independently associated with the incidence of histoplasmosis, and death within six months for patients with histoplasmosis. Some studies have shown that CD8 cell depletion affected the course of fungal infections [16,17]. CD8 depletion could have resulted from the dissemination of the fungal pathogen, or from HIV itself [18,19,20]. CD8 counts often have a

Table 2. Independent predictors of a first episode of disseminated histoplasmosis in a cohort of HIV-infected patients in French Guiana: 1996–2008.

Variable	Incidence rate (per 100 person-years)	Crude hazard ratio	Adjusted hazard ratio* (95% CI)	*P*
Age (years)				
18–30	0.9	1	1	
31–40	2.1	2.7 (1.5–4.6)	1 (0.5–1.9)	0.9
41–60	1.4	1.8 (1–3.2)	0.8 (0.4–1.6)	0.6
61-max	1.2	1.5 (0.7–3.7)	0.9 (0.3–2.6)	0.8
Sex				
Men	2.1	1.4 (1.2–1.7)	1.4 (1.1–1.7)	0.004
Women	1			
CD4 count (per mm3)				
[0–50[	11.8	118.8 (29–485)	47.2 (5.8–380)	<0.001
[50–200[	2.4	23.8 (5.7–98.6)	16.9 (2.2–128)	0.006
[200–350[	0.6	6.1 (1.4–27)	7.1 (0.9–55)	0.06
[350–500[	0.1	1.1 (0.1–7.8)	1.8 (0.16–20)	0.6
[500-max]	0.1	1	1	
CD4 nadir <50/mm3				
Yes	4.7	2.1 (1.1–3.9)	1.9 (1–3.6)	0.05
No	0.6			
CD8 count in the lowest quartile (<643 per mm3)				
Yes	3.5	1.9 (1.3–2.7)	1.8 (1.2–2.9)	0.008
No	0.7			
Antiretroviral treatment				
Yes	0.7	0.4 (0.2–0.5)	0.2 (0.1–0.4)	<0.001
No	2.4			
First six months of antiretroviral treatment				
Yes	11.1	2.8 (2–4)	2.4 (1.1–5)	0.01
No	2.3	0.7 (0.5–0.9)	0.5 (0.2–0.9)	0.03
No treatment	3.9	1		
History of herpes				
Yes	17.1	10.3 (5.7–18.6)	6.4 (3.1–13.2)	<0.001
No	1.4			
History of Pneumocystosis				
Yes	8.7	4.3 (1.8–10.6)	0.1 (0.0–0.5)	0.003
No	1.4			

*Cox multiple model in HIV positive patients with first episode of disseminated histoplasmosis as failure event. Model with 1404 subjects and 94 single failures.

doi:10.1371/journal.pntd.0002638.t002

murky significance for clinicians. The present finding possibly offers a coarse glimpse on the nature of the immune response, but in practice seems unlikely to be very helpful for clinicians.

As observed elsewhere, after adjustments in Cox multiple models, antiretroviral treatment was independently associated with protection from disseminated histoplasmosis [6]. There was, as described before [9], a transient increase in the incidence within 6 months of antiretroviral treatment initiation presumably reflecting a surge of diagnoses following immune reconstitution. Oral fluconazole, although it is not as effective as itraconazole against HC, was also associated with decreased incidence of disseminated histoplasmosis but not with differences in mortality within 6 months of diagnosis. This is consistent with some previous observations [6] but not with other studies in the USA that did not observe any benefits of fluconazole in preventing histoplasmosis [3,21,22]. However, the present study involved a relatively large number of patients and may have had more power to detect moderate protective effects.

In 2001, Hajjeh *et al.* reported that pneumocystosis was associated with a lower risk of histoplasmosis and that herpes was associated with a poor outcome of histoplasmosis [6]. The present study also found that pneumocystosis was associated with a lower risk of histoplasmosis and found that patients with a history of herpes had an increased risk of histoplasmosis. The explanation for this is not clear. Perhaps pneumocystosis occurs earlier in the course of the HIV infection and may lead to initiate a better follow up and treatment, thereby preventing further loss of CD4 cells and the risk of severe immunodepression and disseminated histoplasmosis. Cotrimoxazole was not associated with a modified incidence of first episodes of disseminated histoplasmosis. Cerebral

Table 3. Azoles and incidence of a first episode of histoplasmosis.

	Incidence rate (per 100 person-years)	Crude hazard ratio	Adjusted hazard ratio* (95% CI)	*P*
Oral Fluconazole				
Yes	4.3	2.8 (1.5–5.3)	0.4 (0.1–1)	0.05
No	1.4			
Cotrimoxazole prophylaxis				
Yes	3.9	4.9 (3.5–6.8)	1.3 (0.8–2.1)	0.2
No	0.8			

*Cox multiple model in HIV positive patients with first episode of disseminated histoplasmosis as failure event. Model with 1404 subjects and 94 single failures.
doi:10.1371/journal.pntd.0002638.t003

Table 4. Predictors of death within 6 months in HIV infected patients with disseminated histoplasmosis in French Guiana: 1996–2008.

Variables	Crude hazard ratio (95% CI)	Adjusted hazard ratio* (95% CI)	*P*
Male gender	1.9 (1.3–2.7)	1.9 (1.2–3)	0.005
Antiretroviral treatment	0.1 (0.0–0.5)	0.2 (0.0–0.5)	0.003
CD8 count in the lowest quartile (<643 per mm3)	9.6 (4–22.6)	4.3 (1.1–7.5)	0.002
CD4<50 per mm3	30 (13–67)	14.6 (5.7–37)	<0.001

*Cox model in HIV positive patients with disseminated histoplasmosis with death at 6 months as failure event adjusted for sex, antiretroviral treatment, CD4 count (below 50/mm3 or not) and CD8 count (below first quartile or not). Oral fluconazole or cotrimoxazole were not significantly linked to outcome, and thus removed from the final model with 156 subjects and 28 failures.
doi:10.1371/journal.pntd.0002638.t004

toxoplasmosis, which occurs at similar levels of immunodepression and often leads to similar prophylactic treatment, was not related to the incidence of disseminated histoplasmosis, or to its outcome as reported elsewhere [6]. Finally, pneumocystosis could influence the bronchial mucosal defences against *Histoplasma*, but this broad speculation should be tested in prospective studies.

The association of herpes with histoplasmosis may reflect the fact that clinical herpes lesions were triggered by latent histoplasmosis, or that clinical herpes reflected growing immunodepression. The single failure multiple Cox model using only the first episode of histoplasmosis did not show any link between a history of tuberculosis and histoplasmosis and was removed from the final model. However, the multiple failure model, showing relapses or reinfections showed that tuberculosis, as reported in the literature [5], was associated with disseminated histoplasmosis.

Previous studies in the American middle west had shown the occupational and environmental risk factors of HIV-associated histoplasmosis [3,6] and some variables such as CD4 count [6], and past medical history and treatments [3]. The data collected for the FHDH does not include environmental and occupational data. Therefore, the present study could not explore these risk factors in the context of French Guiana. Most of the usual prognostic factors are not recorded in the FHDH, a cohort that does not go into fine clinical and biological detail. Therefore, the variables used are not of major importance for clinicians to identify prognostic elements influencing treatment [23]. However, the main objective of the present study was not to study prognosis. Despite these limitations, the present study provides additional information using longitudinal data from HIV patients in an Amazonian environment during the HAART era. The harzard ratios in the single models were often confounded, mostly by the CD4 count, as shown by the difference with the multiple models.

In conclusion, immunological factors such as low CD4 count, low CD8 count, low CD4 counts at the Nadir, the absence of antiretroviral treatment and/or oral fluconazole, and male gender were associated with an increased risk of histoplasmosis. Regarding mortality, low CD4 count, low CD8 count, absence of antiretroviral treatment, male gender and an age under 30 years were associated with death within 6 months.

Author Contributions

Conceived and designed the experiments: MN. Performed the experiments: MN PC AA. Analyzed the data: MN AA CB. Contributed reagents/materials/analysis tools: MN. Wrote the paper: MN AA DB VV MD CB EGM JD CA BC PC.

References

1. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M (2011) Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol 49: 785–798.
2. Guimaraes AJ, Nosanchuk JD, Zancope-Oliveira RM (2006) Diagnosis of Histoplasmosis. Braz J Microbiol 37: 1–13.
3. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, et al. (1997) Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. Clin Infect Dis 24: 1195–1203.
4. Wheat LJ, Connolly-Stringfield P, Kohler RB, Frame PT, Gupta MR (1989) Histoplasma capsulatum polysaccharide antigen detection in diagnosis and

management of disseminated histoplasmosis in patients with acquired immunodeficiency syndrome. Am J Med 87: 396–400.
5. Couppie P, Aznar C, Carme B, Nacher M (2006) American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis 19: 443–449.
6. Hajjeh RA, Pappas PG, Henderson H, Lancaster D, Bamberger DM, et al. (2001) Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. Clin Infect Dis 32: 1215–1220.
7. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, et al. What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. Am J Trop Med Hyg 84: 239–240.
8. Nacher M, Aznar C, Blanchet D, Demar M, El Guedj M, et al. (2006) AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? AIDS 20: 951–952.
9. Nacher M, Sarazin F, El Guedj M, Vaz T, Alvarez F, et al. (2006) Increased incidence of disseminated histoplasmosis following highly active antiretroviral therapy initiation. J Acquir Immune Defic Syndr 41: 468–470.
10. Manos NE, Ferebee SH, Kerschbaum WF (1956) Geographic variation in the prevalence of histoplasmin sensitivity. Dis Chest 29: 649–668.
11. Floch H (1953) [Histoplasmosis Darling in research in French Guiana]. Bull Soc Pathol Exot Filiales 46: 288–290.
12. Hay RJ, White HS, Fields PE, Quamina DB, Dan M, et al. (1981) Histoplasmosis in the eastern Caribbean: a preliminary survey of the incidence of the infection. J Trop Med Hyg 84: 9–12.
13. Nacher M, Huber F, El Guedj M, Vaz T, Magnien C, et al. (2007) Risk factors for death among patients in French Guiana: 1996-2005. HIV Med 8: 472–474.
14. Jarrin I, Geskus R, Bhaskaran K, Prins M, Perez-Hoyos S, et al. (2008) Gender differences in HIV progression to AIDS and death in industrialized countries: slower disease progression following HIV seroconversion in women. Am J Epidemiol 168: 532–540.
15. Miller V, Mocroft A, Reiss P, Katlama C, Papadopoulos AI, et al. (1999) Relations among CD4 lymphocyte count nadir, antiretroviral therapy, and HIV-1 disease progression: results from the EuroSIDA study. Ann Intern Med 130: 570–577.
16. Schnizlein-Bick C, Durkin M, Kohler S, Connolly P, LeMonte A, et al. (2003) Effects of CD4 and CD8 T lymphocyte depletion on the course of histoplasmosis following pulmonary challenge. Med Mycol 41: 189–197.
17. Wuthrich M, Filutowicz HI, Warner T, Deepe GS, Jr., Klein BS (2003) Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to CD8+ T cells: implications for vaccine development in immune-deficient hosts. J Exp Med 197: 1405–1416.
18. Anderson RE, Shiboski SC, Royce R, Jewell NP, Lang W, et al. (1991) CD8+ T lymphocytes and progression to AIDS in HIV-infected men: some observations. AIDS 5: 213–215.
19. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA (1995) CD8 naive T cell counts decrease progressively in HIV-infected adults. J Clin Invest 95: 2061–2066.
20. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, et al. (1997) Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol 16: 83–92.
21. Nightingale SD, Cal SX, Peterson DM, Loss SD, Gamble BA, et al. (1992) Primary prophylaxis with fluconazole against systemic fungal infections in HIV-positive patients. AIDS 6: 191–194.
22. Powderly WG, Finkelstein D, Feinberg J, Frame P, He W, et al. (1995) A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. NIAID AIDS Clinical Trials Group. N Engl J Med 332: 700–705.
23. Couppie P, Sobesky M, Aznar C, Bichat S, Clyti E, et al. (2004) Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin Infect Dis 38: 134–138.

4. Aspects cliniques

Nous avons vu en introduction que les caractéristiques cliniques, paracliniques et de prise en charge de 200 cas incidents d'histoplasmose diagnostiqués chez des patients infectés par le VIH de Guyane française avaient fait l'objet d'une description détaillée en 2008 (69). A laquelle s'ajoutait une publication de 2015 à propos d'une description complémentaire des cas diagnostiqués uniquement à Saint Laurent du Maroni (70).

En Guyane française, l'expérience clinique de routine pour la prise en charge hospitalière d'un patient infecté par le VIH à un stade avancé de la maladie (taux de CD4<200/mm^3) était marquée par un problème fréquent de diagnostic différentiel entre l'histoplasmose et la tuberculose, quand les deux maladies n'étaient pas présentes concomitamment. Enjeu de taille car, après avoir réalisé tous les prélèvements microbiologiques, si les examens directs étaient négatifs, il fallait attendre plusieurs jours ou semaines pour avoir les résultats des cultures fongiques ou mycobactériologiques. Dans cette situation fréquente, deux attitudes étaient rapportées, soit on attendait la preuve microbiologique avant d'initier un traitement ciblé, soit on initiait un traitement empirique à visée antifongique ou antituberculeux sur la seule base de l'expérience clinique des praticiens. Ce qui dans un cas comme dans l'autre n'était pas satisfaisant et pouvait constituer une perte de chance pour les patients.
Ceci s'ajoutait aux nombreuses publications faisant état d'une tuberculose mimant une authentique histoplasmose chez des patients infectés par le VIH. Les auteurs rapportaient une perte de chance pour ces patients qui le plus souvent décédaient sous antituberculeux, sans avoir reçu de traitement antifongique ou alors initié trop tardivement.
Il convenait de tester l'hypothèse heuristique, répandue dans la littérature et l'esprit des cliniciens au lit du malade, selon laquelle histoplasmose et tuberculose étaient indiscernables ou difficilement discernables au stade avancé de l'infection par le VIH (ARTICLE 9).

Pour mémoire, dans le cadre de la direction de thèses d'exercice de médecine, deux travaux portant sur les données cliniques de Guyane française on été menés en 2016: le travail du Dr D. Nguyen à propos de la description des syndromes hémophagocytaires réactionnels au cours de l'infection par le VIH et le travail du Dr R. de Reynal à propos des syndromes inflammatoires de reconstitution immunitaire (SRIS) à *H. capsulatum*. Ces travaux seront prochainement soumis pour publication.

ARTICLE 9 : Tuberculosis and Histoplasmosis among Human Immunodeficiency Virus-Infected Patients: A Comparative Study

Author: Adenis, A., Nacher, M., Hanf, M., Basurko, C., Dufour, J., Huber, F., Aznar, C., Carme, B. and Couppie, P.

Year: 2014 **Journal:** Am J Trop Med Hyg

ISSN: 1476-1645 (Electronic) **DOI:** 10.4269/ajtmh.13-0084

Abstract: In disease-endemic areas, histoplasmosis is the main differential diagnosis for tuberculosis among human immunodeficiency virus (HIV)-infected patients. However, no study has compared the two diseases. Thus, the objective of this study was to compare tuberculosis and histoplasmosis in HIV-infected patients. A population of 205 HIV-infected patients (99 with tuberculosis and 106 with histoplasmosis) hospitalized in Cayenne, French Guiana during January 1, 1997-December 31, 2008 were selected retrospectively from the French Hospital Database on HIV. Multivariate analysis showed that tuberculosis was associated with cough (adjusted odds ratio [AOR] = 0.20, 95% confidence interval [CI] = 0.05-0.73) and a C-reactive protein level > 70 mg/L (AOR = 0.98, 95% CI = 0.97-0.99). Variables suggesting an association with disseminated histoplasmosis were a gamma-glutamyl transferase level > 72 IU/L (AOR = 4.99, 95% CI = 1.31-18.99), origin from French Guiana (AOR = 5.20, 95% CI = 1.30-20.73), disseminated localization (AOR = 6.40, 95% CI = 1.44-28.45), a concomitant opportunistic infection (AOR = 6.71, 95% CI = 1.50-29.96), a neutrophil count < 2,750 cells/mm 3 (AOR = 10.54, 95% CI = 2.83-39.24), CD4 cell count < 60 cells/mm3 (AOR = 11.62, 95% CI = 2.30-58.63), and a platelet count < 150,000/mm3 (AOR = 19.20, 95% CI = 3.35-110.14). Tuberculosis and histoplasmosis have similarities, but some factors show a greater association with one of these diseases. Thus, adapted therapeutic choices can be made by using simple clinical and paraclinical criteria.

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24394475

Am. J. Trop. Med. Hyg., 90(2), 2014, pp. 216–223
doi:10.4269/ajtmh.13-0084

Tuberculosis and Histoplasmosis among Human Immunodeficiency Virus–Infected Patients: A Comparative Study

Antoine Adenis,* Mathieu Nacher, Matthieu Hanf, Célia Basurko, Julie Dufour, Florence Huber, Christine Aznar, Bernard Carme, and Pierre Couppie

Centre d'Investigation Clinique Epidémiologie Clinique Antilles-Guyane, Service de Dermatologie Vénérologie, et Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Inserm CIE 802, Centre Hospitalier de Cayenne, Cayenne, French Guiana; Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana

Abstract. In disease-endemic areas, histoplasmosis is the main differential diagnosis for tuberculosis among human immunodeficiency virus (HIV)–infected patients. However, no study has compared the two diseases. Thus, the objective of this study was to compare tuberculosis and histoplasmosis in HIV-infected patients. A population of 205 HIV-infected patients (99 with tuberculosis and 106 with histoplasmosis) hospitalized in Cayenne, French Guiana during January 1, 1997–December 31, 2008 were selected retrospectively from the French Hospital Database on HIV. Multivariate analysis showed that tuberculosis was associated with cough (adjusted odds ratio [AOR] = 0.20, 95% confidence interval [CI] = 0.05–0.73) and a C-reactive protein level > 70 mg/L (AOR = 0.98, 95% CI = 0.97–0.99). Variables associated with disseminated histoplasmosis were a γ-glutamyl transferase level > 72 IU/L (AOR = 4.99, 95% CI = 1.31–18.99), origin from French Guiana (AOR = 5.20, 95% CI = 1.30–20.73), disseminated localization (AOR = 6.40, 95% CI = 1.44–28.45), a concomitant opportunistic infection (AOR = 6.71, 95% CI = 1.50–29.96), a neutrophil count < 2,750 cells/mm^3 (AOR = 10.54, 95% CI = 2.83–39.24), a CD4 cell count < 60 cells/mm^3 (AOR = 11.62, 95% CI = 2.30–58.63), and a platelet count < 150,000/mm^3 (AOR = 19.20, 95% CI = 3.35–110.14). Tuberculosis and histoplasmosis have similarities, but some factors show a greater association with one of these diseases. Thus, adapted therapeutic choices can be made by using simple clinical and paraclinical criteria.

INTRODUCTION

With an acquired immunodeficiency syndrome (AIDS) incidence 10 times higher than the incidence in mainland France, and a prevalence > 1%, French Guiana is the French territory where the human immunodeficiency virus (HIV) epidemic is the most preoccupying.[1] Histoplasmosis and tuberculosis are among the top four AIDS-defining illnesses. Disseminated histoplasmosis was the first AIDS-related cause of death in 2005.[2,3]

Histoplasmosis and tuberculosis during the HIV infection are often seen as disseminated infections. With the aggravation of immunodeficiency, dissemination of the pathogen causes a rapid and fatal evolution in the absence of treatment.[4,5] At this stage, invasive diagnostic methods are necessary. Biological confirmation through pathogen identification by culture is long and sometimes difficult in cases of profound immunosuppression. Rapid and sensitive antigenic detection techniques are not available in most countries.[6]

The non-specific nature of the clinical, biologic, histologic, and radiologic findings for these two diseases makes differential diagnosis of histoplasmosis difficult in disease-endemic areas. Thus, numerous publications report cases of histoplasmosis mimicking tuberculosis, most often because of the absence of diagnostic facilities or because a diagnosis of histoplasmosis was not considered.[7–11]

Although studies before the HIV era reported a high prevalence of positive histoplasmin test results there have been few publications on this disease in the Caribbean and the Guianas.[3,12,13] In low-resource countries, a number of AIDS cases may die of histoplasmosis mistaken for multidrug-resistant tuberculosis.[10,14,15]

In French Guiana, because of their high incidence, clinicians generally suspect histoplasmosis and tuberculosis in immunosuppressed patients at admission. Prolonged hospitalizations are often necessary for identification of infecting pathogens by invasive procedures. Mycologic and mycobacteriologic screening are systematically performed. Frequently, a presumptive treatment is initiated before culture results are obtained.[16]

In this context, a comparative study between tuberculosis and histoplasmosis seems straightforward and might be useful. A systematic review of the literature showed that since 1906, studies on the differential diagnosis of tuberculosis and histoplasmosis have not been conducted.[17] Although it is assumed that these two diseases are similar, there is little basis for making this conclusion.

The objective of this study was to compare tuberculosis and histoplasmosis in HIV-infected patients to identify epidemiologic, clinical, biologic, and radiologic differences. A secondary objective was to help therapeutic decisions in life-threatening situations.

MATERIALS AND METHODS

This retrospective study was conducted at Cayenne Hospital (Cayenne, French Guiana) during January 1997–December 2008. The study population consisted of HIV-infected patients from the French Hospital Database on HIV, a national cohort for which data have been routinely collected since 1992. This database was approved by the Commission Nationale Informatique et Libertés. The study database and protocol were approved by the Institut National de la Santé et de la Recherche Médicale (INSERM (IRB00000388, FWA00005831). Patients provided written informed consent.

Inclusion criteria for study participants were an age ≥ 18 years), admission to Cayenne Hospital or a visit to

*Address correspondence to Antoine Adenis, Centre d'Investigation Clinique Epidémiologie Clinique Antilles-Guyane, INSERM CIE 8202, Centre Hospitalier de Cayenne, Avenue des Flamboyants, BP 6006, 97 300 Cayenne, French Guiana. E-mail: antoine.adenis@gmail.com

the outpatient department before admission (the inclusion date corresponding to the date of treatment initiation for tuberculosis or histoplasmosis), inclusion in the French Hospital Database, confirmed HIV infection (by Western blotting), confirmed tuberculosis (culture and identification of *Mycobacterium tuberculosis*) or histoplasmosis (direct examination and/or culture of *Histoplasma capsulatum var. capsulatum*), and pre-treatment biologic screening < 7 days before treatment initiation. Exclusion criteria were concomitant tuberculosis and histoplasmosis, a history of tuberculosis or histoplasmosis, immune reconstitution disease caused by tuberculosis or histoplasmosis, and a diagnosis of tuberculosis or histoplasmosis by polymerase chain reaction only, which is not a valid reference method for diagnosis.

Data were collected on a standardized form and included sociodemographic, clinical, biologic, radiologic, and therapeutic information. Clinical evaluation of the patients' general condition upon admission used the Eastern Cooperative Oncology Group score,[18] also known as the World Health Organization performance status score. These data were then entered in an anonymized database.

Statistical analysis was performed by using Stata version 10.0 (StataCorp LP, College Station, TX).[19] Descriptive and comparative analysis was performed by using Pearson's chi-square test or Fischer's exact test for categorical variables, Student's *t* test for normally distributed quantitative variables, or Mann-Whitney test for non-Gaussian distributions. Statistical significance was set at $P < 0.05$.

To identify criteria independently associated with tuberculosis and histoplasmosis, stepwise unconditional logistic regression was used.[20] Forward and backward models were used to verify convergence. The dependent variable was tuberculosis or histoplasmosis, coded 0 and 1 respectively. Odds ratios (ORs) and 95% confidence intervals were obtained. For an OR < 1, the variable was associated with tuberculosis, and for an OR > 1, the variable was associated with histoplasmosis.

To select variables for the multivariate model, bivariate logistic regression models used categorical independent variables. In the absence of similar studies in the literature, the cut-off value was the median or mean according to the distribution of continuous variables. For some variables, the reference laboratory threshold was used because it was clinically meaningful (i.e., for platelet counts, the median was 222,000/mm^3, but categorization used a threshold < 150,000/mm^3). For ferritinemia, some ferritin concentrations were > 1,000 μg/L. To avoid confusion, the variable was categorized as > or < 1,000 μg/L.

Age, sex, and variables that had a $P < 0.2$ were used in the multivariate model. Variables with a $P < 0.2$ (systolic and diastolic blood pressure, chest computed tomography scan, abdominal ultrasonography, albuminemia, serum iron level, ferritinemia, triglyceridemia, and fibrinogenemia) were not included in this model because of a large number of missing values and to avoid a large proportion of persons who were dropped from the final model. The saturated multivariate model included 156 patients.

RESULTS

Results of univariate and bivariate analysis are shown in Table 1. The study population consisted of 205 HIV-positive persons. Two groups were defined: 99 persons with tuberculosis and 106 persons with histoplasmosis. There was a predominance of males in both groups. The mean ± SD age was similar in both groups (39.3 ± 11.6 for persons with tuberculosis and 41.6 ± 10.6 for persons with histoplasmosis) ($P = 0.151$).

The proportion of patients from Guyana and Brazil was greater for those with tuberculosis than for those with histoplasmosis. The proportion of patients originating from French Guiana and Suriname was significantly greater for those with histoplasmosis than for those with tuberculosis. Similarly, the proportion of patients having spent > 18 years in French Guiana was greater for persons with histoplasmosis than for those with tuberculosis. The proportion of patients residing outside the Cayenne urban community (residing mostly in western French Guiana along the Maroni River) was significantly greater for person with histoplasmosis than for those with tuberculosis. More specifically, patients residing along the Maroni River represented 17% of persons with histoplasmosis and 7% of those with tuberculosis ($P = 0.047$).

Tuberculosis and histoplasmosis were AIDS-defining events for most persons. The proportion of persons receiving antiretroviral treatment and/or taking trimethoprim-sulfamethoxazole primary prophylaxis on admission was low in both groups. A history of opportunistic infections was in similar proportions in both groups. A major proportion of patients were febrile on admission in both groups. Dyspnea, abdominal pain, diarrhea, and neurologic signs were frequent in both groups, but there was no significant difference.

The presence of concomitant opportunistic infections was more frequent in persons with histoplasmosis than in those with tuberculosis. Tobacco addiction was more frequent in persons with tuberculosis than in those with histoplasmosis. Similarly, multiple addictions were significantly more frequent in persons with tuberculosis than in those with histoplasmosis.

The proportion of patients with a Eastern Cooperative Oncology Group/World Health Organization performance status score > 2 was significantly greater for persons with histoplasmosis than for those with tuberculosis. Although most patients were febrile at admission, the mean ± SD temperature was significantly higher for person with histoplasmosis (39.5 ± 0.9°C) than for persons with tuberculosis (39.0 ± 1.0°C) ($P = 0.021$). The median (interquartile range [IQR]) evolution duration of fever was 30 (8–60) days for persons with tuberculosis and 21 (10–30) days for persons with histoplasmosis ($P = 0.390$). The proportion of persons with a systolic blood pressure < 90 mm Hg and a diastolic blood pressure < 60 mm Hg was significantly higher in persons with histoplasmosis than in those with tuberculosis.

Initial physical examination showed that symptoms involving the pleuro-pulmonary sphere were more frequent in those with tuberculosis (78%) than in those with histoplasmosis (49%) ($P < 0.001$) (Figure 1). The proportion of patients with cough and/or chest pain was higher in persons with tuberculosis than in those with histoplasmosis. Symptoms involving the abdominal sphere were more frequent in persons with histoplasmosis (70%) than in those with tuberculosis (48%) ($P = 0.001$) (Figure 1). Hepatomegaly

TABLE 1
Univariate and bivariate analyses of tuberculosis and histoplasmosis in 205 human immunodeficiency virus–infected patients, French Guiana*

Variable	Tuberculosis, no. positive/ no. tested (%)	Histoplasmosis, no. positive/ no. tested (%)	Bivariate analysis	
			OR (95% CI)	*P*
Sex†				
M	70/99 (71)	69/106 (65)	1	
F	29/99 (29)	37/106 (35)	1.29 (0.72–2.33)	0.391
Age < 40 years†	50/99 (50)	51/106 (48)	0.91 (0.52–1.57)	0.732
Geographic origin†‡				
Haiti	42/96 (44)	33/105 (31)	1	
French Guiana	12/96 (12)	33/105 (31)	3.50 (1.57–7.81)	0.002
Europe	5/96 (5)	2/105 (2)	0.51 (0.09–2.79)	0.437
Guadeloupe	3/96 (3)	6/105 (6)	2.54 (0.59–10.95)	0.209
Guyana	19/96 (20)	6/105 (6)	0.40 (0.14–1.12)	0.081
Suriname	1/96 (1)	17/105 (16)	21.64 (2.74–171.07)	0.004
Brazil	12/96 (12)	6/105 (6)	0.64 (0.22–1.87)	0.412
Africa	2/96 (2)	1/105 (1)	0.64 (0.05–7.33)	0.717
Time spent in French Guiana > 18 years†§	31/76 (41)	60/99 (61)	2.23 (1.21–4.11)	0.010
Residence location†‡				
Cayenne urban community	60/84 (72)	64/101 (63)	1	
Homeless	12/84 (14)	8/101 (8)	0.62 (0.24–1.63)	0.338
Outside Cayenne urban community	12/84 (14)	29/101 (29)	2.27 (1.06–4.84)	0.035
AIDS-defining event	77/92 (84)	87/106 (82)	0.89 (0.42–1.87)	0.763
Treatment on admission				
Antiretroviral drugs	15/98 (15)	16/105 (15)	0.99 (0.46–2.14)	0.989
Primary prophylaxis	20/98 (20)	23/98 (23)	2.00 (0.61–2.36)	0.605
Opportunistic infection				
History†	15/98 (15)	18/106 (17)	1.13 (0.54–2.39)	0.746
Concomitant†	17/98 (17)	45/106 (42)	3.51 (1.84–6.73)	< 0.001
Drug addictions				
Tobacco†	14/98 (14)	1/104 (1)	0.06 (0.01–0.46)	0.007
Alcohol	11/98 (11)	9/104 (9)	0.76 (0.30–1.91)	0.557
Marijuana	2/98 (2)	1/104 (1)	0.47 (0.04–5.27)	0.541
Crack cocaine†	14/98 (14)	7/104 (7)	0.43 (0.17–1.12)	0.085
Multiple addictions (≥ 2)	11/98 (11)	2/103 (2)	0.16 (0.03–0.73)	0.18
Isolate localization				
Pleuro-pulmonary	91/99 (92)	28/106 (26)	0.03 (0.01–0.07)	< 0.001
Lymph node	18/99 (18)	21/106 (20)	1.11 (0.55–2.24)	0.766
Liver	8/99 (8)	28/106 (26)	4.08 (1.76–9.48)	0.001
Peripheral blood	6/99 (6)	16/106 (15)	2.76 (1.03–7.36)	0.043
Gastrointestinal	4/99 (4)	17/106 (16)	4.54 (1.47–14.00)	0.009
Bone marrow	1/99 (1)	46/106 (43)	75.13 (10.10–559.10)	< 0.001
Disseminated†	37/99 (37)	98/106 (92)	20.53 (8.97–46.97)	< 0.001
Physical condition at admission				
WHO performance status score > 2†	23/97 (24)	42/52 (81)	13.51 (5.87–31.10)	< 0.001
Weight loss	67/88 (76)	35/43 (81)	1.37 (0.55–3·41)	0.497
Physical examination				
Fever	83/97 (86)	93/106 (88)	1.21 (0.54–2.71)	0.650
Systolic blood pressure < 90 mm Hg	4/64 (6)	10/29 (34)	7.89 (2.22–28.09)	0.001
Diastolic blood pressure < 60 mm Hg	7/64 (11)	10/29 (34)	4.29 (1.43–12.83)	0.009
Cough†	70/98 (71)	45/105 (43)	0.30 (0.17–0.54)	< 0.001
Dyspnea†	13/98 (13)	21/105 (20)	1.63 (0.79–3.48)	0.202
Chest pain†	20/98 (20)	2/105 (2)	0.08 (0.02–0.33)	0.001
Abdominal pain	30/98 (31)	34/105 (32)	1.09 (0.60–1.96)	0.786
Diarrhea	22/98 (22)	29/105 (28)	1.32 (0.70–2.50)	0.397
Ascitis	1/98 (1)	3/105 (3)	2.85 (2.92–27.90)	0.368
Hepatomegaly†	19/98 (19)	41/105 (39)	2.62 (1.39–4.95)	0.003
Splenomegaly†	4/98 (4)	21/105 (20)	5.81 (1.92–17.59)	0.002
Lower digestive bleeding†	1/98 (1)	7/105 (7)	6.93 (0.84–57.38)	0.073
Headache	9/98 (9)	10/105 (9)	1.04 (0.40–2.68)	0.934
Confusion	7/98 (7)	5/105 (5)	0.65 (0.20–2.12)	0.475
Cognitive and/or motor dysfunction	5/98 (5)	2/105 (2)	0.36 (0.07–1.90)	0.230
Lymphadenopathy > 2 cm†	15/97 (15)	31/105 (29)	2.29 (1.15–4.57)	0.019
Chest radiograph				
Interstitial syndrome	35/92 (38)	38/104 (36)	0.94 (0.52–1.67)	0.828
Alveolar syndrome†	30/92 (33)	2/104 (2)	0.04 (0.01–0.17)	< 0.001
Pleural effusion†	12/92 (13)	1/104 (1)	0.06 (0.01–0.51)	0.009
Pulmonary infiltrate†	10/92 (11)	1/104 (1)	0.08 (0.01–0.63)	0.017

(continued)

TABLE 1
Continued

Variable	Tuberculosis, no. positive/ no. tested (%)	Histoplasmosis, no. positive/ no. tested (%)	Bivariate analysis	
			OR (95% CI)	*P*
Chest CT scan				
Interstitial syndrome	16/30 (53)	2/11 (18)	0.19 (0.04–1.06)	0.058
Alveolar syndrome	11/30 (37)	2/11 (18)	0.38 (0.07–2.11)	0.270
Pleural effusion	6/30 (20)	1/11 (9)	0.40 (0.04–3.76)	0.423
Mediastinal adenopathy	16/30 (53)	4/11 (36)	0.50 (0.12–2.07)	0.340
Abdominal ultrasonography				
Hepatomegaly	14/58 (24)	20/34 (59)	4.49 (1.81–11.15)	0.001
Splenomegaly	4/58 (7)	12/34 (35)	7.36 (2.14–25.33)	0.002
Adenopathy	19/58 (33)	12/34 (35)	1.12 (0.46–2.73)	0.804
Ascitis	2/58 (3)	1/34 (3)	0.85 (0.07–9.72)	0.895
Abdominal CT scan				
Hepatomegaly	3/15 (20)	2/6 (33)	2.00 (0.24–16.61)	0.521
Splenomegaly	2/15 (13)	1/6 (17)	1.30 (0.09–17.73)	0.844
Adenopathy	7/15 (47)	3/6 (50)	1.14 (0.17–7.60)	0.890
Standard biology				
CD4 cell count < 60 cells/mm^3†	30/95 (32)	71/105 (68)	4.52 (2.49–8.20)	< 0.001
CD8 cell count < 650 cells/mm^3†	34/93 (37)	58/98 (59)	2.52 (1.40–4.51)	0.002
Hemoglobin level < 9 g/dL†	41/97 (42)	63/105 (60)	2.05 (1.17–3.59)	0.012
Neutrophil count < 2,750 cells/mm^3†	22/92 (24)	75/102 (74)	8.84 (4.61–16.94)	< 0.001
Platelet count < 150,000/mm^3†	9/96 (9)	45/104 (43)	7.37 (3.35–16.22)	< 0.001
Creatinine level > 132.74 μmol/L†	8/95 (8)	15/101 (15)	1.90 (0.76–4.70)	0.167
Protein level > 83 g/L	33/91 (36)	14/35 (40)	1.17 (0.53–2.61)	0.698
Albumin level < 35 g/L	4/6 (67)	14/15 (93)	7.00 (0.50–98.60)	0.149
AST level > 34 IU/L†	43/96 (45)	79/103 (77)	4.06 (2.21–7.46)	< 0.001
ALT level > 44 IU/L†	15/96 (16)	30/102 (29)	2.25 (1.12–4.51)	0.022
γ-Glutamyl transferase level > 72 IU/L†	37/94 (39)	64/102 (63)	2.59 (1.46–4.62)	0.001
Alkaline phosphatase level > 100 IU/L†	39/95 (41)	64/103 (62)	2.36 (1.33–4.17)	0.003
Lactate dehydrogenase level > 385 IU/L†	32/80 (40)	55/96 (57)	2.01 (1.10–3.68)	0.023
C-reactive protein level > 70 mg/L†	52/90 (58)	43/99 (43)	0.56 (0.31–1.00)	0.050
Serum iron level < 9 μg/L	27/33 (82)	19/25 (76)	0.70 (0.20–2.52)	0.589
Ferritin level > 1,000 μg/L	11/39 (28)	22/31 (71)	6.22 (2.19–17.66)	0.001
Triglyceride level > 1.72 mmol/L	7/37 (19)	11/21 (52)	4.71 (1.44–15.46)	0.010
Fibrinogen level > 4.2 g/L	12/21 (57)	4/20 (20)	0.19 (0·05–0.76)	0.019
Prothrombin ratio < 70%	20/55 (36)	15/30 (50)	1.75 (0.71–4.31)	0.224

*OR = odds ratio; CI = confidence interval; AIDS = acquired immunodeficiency syndrome; WHO = World Health Organization; CT = computed tomography; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Bivariate analysis was conducted by using a logistic regression model.
†Variables selected for the multivariate analysis model.
‡Dummy variables were created for the geographic origin and the residence location with Haiti and the Cayenne urban community as the respective referent population coded as 1.
§Threshold of 18 years corresponds to the median.

and/or splenomegaly was significantly more frequent in persons with histoplasmosis than in those with tuberculosis. Lymphadenopathy > 2 cm was greater in persons with histoplasmosis (29%) than in those with tuberculosis (15%) (Figure 1).

Chest radiographs showed an interstitial syndrome that was comparable in both groups. The proportion of patients with deep lymphadenopathy and/or ascitis was also similar in both groups. Chest radiographs also showed that an alveolar syndrome and/or pleural effusions and/or pulmonary infiltrates were greater in persons with tuberculosis than in those with histoplasmosis. Abdominal ultrasonography showed that hepatomegaly and/or splenomegaly were greater in persons with histoplasmosis than in those with tuberculosis.

Blood counts showed that the median CD4 cell count was low in both groups (111 cells/mm^3, IQR = 48–259 for persons with tuberculosis and 37/mm^3, IQR = 15–84 for persons with histoplasmosis). The average hemoglobin level was decreased in both groups. There was no significant difference in prothrombin rate for both groups.

Immunosuppression was more advanced for persons with histoplasmosis than for those with tuberculosis. Anemia (hemoglobin level < 9 g/dL) was significantly more pronounced for persons with histoplasmosis than for those with tuberculosis. Neutrophil count was significantly lower in persons with histoplasmosis than in those with tuberculosis. Thrombocytopenia (platelet count < 150,000/mm) was more frequent in persons with histoplasmosis than in those with tuberculosis (Figure 1). The proportion of patients with fibrinogenemia > 4.2 g/L (laboratory threshold) was significantly higher for persons with tuberculosis than for those with histoplasmosis.

Median levels of lactate dehydrogenase (LDH) were increased in both groups (328 IU/L, IQR = 231–491 for persons with tuberculosis and 446 IU/L, IQR = 316–1,216 for those with histoplasmosis). Renal failure or hypoalbuminemia was observed in both groups.

Abnormal liver test results were more frequent in persons with histoplasmosis than in those with tuberculosis (Figure 1). Hepatic cytolysis (aspartate aminotransferase levels > 34 IU/L and alanine aminotransferase levels > 44 IU/L) and cholestasis (γ-glutamyl transferase levels > 72 IU/L and alkaline phosphatase levels > 100 IU/L) were significantly more frequent in persons with histoplasmosis. Lactate dehydrogenase levels > 365 IU/L were more frequent in persons with histoplasmosis (57%) than in those with tuberculosis (40%). C-reactive protein levels > 70 mg/L were more frequent in

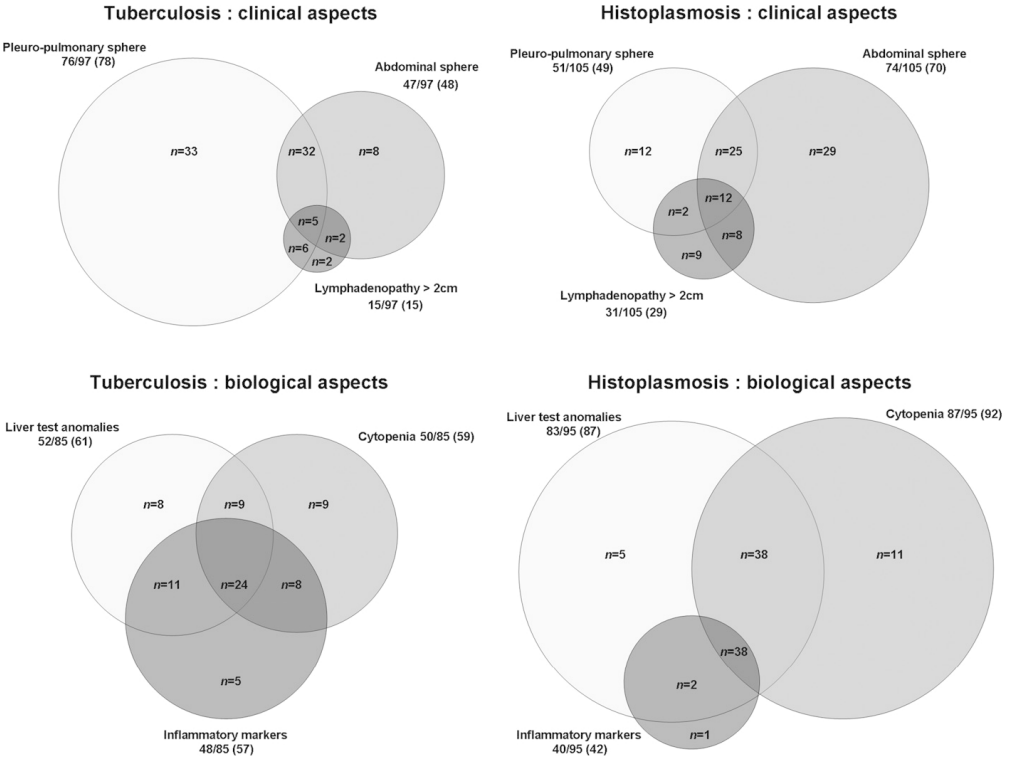


FIGURE 1. Comparison of clinical and biological aspects of tuberculosis and histoplasmosis, French Guiana. Circles indicate no. positive patients/no. tested (%). The scale used proportions to avoid distortion of data. Circles intersections indicate n, which corresponded to the number of patients concerned. Because of missing values, the total for each group is different from the 99 patients with tuberculosis and the 106 patients with histoplasmosis. Anomalous liver test results were defined by an aspartate aminotransferase level > 34 IU/L, an alanine aminotransferase level > 44 IU/L, a γ-glutamyl transferase level > 72 IU/L, or an alkaline phosphatase level > 100 IU/L. Cytopenia was defined by a hemoglobin level < 9 g/dL, a neutrophil count < 2,750 cells/mm^3, or a platelet count < 150,000/mm^3. Inflammatory markers were defined by a C-reactive protein level > 70 mg/L.

persons with tuberculosis than in those with histoplasmosis (Figure 1). Ferritinemia > 1,000 μg/L and/or triglyceridemia >1.72 mmol/L (laboratory threshold) were significantly more frequent in persons with histoplasmosis than in those with tuberculosis.

Lymph node localizations of microorganisms were similar in both groups (18% for persons with tuberculosis and 20% for those with histoplasmosis). Isolates from pleural and pulmonary localizations were more frequent in persons with tuberculosis than in those with histoplasmosis. Isolates from localizations, such as bone marrow, liver, gastrointestinal tract and peripheric blood, were significantly more frequent in persons with histoplasmosis than in those with tuberculosis.

A subgroup of patients who did not have concomitant opportunistic infections was analyzed separately to verify that the results were not affected by concurrent opportunistic infections. There were 91 cases of tuberculosis and 80 cases of histoplasmosis. Replication of the above analyses in this subgroup showed identical differences, suggesting that concomitant infections did not confound the above results.

Multivariate analysis. Forward and backward stepwise logistic regression converged on identical models. Results from multivariate analysis are shown in Table 2 and significant results according to the forward model are shown in Figure 2.

Variables significantly associated with tuberculosis were cough and a C-reactive protein level > 70 mg/L. Variables significantly associated with histoplasmosis were γ-glutamyl transferase level > 72 IU/L, origin from French Guiana, disseminated localizations, concomitant opportunistic infections, neutrophil count < 2,750 cells/mm^3, CD4 cell count <60 cells/mm^3, and platelet count < 150,000/mm^3.

DISCUSSION

Tuberculosis and histoplasmosis have similar symptoms.[8,21,22] This study tested whether histoplasmosis and tuberculosis are

TABLE 2
Multivariate analysis of tuberculosis and histoplasmosis in 205 human immunodeficiency virus–infected patients, French Guiana*

Variable	AOR (95% CI)	*P*
Geographic origin†		
French Guiana	5.20 (1.30–20.73)	0.020
Opportunistic infection		
Concomitant	6.71 (1.50–29.96)	0.013
Isolate localization		
Disseminated	6.40 (1.44–28.45)	0.015
Physical condition on admission		
WHO performance status score > 2	0.29 (0.07–1.24)	0.095
Physical examination		
Cough	0.20 (0.05–0.73)	0.015
Lymphadenopathy > 2 cm	2.89 (0.64–12.95)	0.166
Standard biology		
CD4 cell count < 60 cells/mm^3	11.62 (2.30–58.63)	0.003
CD8 cell count < 650 cells/mm^3	0.31 (0.08–1.29)	0.107
Neutrophil count < 2,750 cells/mm^3	10.54 (2.83–39.24)	< 0.001
Platelet count < 150,000/mm^3	19.20 (3.35–110.14)	0.001
γ-glutamyl transferase level > 72 IU/L	4.99 (1.31–18.99)	0.018
C-reactive protein level > 70 mg/L	0.98 (0.97–0.99)	0.008

*AOR = adjusted odds ratio; CI = confidence interval; WHO = World Health Organization. Multivariate analysis was conducted by using a forward stepwise unconditional logistic regression model.
†A dummy variable was created for the geographic origin with Haiti as the respective referent population coded as 1.

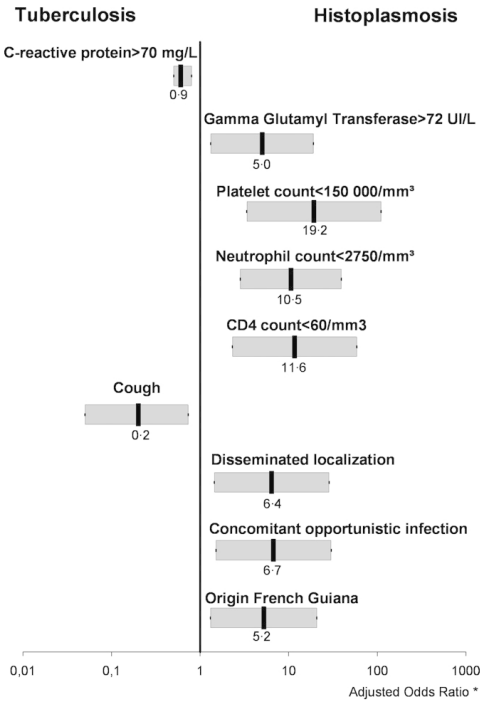


FIGURE 2. Comparison of significant results ($P < 0.05$) in multivariate analysis for tuberculosis and histoplasmosis, French Guiana. Adjusted odds ratios and 95% confidence intervals (horizontal gray bars) are indicated on a logarithmic scale.

indiscernible in HIV-infected patients. Although these pathologies share certain similarities, there are clinical and laboratory differences. Tuberculosis was more frequent among patients from Guyana and Brazil, and histoplasmosis was more frequent among patients from French Guiana and Suriname. Residence duration in French Guiana and residence location outside urban was more frequent in persons with histoplasmosis. Thus, tuberculosis seemed to be an urban pathology and histoplasmosis seemed more to be a rural pathology, notably near the Maroni River.

Most patients did not take any treatment (antiretroviral or primary prophylaxis) at the time of diagnosis. It was therefore not surprising to observe concurrent opportunistic infections, notably in patients with histoplasmosis.

The initial clinical presentation in both groups was dominated by prolonged fever in a context of poor general condition, notably weight loss. However, this condition was more pronounced in persons with histoplasmosis.

Symptoms involving the pleuro-pulmonary sphere were more frequent in persons with tuberculosis, and *M. tuberculosis* was more frequently identified at the pleuro-pulmonary level (Figure 1). Similarly, cough or chest pain associated with abnormal chest radiograph results were more frequent in persons with tuberculosis. Although an interstitial syndrome was observed in both groups, alveolar syndrome and pulmonary infiltrate were more evocative of tuberculosis.

Conversely, symptoms or abnormal medical imagery results involving the abdominal sphere were more frequent in persons with histoplasmosis. *Histoplasma capsulatum* was identified more frequently at the abdominal level (liver and gastrointestinal tract). Hepatomegaly, splenomegaly, and lower digestive bleeding associated with abnormal abdominal ultrasonographic results were more frequent in persons with histoplasmosis. Although the presence of deep lymphadenopathy was similar in both groups, hepatomegaly or splenomegaly were more frequent in persons with histoplasmosis. Lymph node localizations were frequently observed in both groups, but lymphadenopathy > 2 cm seemed more evocative of histoplasmosis. Most cases of histoplasmosis were disseminated forms, presumably because of greater immunodepression than in tuberculosis cases, facilitating dissemination of the pathogen.

Results of standard biologic tests showed numerous differences. Although anemia was common in both groups, it was more frequent in persons with histoplasmosis. It was generally part of pancytopenia, which was also significantly more frequent in persons with histoplasmosis. Moreover, abnormal liver test results (hepatic cytolysis and cholestasis), increased LDH levels, and hypertriglyceridemia were more frequent in persons with histoplasmosis than in those with tuberculosis. Conversely and independent of CD4 cell counts, inflammatory markers were more frequent in persons with tuberculosis than in those with histoplasmosis (increased levels of C-reactive protein and fibrinogen), but ferritinemia (ferritin concentration > 1,000 μg/L) was more frequent in persons with histoplasmosis.

Increased LDH levels have been reported as an indicator for diagnosis of tuberculosis and histoplasmosis[16,23,24] and as a prognostic factor in severe disseminated forms of histoplasmosis.[6] In the present study, after adjusting for CD4 cell counts, we found no significant difference in LDH levels between persons with tuberculosis and those

with histoplasmosis. Thus, increased LDH levels could not discriminate tuberculosis from histoplasmosis.

Ferritin concentration has also been described as an indicator for the diagnosis of severe disseminated histoplasmosis.[25] However, because of a large number of missing values, this variable was not selected for multivariate analysis.

This study had limitations. Data were collected retrospectively, which might have led to selection biases. Missing values for certain variables that were not included in the regression model may not have been missing randomly and were most likely influenced by clinical experience of the physicians. Histoplasmosis and tuberculosis represent only 50% of AIDS-defining illnesses in French Guiana, a finding that does not enable definition of decisional algorithms adapted to clinical situations.

Despite its limitations, this study is the first to compare tuberculosis and histoplasmosis among HIV-positive patients. A study reported excessive mortality in patients receiving antituberculosis treatment because of lack of knowledge about histoplasmosis or diagnostic difficulties.[10] Thus, not knowing the differences between tuberculosis and histoplasmosis is a serious concern because it should strongly encourage clinicians in disease-endemic areas to conduct invasive diagnostic procedures or initiate presumptive treatment, notably in the presence of severity criteria.[16,26]

Nevertheless, in addition to a high clinical suspicion index, there is a need for an affordable, specific, sensitive, user friendly, robust, rapid, equipment free, and deliverable diagnostic tool to help clinicians in low-resource countries diagnose histoplasmosis.

The question initiating this study was whether tuberculosis and histoplasmosis in HIV-infected patients are clinically similar. The answer to this question is no. Although tuberculosis and histoplasmosis have similarities, a certain number of particularities described in this report can aid therapeutic decisions by clinicians, notably when a short-term prognosis is at risk.

Received February 12, 2013. Accepted for publication September 4, 2013.

Published online January 6, 2014.

Financial support: This study was supported by the grant Investissement d'Avenir from the Agence Nationale de la Recherche (CEBA reference no. ANR-10-LABX-0025).

Authors' addresses: Antoine Adenis, Mathieu Nacher, Matthieu Hanf, and Célia Basurko, Centre d'Investigation Clinique Epidémiologie Clinique Antilles-Guyane, INSERM CIE 802, Centre Hospitalier de Cayenne, Cayenne, French Guiana, and Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana, E-mails: antoine.adenis@gmail.com, mathieu.nacher@ch-cayenne.fr, matthieu.hanf@ch-cayenne.fr, and c.basurko@free.fr. Julie Dufour and Florence Huber, Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana, and Service de Dermatologie Vénérologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mails: dufour-julie@orange.fr and flottehuber@yahoo.fr. Christine Aznar, Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana, and Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mail: christine.aznar1@wanadoo.fr. Bernard Carme, Centre d'Investigation Clinique Epidémiologie Clinique Antilles-Guyane, INSERM CIE 802, et Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, and et Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana, E-mail: bernard.carme@ch-cayenne.fr. Pierre Couppie, Equipe EA3593, Epidémiologie des Parasitoses et des Mycoses Tropicales, Université des Antilles et de la Guyane, Cayenne, French Guiana, and et Service de Dermatologie Vénérologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mail: couppie.pierre@voila.fr.

REFERENCES

1. Nacher M, El Guedj M, Vaz T, Nasser V, Randrianjohany A, Alvarez F, Sobesky M, Magnien C, Couppié P, 2005. Risk factors for late HIV diagnosis in French Guiana. *AIDS 19:* 727–729.
2. Lewden C, Sobesky M, Cabié A, Couppié P, Boulard F, Bissuel F, May T, Morlat P, Chêne G, Lamaury I, Salmon D; Group d'Étude Mortalité 2000, 2004. Causes of death among HIV infected adults in French Guyana and the French West Indies in the era of highly active antiretroviral therapy (HAART [in French]. *Med Mal Infect 34:* 286–292.
3. Nacher M, Aznar C, Blanchet D, Demar M, El Guedy M, Vaz T, Clyti E, Carme B, Couppié P, 2006. AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? *AIDS 20:* 951–952.
4. Wheat LJ, Connolly-Stringfield PA, Baker RL, Curfman MF, Eads ME, Israel KS, Norris SA, Webb DH, Zeckel ML, 1990. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and review of the literature. *Medicine (Baltimore) 69:* 361–374.
5. Jones BE, Young SM, Antoniskis D, Davidson PT, Kramer F, Barnes PF, 1993. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. *Am Rev Respir Dis 148:* 1292–1297.
6. Couppié P, Aznar C, Carme B, Nacher M, 2006. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. *Curr Opin Infect Dis 19:* 443–449.
7. Chan KS, Looi LM, Chan SP, 1993. Disseminated histoplasmosis mimicking miliary tuberculosis: a case report. *Malays J Pathol 15:* 155–158.
8. Tong P, Tan WC, Pang M, 1983. Sporadic disseminated histoplasmosis simulating miliary tuberculosis. *Br Med J (Clin Res Ed) 287:* 822–823.
9. Greene L, Peters B, Lucas SB, Pozniak AL, 2000. Extrapulmonary tuberculosis masking disseminated histoplasmosis in AIDS. *Sex Transm Infect 76:* 54–56.
10. Bulmer AC, Bulmer GS, 2001. Incidence of histoplasmin hypersensitivity in the Philippines. *Mycopathologia 149:* 69–71.
11. Rezende RE, Brunaldi MO, Girão MS, Zucoloto S, Garcia SB, Machado AA, Módena JL, 2009. Case report: esophageal histoplasmosis associated with disseminated tuberculosis in acquired immunodeficiency syndrome. *Am J Trop Med Hyg 80:* 347–350.
12. Hay RJ, White HS, Fields PE, Quamina DB, Dan M, Jones TR, 1981. Histoplasmosis in the eastern Caribbean: a preliminary survey of the incidence of the infection. *J Trop Med Hyg 84:* 9–12.
13. Floch H, 1953. Histoplasmosis in French Guiana [in French]. *Ann Parasitol Hum Comp 28:* 108–125.
14. Eza D, Cerrillo G, Moore DA, Castro C, Ticona E, Morales D, Cabanillas J, Barrantes F, Alfaro A, Benavides A, Rafael A, Valladares G, Arevalo F, Evans CA, Gilman RH, 2006. Postmortem findings and opportunistic infections in HIV-positive patients from a public hospital in Peru. *Pathol Res Pract 202:* 767–775.
15. Souza SL, Feitoza PV, Araújo JR, Andrade RV, Ferreira LC, 2008. Causes of death among patients with acquired immunodeficiency syndrome autopsied at the Tropical Medicine Foundation of Amazonas [in Portuguese]. *Rev Soc Bras Med Trop 41:* 247–251.
16. Huber F, Nacher M, Aznar C, Pierre-Damar M, El Guedj M, Vaz T, Vantilcke V, Mahamat A, Magnien C, Chauvet C, Carme A, Couppié P, 2008. AIDS-related *Histoplasma*

capsulatum var. capsulatum infection: 25 years experience of French Guiana. *AIDS 22:* 1047–1053.
17. Darling ST, 1906. A protozoon general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymph nodes. *JAMA 46:* 1283–1285.
18. Oken MM, Creech RH, Tormey DC, Horton J, Davies TE, McFAdden ET, Carbone RP, 1982. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol 5:* 649–655.
19. StataCorp, 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP.
20. Hosmer DW Jr, Wang CY, Lin IC, Lemeshow S, 1978. A computer program for stepwise logistic regression using maximum likelihood estimation. *Comput Programs Biomed 8:* 121–134.
21. Walls K, Furcolow ML, Lehan PH, 1958. Histoplasmosis as a problem in tuberculosis sanatoriums throughout the United States. *J Lab Clin Med 51:* 266–270.
22. Furcolow ML, Brasher CA, 1956. Chronic progressive (cavitary) histoplasmosis as a problem in tuberculosis sanatoriums. *Am Rev Tuberc 73:* 609–619.
23. Corcoran GR, Al-Abdely H, Flanders CD, Geimer J, Patterson TF, 1997. Markedly elevated serum lactate dehydrogenase levels are a clue to the diagnosis of disseminated histoplasmosis in patients with AIDS. *Clin Infect Dis 24:* 942–944.
24. Dos Santos RP, Scheid KL, Goldani LZ, 2008. Laboratory features for presumptive diagnosis of disseminated tuberculosis in HIV-infected patients. *Int J Tuberc Lung Dis 12:* 1340–1343.
25. Guturu P, Sarria JC, 2008. Extreme hyperferritenemia in an AIDS patient with disseminated histoplasmosis. *J Infect 57:* 356–357.
26. Couppié P, Sobesky M, Aznar C, Bichat S, Clyti E, Bissuel F, El Guedj M, Alvarez F, Demar M, Louvel D, Pradinaud R, Carme B, 2004. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. *Clin Infect Dis 38:* 134–138.

5. Aspects paracliniques

En Guyane française, les options pour le diagnostic de l'histoplasmose reposaient sur l'examen direct et la culture fongique, l'examen antomopathologique, un examen sérologique de type immunodiffusion et une méthode de Real-Time Polymérase Chain Reaction (RT-PCR). Si les deux premières méthodes pouvaient être pratiquées sur tout type de tissus ou fluide corporel, sérologie et RT-PCR étaient essentiellement réalisées à partir de prélèvements sanguins.

Malgré des résultats satisfaisants en terme de réduction de la mortalité avec ces méthodes diagnostiques (ARTICLE 5), il semblait que les méthodes rapides non invasives uniquement développées aux USA pourraient aider à aller plus loin dans la précocité de la prise en charge thérapeutique et la réduction de la mortalité.

Comme nous le verrons dans la deuxième partie de ce travail, les regards se portaient vers les méthodes ELISA de détection polyclonale d'antigènes urinaires d'*H. capsulatum*, simples, non invasives et peu coûteuses (moins coûteuses qu'une RT-PCR tout du moins).

Dans le même temps, quelques auteurs rapportaient une fréquence importante de faux positifis, liés à des réactions croisées avec *H. capsulatum*, lors de l'utilisation du test de détection d'antigène galactomananne d'*Aspergillus* sp. dans le sérum de patients infectés par le VIH. Selon les auteurs, en l'absence d'autre test antigénique commercialisé, ce test pouvait s'envisager comme une option pertinente pour orienter le diagnostic et, pour certains, utile pour le suivi de la décroissance de la fongémie sous traitement antifongique.

Afin d'évaluer les capacités de ce test de détection d'antigène galactomananne pour le diagnostic de l'histoplasmose chez les patients infectés par le VIH en Guyane française, nous avons collaboré à un travail mené par le laboratoire hospitalo-universitaire de parasitologie-mycologie du centre hospitalier de Cayenne (ARTICLE 10).

ARTICLE 10 : A complementary tool for management of disseminated Histoplasma capsulatum var. capsulatum infections in AIDS patients.

Author: Iriart, Xavier, Blanchet, Denis, Menard, Sandie, Lavergne, Rose-Anne, Chauvin, Pamela, **Adenis, Antoine**, Cassaing, Sophie, Fillaux, Judith, Magnaval, Jean-François, Demar, Magalie, Carme, Bernard, Bessieres, Marie-Hélène, Couppie, Pierre, Nacher, Mathieu, Berry, Antoine and Aznar, Christine

Year: 2014 **Journal:** International Journal of Medical Microbiology

ISSN: 1438-4221 **DOI:** http://dx.doi.org/10.1016/j.ijmm.2014.07.016

Abstract: In South America, disseminated histoplasmosis due to Histoplasma capsulatum var. capsulatum (H. capsulatum), is a severe and frequent opportunistic infection in AIDS patients. In areas outside the USA where specific-Histoplasma antigen detection is not available, the diagnosis is difficult. With the galactomannan antigen (GM) detection, a test commonly used for invasive aspergillosis diagnosis, there is a cross-reactivity with H. capsulatum that can be helpful for the diagnosis of histoplasmosis. The aim of this study was to evaluate the GM detection for the diagnosis of disseminated histoplasmosis in AIDS patients. The performance of the GM detection was evaluated with serum collected in French Guiana where H. capsulatum is highly endemic. Sera from AIDS patients with disseminated histoplasmosis occurring from 2002 to 2009 and from control HIV-positive patients without histoplasmosis were tested with the GM detection and Histoplasma-specific antibody detection (IEP). In 39 AIDS patients with proven disseminated histoplasmosis, the sensitivity of the Histoplasma IEP was only 35.9% and was linked to the TCD4+ lymphocyte level. For the GM detection, the sensitivity (Se) was 76.9% and specificity (Sp) was 100% with the recommended threshold for aspergillosis diagnosis (0.5). The test was more efficient with a 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−: 0.18). This study confirms that the GM detection can be a surrogate marker for the diagnosis of disseminated histoplasmosis in AIDS patients in endemic areas where Histoplasma EIA is not available.

URL: http://www.sciencedirect.com/science/article/pii/S1438422114000988

International Journal of Medical Microbiology 304 (2014) 1062–1065

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Short Communication

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

Xavier Iriart[a,f,**], Denis Blanchet[b], Sandie Menard[f], Rose-Anne Lavergne[a,f], Pamela Chauvin[a], Antoine Adenis[d], Sophie Cassaing[a], Judith Fillaux[a], Jean-François Magnaval[a], Magalie Demar[b,e], Bernard Carme[b,e], Marie-Hélène Bessieres[a], Pierre Couppie[c,e], Mathieu Nacher[d,e], Antoine Berry[a,f], Christine Aznar[b,e,*]

[a] *Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Toulouse, Toulouse, France*
[b] *Laboratoire Hospitalier et Universitaire Parasitologie Mycologie, Centre Hospitalier de Cayenne, BP 6006, 97300 Cayenne, French Guiana*
[c] *Service de dermatologie, Centre Hospitalier de Cayenne, BP 6006, 97300 Cayenne, French Guiana*
[d] *CIC-EC Antilles-Guyane, CIE 802 Inserm, Centre Hospitalier de Cayenne, BP 6006, 97300 Cayenne, French Guiana*
[e] *Equipe EA 3593 Épidémiologie des Parasitoses et Mycoses Tropicales, Université Antilles Guyane, Campus Saint Denis, Cayenne, French Guiana*
[f] *INSERM UMR1043/CNRS UMR5282/Université de Toulouse UPS, Centre de Physiopathologie de Toulouse Purpan (CPTP), Toulouse F-31300, France*

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 21 July 2014
Accepted 28 July 2014

Keywords:
Histoplasmosis
AIDS
Histoplasma capsulatum
Diagnosis
Platelia
Galactomannan
Antibody
Cross-reactivity

ABSTRACT

In South America, disseminated histoplasmosis due to *Histoplasma capsulatum* var. *capsulatum* (*H. capsulatum*), is a severe and frequent opportunistic infection in AIDS patients. In areas outside the USA where specific-*Histoplasma* antigen detection is not available, the diagnosis is difficult. With the galactomannan antigen (GM) detection, a test commonly used for invasive aspergillosis diagnosis, there is a cross-reactivity with *H. capsulatum* that can be helpful for the diagnosis of histoplasmosis. The aim of this study was to evaluate the GM detection for the diagnosis of disseminated histoplasmosis in AIDS patients. The performance of the GM detection was evaluated with serum collected in French Guiana where *H. capsulatum* is highly endemic. Sera from AIDS patients with disseminated histoplasmosis occurring from 2002 to 2009 and from control HIV-positive patients without histoplasmosis were tested with the GM detection and *Histoplasma*-specific antibody detection (IEP). In 39 AIDS patients with proven disseminated histoplasmosis, the sensitivity of the *Histoplasma* IEP was only 35.9% and was linked to the TCD4+ lymphocyte level. For the GM detection, the sensitivity (Se) was 76.9% and specificity (Sp) was 100% with the recommended threshold for aspergillosis diagnosis (0.5). The test was more efficient with a 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−: 0.18). This study confirms that the GM detection can be a surrogate marker for the diagnosis of disseminated histoplasmosis in AIDS patients in endemic areas where *Histoplasma* EIA is not available.

Introduction

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

* Corresponding author at: Laboratoire Hospitalier et Universitaire Parasitologie Mycologie, CH Andrée Rosemon, Cayenne 97306, French Guiana.
** Corresponding author at: Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Toulouse, Hôpital Purpan, Institut Fédératif de biologie (IFB), 330 avenue de Grande Bretagne, TSA 40031, 31059 Toulouse Cedex 9, France.
E-mail addresses: iriart.x@chu-toulouse.fr (X. Iriart), christine.aznar1@wanadoo.fr (C. Aznar).

http://dx.doi.org/10.1016/j.ijmm.2014.07.016
1438-4221/

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

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

Patients and methods

Study population

Disseminated histoplasmosis cases occurring in AIDS patients followed in the Cayenne hospital (French Guiana) were selected from 2002 to 2009. Among this cohort, all patients for whom sera were collected at the time of diagnosis of histoplasmosis (±10 days = baseline) and available for analysis (stored at −20 °C) were included in the study. HIV-patients were considered as histoplasmosis cases, if there was microbiological evidence of *Histoplasma* infection (positive direct examination and/or culture and/or polymerase chain reaction (Simon et al., 2010) for *Histoplasma capsulatum*). Thirty HIV-positive patients who had never been in an endemic area for *Histoplasma* were included as negative controls. For these controls, no critical antibiotics (Piperacillin-tazobactam), polyvalent immunoglobulins or dialysis were present when GM detection was performed.

GM detection and Histoplasma-specific antibody detection

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

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

Analysis and statistical methods

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

Results

Patient characteristics

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

Anti-Histoplasma antibody detection

For AIDS-patients with disseminated histoplasmosis, the sensitivity (Se) of *Histoplasma* IEP was only 35.9% [95% CI: 21.7–52.8] while the specificity (Sp) was 100% [95% CI: 85.9–100] (Table 1). The sensitivity was linked to the TCD4+ lymphocyte level as the counts of these cells was statistically higher in patients with positive *Histoplasma*-specific antibody detection than in patients with negative serology (26 [5; 37] vs 84 [77; 90]) (p = 0.007; Mann–Whitney rank sum test). On the contrary, there was no statistical difference for TCD8+ lymphocyte levels (data not shown).

GM detection for diagnosis of histoplasmosis

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

The area under the curve was 0.963 on the ROC curve (Fig. 1). Two other thresholds (0.4 and 0.35) appeared to be potentially more interesting than the recommended threshold 0.5, with sensitivities of 82.1% [95% CI: 65.9–91.9] and 87.2% [95% CI: 71.8–95.1] and specificities of 100% [95% CI: 85.9–100] and 93.3% [95% CI:

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

	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%)	–	–
- Hematologic (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 ≥ 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 ≥ 0.5) or antibodies, No. (%):	**32** (82.1%)	**0** (0.0%)	**<0.001**[b]

BAL, broncho-alveolar lavage; CSF, cerebro-spinal fluid.
[a] Calculated by Mann–Whitney rank sum test.
[b] Calculated by χ^2 test.

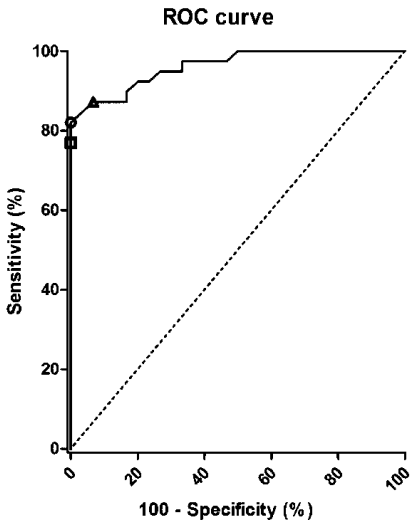


Fig. 1. Performance of GM detection according to the threshold. Receiver Operating Characteristics (ROC) curve for determination of cut-off and assay sensitivity (Se) and specificity (Sp). The thresholds 0.35, 0.4 and 0.5 are shown on the ROC curve with a triangle, circle and square, respectively.

76.5–98.8], respectively. Positive likelihood ratios (LR+) were 13.08 [95% CI: 3.41–50.2], +∞ [95% CI: non-calculable], +∞ [95% CI: non-calculable] for thresholds of 0.35, 0.4, 0.5, respectively, and negative likelihood ratios (LR−) 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 thresholds of 0.35, 0.4, 0.5, respectively.

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

Discussion

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

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

In two other studies (Wheat et al., 2007; Xavier et al., 2009), the sensitivity of the GM detection was about 48% and 67% in histoplasmosis diagnosis but these studies were not only limited to AIDS patients and the clinical presentation was not specified (e.g., fungemia vs non-disseminated disease). As the GM detection cross-reactivity seems to occur with high levels of *Histoplasma* antigens (Wheat et al., 2007), the sensitivity could be better in AIDS patients with disseminated disease because of a high *Histoplasma* burden (Ranque et al., 2007). For this reason, it seems to be essential to reserve the GM detection for disseminated histoplasmosis diagnosis in AIDS patients on the basis of epidemiological, clinical, and laboratory arguments. Moreover, as the incidence of

invasive aspergillosis in HIV-positive patients is generally <0.5% (Tong et al., 2009), the risk of misdiagnosis with aspergillosis is low. The positivity of the GM detection seems to be also very useful in the diagnosis and the monitoring of African histoplasmosis due to *Histoplasma capsulatum* var *duboisii* (Therby et al., 2014). Similarly, the cross-reactivity of the GM detection exists with *Cryptococcoccus* (Dalle et al., 2005) but the incidence of this infection was lower in French Guiana with about 0.25 per 100 HIV/AIDS patients-years (Debourgogne et al., 2011). Nevertheless, an important limitation of this test concerns its significant cost. Moreover, the test requires a large number of controls which does not make its use consistent with small series. In South America, where histoplasmosis should be considered as neglected disease, the price of this test does not easily allow its use outside the rich countries.

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

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

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

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the technicians of Toulouse and Cayenne Hospital for technical assistance and John Woodley for the revision of the English.

References

Aquino, V.R., Goldani, L.Z., Pasqualotto, A.C., 2007. Update on the contribution of galactomannan for the diagnosis of invasive aspergillosis. Mycopathologia 163, 191–202.

Connolly, P.A., Durkin, M.M., Lemonte, A.M., Hackett, E.J., Wheat, L.J., 2007. Detection of histoplasma antigen by a quantitative enzyme immunoassay. Clin. Vaccine Immunol. 14, 1587–1591.

Couppie, P., Sobesky, M., Aznar, C., Bichat, S., Clyti, E., Bissuel, F., El Guedj, M., Alvarez, F., Demar, M., Louvel, D., Pradinaud, R., Carme, B., 2004. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin. Infect. Dis. 38, 134–138.

Dalle, F., Charles, P.E., Blanc, K., Caillot, D., Chavanet, P., Dromer, F., Bonnin, A., 2005. *Cryptococcus neoformans* galactoxylomannan contains an epitope(s) that is cross-reactive with Aspergillus galactomannan. J. Clin. Microbiol. 43, 2929–2931.

De Pauw, B., Walsh, T.J., Donnelly, J.P., Stevens, D.A., Edwards, J.E., Calandra, T., Pappas, P.G., Maertens, J., Lortholary, O., Kauffman, C.A., Denning, D.W., Patterson, T.F., Maschmeyer, G., Bille, J., Dismukes, W.E., Herbrecht, R., Hope, W.W., Kibbler, C.C., Kullberg, B.J., Marr, K.A., Munoz, P., Odds, F.C., Perfect, J.R., Restrepo, A., Ruhnke, M., Segal, B.H., Sobel, J.D., Sorrell, T.C., Viscoli, C., Wingard, J.R., Zaoutis, T., Bennett, J.E., 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. 46, 1813–1821.

Debourgogne, A., Iriart, X., Blanchet, D., Veron, V., Boukhari, R., Nacher, M., Carme, B., Aznar, C., 2011. Characteristics and specificities of Cryptococcus infections in French Guiana, 1998–2008. Med. Mycol. 49, 864–871.

Desoubeaux, G., Bailly, E., Chandenier, J., 2014. Diagnosis of invasive pulmonary aspergillosis: updates and recommendations. Med. Mal. Infect. 44, 89–101.

Giacchino, M., Chiapello, N., Bezzio, S., Fagioli, F., Saracco, P., Alfarano, A., Martini, V., Cimino, G., Martino, P., Girmenia, C., 2006. Aspergillus galactomannan enzyme-linked immunosorbent assay cross-reactivity caused by invasive *Geotrichum capitatum*. J. Clin. Microbiol. 44, 3432–3434.

Hage, C.A., Ribes, J.A., Wengenack, N.L., Baddour, L.M., Assi, M., McKinsey, D.S., Hammoud, K., Alapat, D., Babady, N.E., Parker, M., Fuller, D., Noor, A., Davis, T.E., Rodgers, M., Connolly, P.A., El Haddad, B., Wheat, L.J., 2011. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin. Infect. Dis. 53, 448–454.

Huang, Y.T., Hung, C.C., Hsueh, P.R., 2007. Aspergillus galactomannan antigenemia in penicilliosis marneffei. AIDS 21, 1990–1991.

Huber, F., Nacher, M., Aznar, C., Pierre-Demar, M., El Guedj, M., Vaz, T., Vantilcke, V., Mahamat, A., Magnien, C., Chauvet, E., Carme, B., Couppie, P., 2008. AIDS-related *Histoplasma capsulatum* var. *capsulatum* infection: 25 years experience of French Guiana. AIDS 22, 1047–1053.

Jones, O., Cleveland, K.O., Gelfand, M.S., 2009. A case of disseminated histoplasmosis following autologous stem cell transplantation for Hodgkin's lymphoma: an initial misdiagnosis with a false-positive serum galactomannan assay. Transpl. Infect. Dis. 11, 281–283.

Kauffman, C.A., 2007. Histoplasmosis: a clinical and laboratory update. Clin. Microbiol. Rev. 20, 115–132.

Kauffman, C.A., 2008. Diagnosis of histoplasmosis in immunosuppressed patients. Curr. Opin. Infect. Dis. 21, 421–425.

Lewden, C., Sobesky, M., Cabie, A., Couppie, P., Boulard, F., Bissuel, F., May, T., Morlat, P., Chene, G., Lamaury, I., Salmon, D., 2004. [Causes of death among HIV infected adults in French Guyana and the French West Indies in the era of highly active antiretroviral therapy (HAART)]. Med. Mal. Infect. 34, 286–292.

Moir, S., Fauci, A.S., 2009. B cells in HIV infection and disease. Nat. Rev. Immunol. 9, 235–245.

Narreddy, S., Chandrasekar, P.H., 2008. False-positive Aspergillus galactomannan (GM) assay in histoplasmosis. J. Infect. 56, 80–81.

Pineau, S., Talarmin, J.P., Morio, F., Grossi, O., Boutoille, D., Leaute, F., Le Pape, P., Gay-Andrieu, F., Miegeville, M., Raffi, F., 2010. [Contribution of molecular biology and Aspergillus galactomannan antigen assay for the diagnosis of histoplasmosis]. Med. Mal. Infect. 40, 541–543.

Ranque, S., Pelletier, R., Michel-Nguyen, A., Dromer, F., 2007. Platelia Aspergillus assay for diagnosis of disseminated histoplasmosis. Eur. J. Clin. Microbiol. Infect. Dis. 26, 941–943.

Riviere, S., Denis, B., Bougnoux, M.E., Lanternier, F., Lecuit, M., Lortholary, O., 2012. Serum Aspergillus galactomannan for the management of disseminated histoplasmosis in AIDS. Am. J. Trop. Med. Hyg. 87, 303–305.

Sathapatayavongs, B., Batteiger, B.E., Wheat, J., Slama, T.G., Wass, J.L., 1983. Clinical and laboratory features of disseminated histoplasmosis during two large urban outbreaks. Medicine (Baltimore) 62, 263–270.

Simon, S., Veron, V., Boukhari, R., Blanchet, D., Aznar, C., 2010. Detection of *Histoplasma capsulatum* DNA in human samples by real-time polymerase chain reaction. Diagn. Microbiol. Infect. Dis. 66, 268–273.

Therby, A., Polotzanu, O., Khau, D., Monnier, S., Greder Belan, A., Eloy, O., 2014. [Aspergillus galactomannan assay for the management of histoplasmosis due to *Histoplasma capsulatum* var. *duboisii* in HIV-infected patients: education from a clinical case]. J. Mycol. Med. 24, 166–170.

Tobon, A.M., Agudelo, C.A., Rosero, D.S., Ochoa, J.E., De Bedout, C., Zuluaga, A., Arango, M., Cano, L.E., Sampedro, J., Restrepo, A., 2005. Disseminated histoplasmosis: a comparative study between patients with acquired immunodeficiency syndrome and non-human immunodeficiency virus-infected individuals. Am. J. Trop. Med. Hyg. 73, 576–582.

Tong, K.B., Lau, C.J., Murtagh, K., Layton, A.J., Seifeldin, R., 2009. The economic impact of aspergillosis: analysis of hospital expenditures across patient subgroups. Int. J. Infect. Dis. 13, 24–36.

Van Der Veer, J., Lewis, R.J., Emtiazjoo, A.M., Allen, S.D., Wheat, L.J., Hage, C.A., 2012. Cross-reactivity in the Platelia™ Aspergillus enzyme immunoassay caused by blastomycosis. Med. Mycol. 50, 396–398.

Vergidis, P., Walker, R.C., Kaul, D.R., Kauffman, C.A., Freifeld, A.G., Slagle, D.C., Kressel, A.K., Wheat, L.J., 2012. False-positive Aspergillus galactomannan assay in solid organ transplant recipients with histoplasmosis. Transpl. Infect. Dis. 14, 213–217.

Wheat, L.J., 2006. Antigen detection, serology, and molecular diagnosis of invasive mycoses in the immunocompromised host. Transpl. Infect. Dis. 8, 128–139.

Wheat, L.J., Hackett, E., Durkin, M., Connolly, P., Petraitiene, R., Walsh, T.J., Knox, K., Hage, C., 2007. Histoplasmosis-associated cross-reactivity in the BioRad Platelia Aspergillus enzyme immunoassay. Clin. Vaccine Immunol. 14, 638–640.

Xavier, M.O., Pasqualotto, A.C., Cardoso, I.C., Severo, L.C., 2009. Cross-reactivity of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Cryptococcus* species in the commercial Platelia Aspergillus enzyme immunoassay. Clin. Vaccine Immunol. 16, 132–133.

6. Aspects thérapeutiques

Deux principales options thérapeutiques sont recommandées pour la prise en charge des cas d'histoplasmose chez les patients infectés par le VIH : soit un traitement oral, soit un traitement intraveineux (ARTICLE 1).Le critère de jugement pour le choix entre ces deux options est représenté par la sévérité de la maladie observée par le clinicien. Si une liste de 15 critères de sévérité a été établie par les CDC, sur la base de deux études sur les facteurs pronostiques de sévérité, elle était peu utilisée et semblait peu pertinente en l'état. En effet, au lit du malade, le choix entre les deux options thérapeutiques reposait principalement sur le jugement et l'expérience des cliniciens.

Si dans certaines situations la sévérité était évidente, on pouvait régulièrement constater une dégradation inattendue et brutale du tableau clinique chez des patients jugés initialement comme non sévères. Cette dégradation était le plus souvent rapidement mortelle malgré l'utlisation des thérapeutiques antifongiques efficaces.

En dépit de quelques études publiées, la définition de la sévérité faisait toujours débat et les recommandations n'évoluaient pas. Les études étaient toujours basées sur un faible nombre d'individus avec une définition hétérogène et parfois discutable de la sévérité étudiée. Toutefois, quelle que soit l'étude, la mortalité associée aux formes sévères était toujours importante (jusqu'à 50% à 70% des cas), justifiant la pertinence de s'intéresser à nouveau à ce sujet.

Dans l'objectif d'étudier les facteurs associés à la sévérité de l'histoplasmose chez les patients infectés par le VIH de Guyane, nous avons complété le travail de 2004 de Couppié et al. sur la base de données Histoplasmose et VIH de Guyane (71).

In fine, selon les résultats observés, la perspective était de travailler au développement d'un score clinique, guide du clinicien dans le choix des antifongiques à prescrire dès l'admission d'un patient infecté par le VIH avec suspicion d'histoplasmose.

Par rapport à 2004, un plus grand nombre de patients a été étudié, avec une définition toujours très restrictive de la sévérité (survenue du décès dans les 30 jours après la mise en route du traitement antifongique), dans l'espoir que l'augmentation de puissance statistique et l'ajustement sur le taux de CD4 autoriseraient une description plus fine.

Les résultats ont fait l'objet d'une communication orale dont le résumé est présenté ci-dessous (COMMUNICATION ORALE 1) (Annexe 2). Le manuscrit était à finaliser au moment de rendre ce manuscrit avec l'espoir de le soumettre rapidement pour publication et de le présenter aux membres du jury le jour de la soutenance.

Pour mémoire, nous avons identifié des partenaires qui souhaiteraient participer à la validation externe d'un score clinique de sévérité, une fois que nous l'aurons validé sur les données de Guyane.

COMMUNICATION ORALE 1 : American histoplasmosis in HIV-infected patients: a study of prognostic factors associated with early death.

Author: Adenis, A., Nacher, M., Hanf, M., Aznar, C.., Lortholary O., Carme, B. and Couppié, P.

Year: 2013

Conference name: 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene

Conference location: Washington DC, USA

Abstract: American histoplasmosis is a endemic fungal infection in French Guiana. In persons with AIDS, it is the most frequent opportunistic infection and the leading cause of death. In order to reduce deaths, it is important to identify prognostic factors associated with early mortality so that appropriate therapy can be given. We looked at a one of the largest series of patients available to determine risk factors for early mortality. A retrospective study was conducted to identify persons with HIV/AIDS infected with Histoplasma capsulatum var. capsulatum and admitted to one of the three main hospitals of French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using multivariable logistic regression models. A total of 274 patients with HIV/AIDS were identified with histoplasmosis during 1992-2011. Forty six patients met the criteria for early death. The final multivariate model found several factors associated with an increased risk of early death: dyspnea OR=11.36 [4.28-30.17], acute renal failure OR=7.23 [1.47-35.71], WHO performance status score > 2 OR=4.05 [1.86-8.82] and platelet count ≤ 100 000/mm3 OR=3.51 [1.34-9.16]. Cases found during 2005-2011, OR=0.02 [0.01-0.12], and those from Cayenne General Hospital, OR=0.13 [0.04-0.47], were associated with a reduced risk of early death. This is the largest case series looking at factors associated with early death for histoplasmosis. For the first time adjusted on CD4 count, these results are consistent with other reports from the Americas. The factors identified can provide clinicians arguments about early and aggressive intervention with antifungal therapy in order to prevent early death due to histoplasmosis.

En zone d'endémie pour *H. capsulatum*, dans l'attente d'un diagnostic de certitude chez des patients suspects d'histoplasmose ou étiquettés fièvre d'origine inconnue, la mise en route d'un traitement antifongique empirique visant *H. capsulatum* chez les patients infectés par le VIH était objet de discussions cliniques récurrentes.

Les difficultés du diagnostic différentiel entre tuberculose et histoplasmose, pathologies le plus souvent disséminées et mortelles au stade avancé de l'infection par le VIH, exposaient les patients à des pertes de chance en cas de retard à la mise en route d'un traitement adapté. Sans compter les cas de coinfection histoplamose et tuberculose pour lesquels des problèmes d'interactions médicamenteuses compliquaient la prise en charge, exposant à nouveau les patients à des pertes de chance. De plus, les traitements antifongiques utilisés n'étaient pas exempts d'effets secondaires ou d'intéractions médicamenteuses.

Peu de données étaient disponbiles dans la littérature pour justifier cette stratégie empirique. En 2008, Huber et al rapportaient les données issues de l'expérience guyanaise. Un traitement antifongique avait été mis en route empiriquement chez 14,3% des patients, en moyenne 29,6 jours avant un diagnostic de certitude. Avec 36% de décès (5 cas sur 14), la mortalité observée chez ces patients était importante. Données à interpréter avec prudence car l'information sur la survie après traitement n'était pas disponible pour l'ensemble des patients pris en charge empiriquement (seuls 14 cas sur 105 cas) (69).

Par la suite, les arguments développés à propos de la fréquence de l'histoplasmose en Guyane (ARTICLE 4 et 5), de la saisonalité de l'incidence (ARTICLE 6 et 7) et les indices pour le diagnsotic différentiel entre l'histoplasmose et la tuberculose (ARTICLE 9) venaient nourrir les discussions cliniques autour de la pertinence probable de mettre en place une stratégie empirique antifongique en routine clinique. Toutefois, face au constat d'une stratégie empirique antituberculose moins discutée et plus établie, localement et chez les cliniciens de la région, il semblait nécessaire de comparer l'incidence et la mortalité de la tuberculose et de l'histoplasmose à l'aide des données de la cohorte de patients infectés par le VIH de Guyane. Une base de comparison objective pourrait-elle donner des arguments plus convaincants pour les cliniciens assurant la prise en charge de l'infection par le VIH dans des zones d'endémie forte de tuberculose et d'histoplamose ? (ARTICLE 11).

ARTICLE 11 : Histoplasmosis or Tuberculosis in HIV-Infected Patients in the Amazon: What Should Be Treated First?

Author: Nacher, Mathieu, **Adenis, Antoine**, Sambourg, Emilie, Huber, Florence, Abboud, Philippe, Epelboin, Loïc, Mosnier, Emilie, Vantilcke, Vincent, Dufour, Julie, Djossou, Félix, Demar, Magalie and Couppié, Pierre

Year: 2014 **Journal:** PLOS Neglected Tropical Diseases

ISSN: NA **DOI:** 10.1371/journal.pntd.0003290

Abstract: NA

URL: https://doi.org/10.1371/journal.pntd.0003290

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Viewpoints

Histoplasmosis or Tuberculosis in HIV-Infected Patients in the Amazon: What Should Be Treated First?

Mathieu Nacher[1,2]*, Antoine Adenis[1,2], Emilie Sambourg[2,3], Florence Huber[4], Philippe Abboud[2,5], Loïc Epelboin[2,5], Emilie Mosnier[5,6], Vincent Vantilcke[7], Julie Dufour[2,3], Félix Djossou[2,5], Magalie Demar[2,8], Pierre Couppié[2,3]

1 Centre d'Investigation Clinique Antilles Guyane, Inserm CIC1424, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **2** Equipe EA3593, Ecosystèmes amazoniens et Pathologie Tropicale, Université de la Guyane, Cayenne, French Guiana, France, **3** Service de Dermatologie Vénérologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **4** Hôpital de Jour Adultes, Consultations Spécialisées, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **5** Unité des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **6** Département des centres délocalisés de prévention et de soins, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **7** Service de Médecine Interne, Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, French Guiana, France, **8** Laboratoire Hospitalo-Universitaire de Parasitologie-Mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, France

Histoplasmosis and tuberculosis are probably among the most frequent AIDS-defining illnesses in the Amazon region and beyond [1]. Whereas tuberculosis is a well-known disease present in clinical algorithms and in specific public health programs, disseminated histoplasmosis is relatively neglected in South and Central America [2,3]. Histoplasmosis and tuberculosis are often presented as clinically and paraclinically similar [4]. Recently, we showed that disseminated histoplasmosis, while having some similarities with tuberculosis, had some marked differences with more pulmonary signs and inflammation in tuberculosis whereas histoplasmosis was more likely to be associated with cytopenia, liver enzyme abnormalities, or symptoms from the abdominal sphere [5].

Histoplasmosis and tuberculosis in HIV patients often are disseminated infections with a fatal evolution in the absence of treatment. For both infections, diagnosis is often slow with cultures that may take weeks to isolate the pathogen [6]. Patients with severe disseminated histoplasmosis are at risk of early death within days of their admission, notably if there is treatment delay. For tuberculosis, early mortality in severely immunocompromised patients is also a problem and has led to promote early rather than late initiation of antiretroviral therapy [7].

In practice, once other common opportunistic infections have been excluded, clinicians facing a severely immunocompromised HIV patient will need to conduct investigations and start a presumptive treatment, which often includes antituberculosis drugs but not antifungal drugs. This heuristic of HIV care does not rely on precise epidemiologic data and should be adapted to the local epidemiology.

In French Guiana, HIV is a major public health problem [8]. Histoplasmosisand tuberculosis incidences in HIV-infected patients are high [1,9,10]. Therefore, clinicians facing a severely immunocompromised patient often need to consider both alternatives and make a decision.

Since what treatment to start and when to start it may lead to different survival chances in this very common differential diagnosis situation, we aimed to gather additional evidence to guide clinicians.

Longitudinal data from the French Hospital Database on HIV infection (FHDH) in French Guiana between 1996–2008, described in [11], allowed us to collect incidence and mortality rates. The diagnosis of histoplasmosis was performed according to the European Organisation for Research and Treatment of Cancer (EORTC) criteria [12]. The diagnosis of tuberculosis relied on confirmed tuberculosis (culture and identification of *Mycobacterium tuberculosis*). All HIV patients in French Guiana can receive free antiretroviral treatments (including the most recent drugs) regardless of their origin or socioeconomic level.

A total of 2,323 patients were included. This amounted to 40,443 records and 9,608 years at risk. There were 141 first episodes of disseminated histoplasmosis observed and 119 cases of confirmed tuberculosis. Figure 1 shows the incidence rates of first episodes of disseminated histoplasmosis and of tuberculosis for different CD4 strata, and the gradual increase of the incidence rate ratio of histoplasmosis/tuberculosis as immunosuppression increases.

Figure 2 shows the respective Kaplan Meier curves for survival for histoplasmosis and tuberculosis in patients with CD4 counts below 200 cells per mm^3 within the first 12 months after the opportunistic infection. Histoplasmosis seemed to lead to more deaths than tuberculosis; however, this difference was not statistically significant. For the 141 patients with a first episode of histoplasmosis, there were 13.5% of deaths at one month, 17.5% at three months, and 22.5% at six months after the date of diagnosis of histoplasmosis. Among 119 first episodes of confirmed tuberculosis, 68 were in patients with CD4 counts less than 200 cells per mm^3. For patients with CD4 counts below 200 cells per mm^3, there was 10% mortality at one

Citation: Nacher M, Adenis A, Sambourg E, Huber F, Abboud P, et al. (2014) Histoplasmosis or Tuberculosis in HIV-Infected Patients in the Amazon: What Should Be Treated First? PLoS Negl Trop Dis 8(12): e3290. doi:10.1371/journal.pntd.0003290

Editor: Gary L. Simon, George Washington University, United States of America

Published December 4, 2014

Data Availability: The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. The data concerns computerized patient records of HIV patients. All elements allowing patient recognition cannot be shared. Our ethical point of contact is Commission Nationale de l'Informatique et des Libertés at the phone number 0153732222.

Funding: This work has benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: mathieu.nacher@ch-cayenne.fr

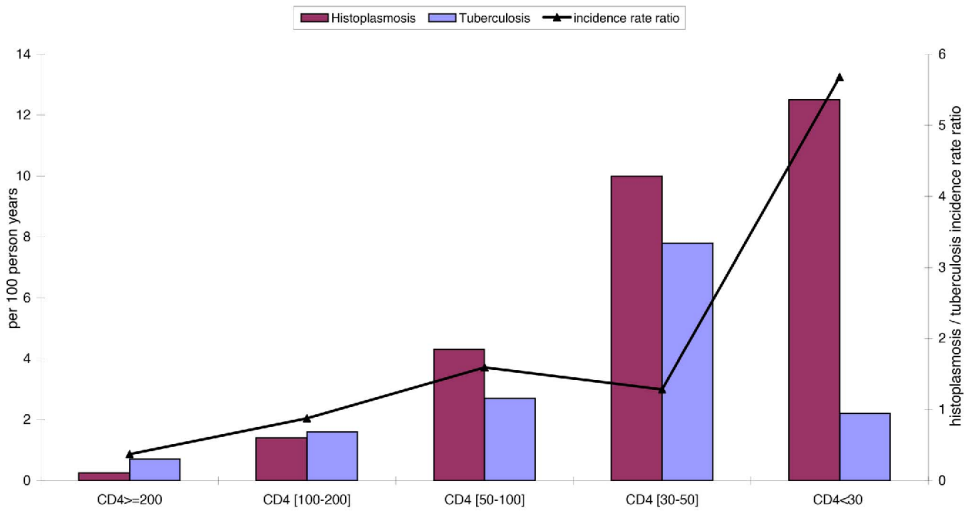


Figure 1. Shows the incidence rate for tuberculosis and histoplasmosis for different CD4 strata.
doi:10.1371/journal.pntd.0003290.g001

month, 19% at three months, and 31% at six months.

For clinicians, the situation where a severely immunocompromized HIV-infected patient is admitted for a "tuberculosis-like" illness is common and requires prompt identification and treatment of both the opportunistic agent and the underlying immunosuppression. Ideally, treatments should be administered once the opportunistic agent has been identified. However, in the Amazon region, invasive diagnostic procedures are often not performed or available, and laboratory facilities are lacking. Thus, empirical treatment remains an important strategy. Despite the potential adverse events or drug interactions, it is common to simultaneously treat different confirmed or suspected opportunistic infections. However, when possible, it is preferable to

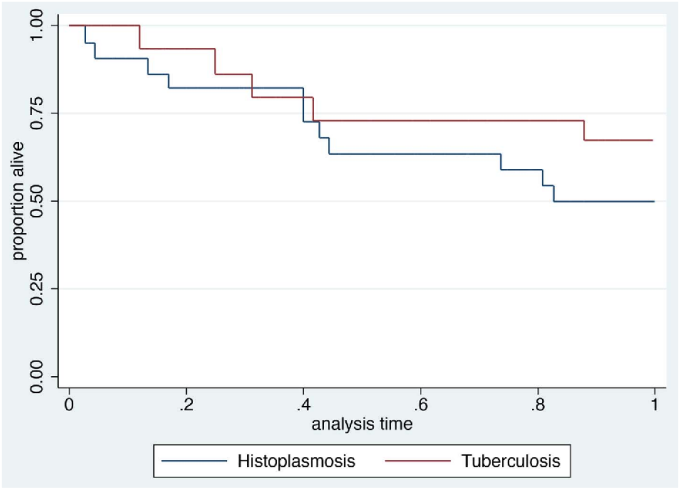


Figure 2. Shows the incidence of death during the first year afer histoplasmosis or tuberculosis among patients with CD4 counts less than 200.
doi:10.1371/journal.pntd.0003290.g002

target the most likely agent than to give numerous drugs, which makes it difficult to know what leads to improvement or what drug leads to adverse events [13].

In an Amazonian context, among immunosuppressed patients, the incidence of histoplasmosis was higher than that of tuberculosis. Despite comparable overall mortality in terms of proportion of patients with histoplasmosis and tuberculosis dying, the number of histoplasmosis-related deaths was higher. Thus, for HIV patients with CD4 counts below 200 with a tuberculosis-like syndrome (histoplasmosis-like may be a more appropriate heuristic in our epidemiological context), clinicians with poor diagnostic facilities may be better inspired, given the differences in incidence rates, to start with amphotericin B (ideally in its liposomal formulation) than antituberculosis drugs and reevaluate the situation 3–7 days later in view of the treatment response [6,14]. As shown elsewhere [15], the data also suggests antiretrovirals should be started without delay in order to minimize the duration of the severe immunosuppression that puts the patient at great risk of dying from other opportunistic agents.

References

1. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, et al. (2011) What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. Am J Trop Med Hyg 84: 239–240.
2. Nacher M, Adenis A, Aznar C, Blanchet D, Vantilcke V, et al. (2014) How Many Have Died from Undiagnosed Human Immunodeficiency Virus-Associated Histoplasmosis, A Treatable Disease? Time to Act. Am J Trop Med Hyg.
3. Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, et al. (2013) Disseminated histoplasmosis in HIV-infected patients in South America: a neglected killer continues on its rampage. PLoS Negl Trop Dis 7: e2319.
4. Krug ES, Glenn HR (1948) Comparative studies in tuberculosis and histoplasmosis among 2000 students entering Pennsylvania State College, September, 1947. J Lancet 68: 206–208.
5. Adenis A, Nacher M, Hanf M, Basurko C, Dufour J, et al. (2014) Tuberculosis and histoplasmosis among human immunodeficiency virus-infected patients: a comparative study. Am J Trop Med Hyg 90: 216–223.
6. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, et al. (2008) AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. AIDS 22: 1047–1053.
7. Blanc FX, Sok T, Laureillard D, Borand L, Rekacewicz C, et al. (2011) Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med 365: 1471–1481.
8. Nacher M, Vantilcke V, Parriault MC, Van Melle A, Hanf M, et al. (2010) What is driving the HIV epidemic in French Guiana? Int J STD AIDS 21: 359–361.
9. Nacher M, Aznar C, Blanchet D, Demar M, El Guedj M, et al. (2006) AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? AIDS 20: 951–952.
10. Guernier V, Guegan JF, Deparis X (2006) An evaluation of the actual incidence of tuberculosis in French Guiana using a capture-recapture model. Microbes Infect 8: 721–727.
11. Nacher M, Adenis A, Blanchet D, Vantilcke V, Demar M, et al. (2014) Risk factors for disseminated histoplasmosis in a cohort of HIV-infected patients in French Guiana. PLoS Negl Trop Dis 8: e2638.
12. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, et al. (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 46: 1813–1821.
13. Couppie P, Sobesky M, Aznar C, Bichat S, Clyti E, et al. (2004) Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin Infect Dis 38: 134–138.
14. Couppie P, Aznar C, Carme B, Nacher M (2006) American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis 19: 443–449.
15. Lawn SD, Little F, Bekker LG, Kaplan R, Campbel E, et al. (2009) Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa. AIDS 23: 335–342.

DEUXIEME PARTIE : COOPERATION REGIONALE ET PROJETS MIS EN ŒUVRE

Le contexte, les connaissances et les perspectives de développement au moment d'initier ce travail de thèse doivent faire l'objet d'un rappel synthétique afin de pouvoir juger de la pertinence des actions décrites dans cette deuxième partie.

En Guyane française, l'infection par le VIH est un problème majeur de santé publique avec des particularités par rapport à la France hexagonale. L'incidence du SIDA reste un problème important, avec l'histoplasmose parmi les principales infections opportunistes pourvoyeuses de cas de SIDA. Cas de SIDA dont la mortalité reste importante alors qu'elle diminue en France hexagonale. Malgré un nombre global de dépistages important, le dépistage reste tardif dans les communautés les plus vulnérables. Les patients dépistés sont souvent perdus de vue, notamment dans les mois qui suivent le diagnostic.

Les infections fongiques invasives sont à l'origine d'une mortalité importante au cours de l'infection par le VIH. Leur symptomatologie est jugée non spécifique, diagnostic différentiel de nombreuses pathologies mieux connues et plus fréquentes. S'ajoutant aux difficultés rencontrées dans l'établissement d'un diagnostic de certitude, essentiellement basé sur une culture fongique lente à obtenir, on constate que les résultats définitifs nécessitent le plus souvent plusieurs semaines lorsque l'examen direct fongique initial est négatif. Selon le contexte épidémiologique, comme décrit précédemment, la suspicion systématique de la possibilité d'une infection fongique invasive et l'initiation précoce, voire empirique en première instance, d'un traitement antifongique pourrait avoir un impact significatif autorisant la guérison d'infections rapidement mortelles.
En Guyane française, l'histoplasmose chez les patients infectés par le VIH n'échappe pas à ces généralités. Première infection opportuniste au stade SIDA, son principal diagnostic différentiel est la tuberculose. Elle est également une des premières causes de décès des patients au stade SIDA. Des méthodes invasives sont nécessaires au diagnostic (myélogramme, ponction-biopsie hépatique, biopsie ganglionnaire, etc.). Seuls les cliniciens sensibilisés évoquent le diagnostic et cherchent à le confirmer. L'identification de l'agent pathogène par la culture, méthode « gold standard » pour le diagnostic de certitude, s'avère longue (médiane d'obtention d'une culture fongique à partir du sang autour de 15 jours) et coûteuse (manipulations dans un laboratoire de niveau de sécurité biologique 3, NSB3), parfois même difficile en cas d'immunodépression profonde. Malgré les développements de méthodes diagnostiques de biologie moléculaire à la recherche d'*H. capsulatum,* localement

disponibles (86, 87), l'accès à ces méthodes rapides et performantes est limité du fait de contingences logistiques et humaines. Et, si les méthodes non invasives de détection antigénique rapides et sensibles d'*H. capsulatum* dans les urines seraient utiles chez les patients infectés par le VIH, elles sont uniquement disponibles aux USA.

Dans ce contexte, il semblait que la mise en place d'une technique diagnostique de l'histoplasmose non invasive, sensible, spécifique, rapide, techniquement simple, abordable sur le plan financier et diffusable facilement serait un apport considérable. La détermination d'un seuil de concentration antigénique associé à la sévérité de l'épisode infectieux pourrait être un outil pronostique précieux pour les cliniciens. Ils pourraient initier sans délais un traitement antifongique efficace et adapté au pronostic, permettant ainsi de réduire la mortalité imputable.

Une publication récente rapportait la mise au point par une équipe de la Mycotic Diseases Branch des Centers for Diseases Control and prevention (CDC-MDB) d'un test de détection d'antigènes urinaires et sériques d'*H. capsulatum* spécifiquement pour cela (88). Lors d'une étude prospective de phase 3, chez des patients infectés par le VIH du Guatemala, la méthode ELISA développée par les CDC a été comparée avec le test de référence représenté par la culture fongique. Les performances du test retrouvaient une sensibilité de 81% et une spécificité de 95% dans les urines et, des résultats encore plus intéressants sur le sérum pour un sous-groupe de patients (88). Les performances intéressantes de ce test simple et rapide, en comparaison à la méthode de référence invasive et requérant un laboratoire NSB3, avaient motivé une équipe colombienne de Medellin à l'utiliser en routine avec une prévalence d'environ 10% de test positifs chez les patients infectés par le VIH (communication personnelle du Dr Chiller, CDC). Cette technologie simple pouvait être pratiquée quel que soit le niveau de ressources du laboratoire. Elle facilitait le diagnostic rapide de l'histoplasmose et la mise en route plus rapide d'un traitement antifongique efficace. Ceci pouvait participer de la réduction de la mortalité imputable à cette maladie dans les régions où, comme en Guyane française, cette mortalité restait problématique, voire totalement méconnue.

Après avoir obtenu un accord de principe de l'équipe des CDC pour la mise en œuvre d'une collaboration scientifique visant à l'utilisation de leur méthode diagnostique dans le contexte du plateau des Guyanes, nous avons mis en œuvre un projet de recherche en France et au Suriname que nous décrirons par la suite.

Il y a deux raisons à la participation du Suriname. Premièrement, il semblait que l'histoplasmose n'était pas ou peu identifiée chez les patients infectés par le VIH, alors que sur la base de l'hypothèse écologique *H. capsulatum* devait être présent dans l'environnement et responsable d'infections

humaines. Ce qui, considérant une problématique similaire de l'infection par le VIH par rapport à la Guyane française, et une forte endémie de la tuberculose au Suriname, laissait supposer qu'un nombre important de patients infectés par le VIH surinamais décédaient sous antituberculeux, les médecins évoquant une tuberculose « résistante » au traitement, alors même qu'ils présentaient une histoplasmose. D'un commun accord avec nos partenaires surinamais, il était nécessaire et pertinent de tester l'hypothèse de la présence d'*H. capsulatum* chez les patients infectés par le VIH au Suriname.

Deuxièmement, considérant la faisabilité scientifique et technique du programme de part et d'autre de la frontière, et la probabilité d'observer un nombre important de patients coinfectés par l'histoplamose et le VIH au Suriname, l'ajout du Suriname augmentait la puissance statistique nécessaire à l'étude des différents objectifs listés avec nos partenaires dans le cadre de ce projet de recherche.

C'est dans ce contexte de développement d'un partenariat de recherche avec les équipes du Suriname et de la CDC-MDB que nous avons confirmé les besoins importants du Suriname en matière de prise en charge des infections fongiques invasives, plus particulièrement chez les patients infectés par le VIH. La coopération régionale transfrontalière en santé était une option pertinente pour répondre à ces besoins, dont la thématique se voyait légitimée par les financements importants et les retombées attendues d'un programme de recherche biomédicale.

L'hypothèse selon laquelle l'appartenance des pays voisins au biome amazonien rendait probable la présence d'*H. capsulatum* dans l'environnement, présence attestée par des études historiques des sols et en population générale, a toujours guidé ces développements régionaux.

Associée au constat d'un grand nombre de cas de SIDA, d'une mortalité hospitalière importante des cas de SIDA, d'une très forte endémie de tuberculose, de l'absence de capacités diagnostiques des infections fongiques invasives, de la méconnaissance de celles-ci par la majorité des professionnels de santé et de l'absence ou de l'impossibilité financière d'accès aux principaux antifongiques efficaces dans le traitement de l'histoplasmose, il était nécessaire de lancer des programmes dont les bénéfices aux populations seront plus rapides que ceux espérés dans le cadre du projet de recherche initial.

Ainsi, un programme de coopération transfrontalière entre la Guyane française et le Suriname dans un premier temps, puis avec l'ajout du Guyana dans un second temps, a été mis en place concomitamment au projet de recherche ANRS 12260 EDIRAPHIS. Les objectifs et les résultats sont décrits au sein de cette deuxième partie.

1. Activité de recherche : le projet ANRS 12260 EDIRAPHIS :

Intitulé « L'histoplasmose à Histoplasma capsulatum sur le plateau des Guyanes et aux Antilles : Evaluation de la prévalence chez les patients infectés par le VIH à l'aide d'une méthode de diagnostic rapide ELISA de détection d'antigènes sériques et urinaires d'Histoplasma », ce projet a été cofinancé par l'Institut national de la santé et de la recherche médicale - Agence Nationale de Recherches sur le Sida et les hépatites virales (Inserm-ANRS) et le Programme Opérationnel du FEDER-FSE 2007-2013 de la Région Guyane (N°présage 31362). Le résumé détaillé de la recherche est disponible à l'annexe 3. Le projet a été mis en place en Guyane française et au Suriname. En l'absence de financements, les Antilles n'ont pas pu participer à cette étude.

L'objectif principal était de mesurer la proportion de patients infectés par le VIH hospitalisés, ou vus en consultation dans l'attente d'une hospitalisation, pour une suspicion de syndrome infectieux ayant un test positif pour la détection d'antigènes sériques et/ou urinaire d'*H. capsulatum*.

Les critères d'inclusion étaient :

- adulte (âge supérieur ou égal à 18 ans) ;
- patient vu en hospitalisation ou en consultation dans l'attente d'une hospitalisation ;
- infection par le VIH1 ou le VIH2 confirmée par les techniques validées en France et au Suriname, soit antérieure à l'épisode concerné, soit de découverte concomitante ;
- patient présentant au minimum un des trois éléments suivants : une altération de l'état général (cotation selon l'échelle OMS de Performance Status [PS OMS] ≥ 1) et/ou une fièvre et/ou des symptômes suspects de syndrome infectieux ;
- prescription médicale dans les 15 jours après l'admission en hospitalisation de prélèvements sanguins et urinaires à la recherche d'un agent infectieux ;
- recueil du consentement écrit d'accord de participation à l'étude.

Au cours d'une période de recrutement, étendue du 01/07/2013 au 01/07/2015, tout patient infecté par le VIH, connu ou non, nécessitant une hospitalisation dans un des 5 centres investigateurs pour une fièvre et/ou une altération de l'état général (PS OMS ≥ 1), associées ou non à des symptômes non spécifiques potentiellement liés à un syndrome infectieux, se voyait proposer de participer à l'étude. Le médecin investigateur de chaque site informait le patient et recueillait son consentement. Il prescrivait les explorations qu'ils jugeaient nécessaires au vu de l'état du patient ainsi que les traitements adaptés suivant les pratiques habituelles propres à chacun des centres d'inclusion. Les données personnelles associées étaient recueillies dans un cahier d'observation anonymisé. La seule différence pour le patient était qu'une fraction des prélèvements sanguins et

urinaires, réalisés habituellement dans un contexte de suspicion de pathologie infectieuse, était utilisée pour l'évaluation en double insu du test ELISA de détection polyclonale d'antigène d'*H. capsulatum* développé par le l'équipe des CDC-MDB (ni le médecin ni le patient n'avaient les résultats du test ELISA à l'étude). La durée de participation pour un patient était de 3 mois. Chaque participant a fait l'objet d'une visite d'inclusion à J0 et, d'un suivi à J 30 et J 90 après l'inclusion (synthèse du déroulement de l'étude et des données recueillies à la figure 14).

Actions		Admission du patient dans le service investigateur	Visite 1	Suivi 1	Suivi 2 Fin d'étude
			Inclusion dans l'étude J 0	Suivi 30 jours après l'inclusion J 30	Suivi 90 jours après l'inclusion J 90
1	**Eligibilité** (critères d'inclusion et de non-inclusion)		X		
2	**Information du patient** (ou du tiers représentant)		X		
3	**Signature du consentement par le patient** (ou le tiers représentant)		X		
4	**Appel du coordinateur du pays** (si patient éligible)		X		
5	**Interrogatoire du patient : ***				
	_ Anamnèse		X		
	_ Antécédents		X		
	_ Exposition environementale		X		
6	**Examen clinique ***		X		
7	**Biologie standard ***		X		
8	**CD4/CD8 et charge virale VIH ***		X		
9	**Imagerie médicale †**		X		
10	**Examens endoscopiques †**		X		
11	**Prélèvement sanguin de l'étude** (tube de 10 mL avec gel séparateur)		X		
12	**Prélèvement urinaire de l'étude** (Flacon vissé de 50 mL)		X		
13	**Examens microbiologiques : ‡**				
	_ Mycobactériologie		X	X	X
	_ Mycologie		X	X	X
	_ Anatomopathologie		X	X	X
14	**Thérapeutiques antifongiques ‡**		X	X	X
15	**Diagnostic principal et secondaire** (avec les critères de sévérité du CDC pour l'histoplasmose) **§**			X	X
16	**Statut Vital** (suivi par contact téléphonique)			X	X

***** Ces données concernent l'interrogatoire, l'examen clinique et le bilan biologique réalisés à l'admission du patient dans le service, de façon concomitante à l'inclusion dans l'étude.
† L'imagerie médicale et les examens endoscopiques concernent les premiers examens réalisés depuis l'admission du patient à l'hôpital.
‡ Ces données concernent les examens microbiologiques et les thérapeutiques antifgongiques prescrits entre l'admission à l'hôpital et 90 jours après l'inclusion, et feront l'objet d'un suivi par l'ARC / TEC.
§ Ces données seront recueillies dans le compte-rendu d'hospitalisation du patient. Si le patient est toujours hospitalisé à J 90, le médecin investigateur statuera sur les diagnostics principal et secondaire concernant l'hospitalisation en cours pour que l'l'ARC / TEC renseigne le cahier d'observation à J 90 au plus tard.

Figure 14 : Déroulement de l'étude et synthèse des données recueillies dans le cadre du projet ANRS 12260 EDIRAPHIS (Présage 31362)

Au moment de rendre ce manuscrit, seuls sont disponibles des résultats préliminaires concernant l'objectif principal. Si toutes les analyses de laboratoire ont été réalisées puis contrôlées deux fois par des partenaires externes au projet, l'ensemble des données du cahier d'observation ne sont pas encore disponibles et font l'objet de contrôles qualité en cours.

Sur une période de recrutement de 24 mois en Guyane et au Suriname, le diagramme des inclusions renseignait 515 patients inclus dont 490 éligibles pour la mesure de l'objectif prinicipal. Les quatre patients pour lesquels nous disposons uniquement d'un des deux prélèvements à récupérer selon le protocole d'étude (sérum et urines) n'ont pas été exclus de l'analyse (figure 15).

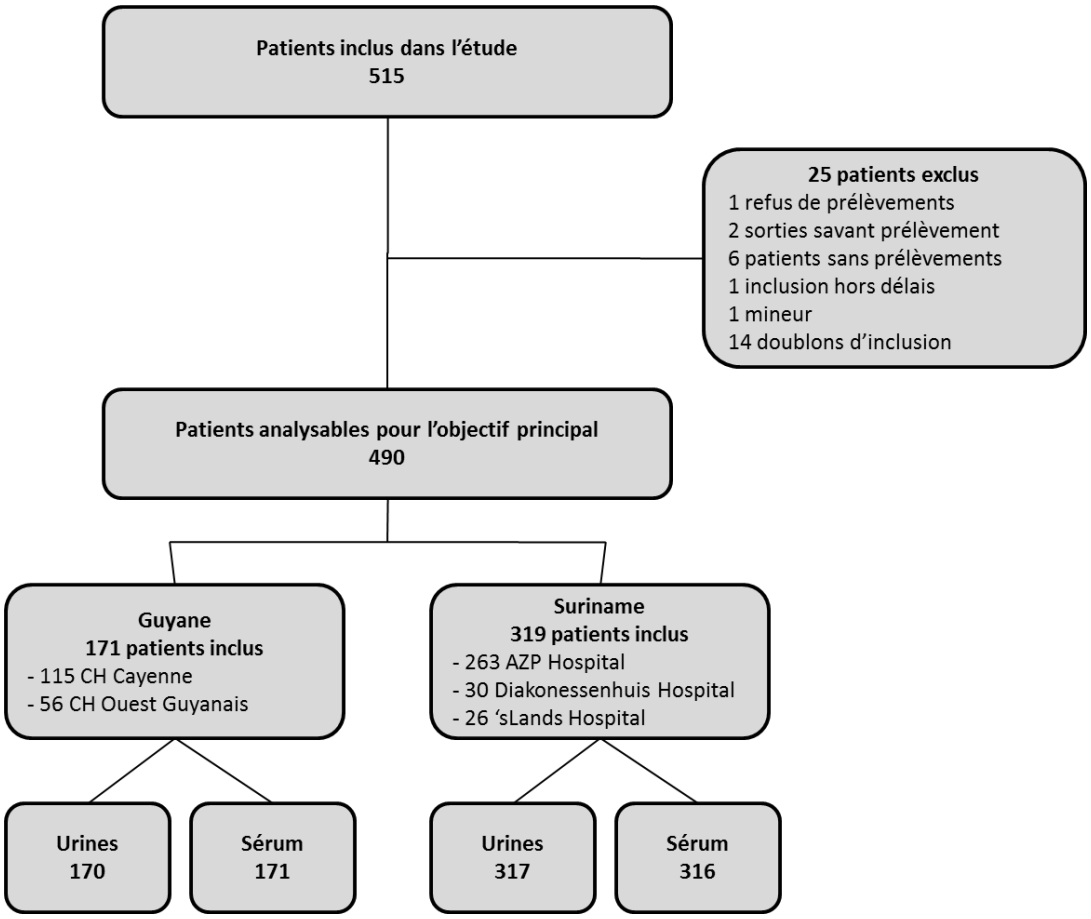


Figure 15 : Diagramme des inclusions du projet ANRS 12260 EDIRAPHIS, du 01/07/2013 au 01/07/2015

Au tableau 1, la prévalence de l'antigénémie et de l'antigénurie d'*H. capsulatum*, chez l'ensemble des patients infectés par le VIH participants à ce projet, étaient respectivement de 20,5% [IC95% : 17,0-24,4] et de 14,2% [11,2-17,6]. Si l'on considère ces résultats au sein de chaque pays participants, on retrouvait des chiffres plus importants au Suriname par rapport à la Guyane française, dans le sérum comme dans les urines des participants, avec respectivement 24,4% versus 13,4% dans le sérum et 17,3% versus 8,2% dans les urines. Les niveaux de prévalence retrouvés dans le sérum étaient

toujours supérieurs à ceux retrouvés dans les urines quel que soit le scénario (total ou par pays). Les résultats de l'ensemble des investigations diagnostiques recherchant des agents infectieux fongiques, dont la prescription n'était pas obligatoire dans le protocole d'étude mais respectait des bonnes pratiques standardisées entre les deux pays, retrouvaient un diagnostic de certitude de l'histoplasmose pour 17,9% des 168 participants ayant bénéficié de ces explorations (25% au Suriname versus 15% en Guyane française). Il était notable que 70% (120/171) des participants de Guyane française avait fait l'objet d'investigations à la recherche d'agents infectieux fongiques par rapport aux 15% (48/319) de participants du Suriname.

Tableau 1 : Synthèse des résultats préliminaires du projet ANRS 12260 EDIRAPHIS

Projet ANRS 12260 EDIRAPHIS	Total % (n/N) [IC95%]	Suriname % (n/N) [IC95%]	Guyane fr. % (n/N) [IC95%]
Test EIA CDC anti-*Histoplasma*			
Serum	20,5 (100/487) [17,0-24,4]	24,4 (77/316) [19,7-29,5]	13,4 (23/171) [8,7-19,5]
Urines	14,2 (69/487) [11,2-17,6]	17,3 (55/317) [13,3-22,0]	8,2 (14/170) [4,6-13,4]
Investigations fongiques *			
***H. capsulatum* positif (selon critères EORTC-MSG)**	17,9 (30/168) [12,4-24,5]	25,0 (12/48) [13,6-39,6]	15,0 (18/120) [9,1-22,7]

EIA : Enzyme Immuno Assay ; CDC : Centers for diseases control and prevention ; Ac : anticorps ; Guyane fr.: Guyane française
* Les résultats d'investigations fongiques positives pour *H. capsulatum* dans les conditions définies par la conférence de consensus sur les conditions du diagnostic de certitude des infections fongiques invasives de l'European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG)

En dehors du constat de la présence d'*H. capsulatum* dans le sérum et les urines de patients infectés par le VIH, dans des proportions plus importantes au Suriname par rapport à la Guyane, dans le sérum par rapport aux urines, nous ne discuterons pas ces résultats. Ces derniers doivent faire l'objet d'analyses plus fines à l'aide de l'ensemble des données recueillies dans les cahiers d'observations de l'étude. Ces analyses devraient débuter fin 2017.

Pour mémoire, un des objectifs du projet ANRS 12260 EDIRAPHIS était de constituer une collection d'échantillons biologiques de sérum et d'urines.

Dans l'attente des données des cahiers d'observations, nous avons développés, sous la supervision du conseil scientifique de l'étude, les projets ancillaires suivants :

- Prévalence d'*H. capsulatum* dans les urines des participants avec une nouvelle méthode ELISA, basée sur une détection monoclonale d'antigène d'*H. capsulatum*, et analyse des capacités diagnostiques (sensibilité, spécificité, valeurs prédictives positives et négatives) en phase clinique (équivalent phase 4) versus la méthode de référence. Ce test a été obtenu dans le cadre d'une collaboration scientifique portant sur une cession gratuite des tests sans autre contrepartie que l'accès aux résultats de laboratoire. La société partenaire était la société IMMY (Norman, Oklahoma, USA), impliquée dans le développement et la commercialisation de tests pour le diagnostic des infections fongiques depuis plus de 50 ans.
- Prévalence de l'histoplasmose dans le sérum des participants avec une méthode sérologique de type immunodiffusion. Cette méthode avait montré des résultats intéressants chez les patients infectés par le VIH en Colombie (89). Ce test a été obtenu dans le cadre d'une collaboration scientifique portant sur une cession gratuite des tests sans aucune contrepartie. Le partenaire était la Corporación para Investigaciones Biológicas (CIB), institut colombien basé à Medellin dont les missions sont la réalisation de diagnostics de routine pour tout type d'infections fongiques, le développement de recherches sur les nouvelles méthodes diagnostiques des infections fongiques et le développement de l'enseignement sur les infections fongiques.
- Prévalence de la positivité dans le sérum d'une sous-population de participants et études des capacités diagnostiques de deux méthodes diagnostiques d'intérêt versus la méthode de référence (critères EORTC/MSG pour le diagnostic d'*H. capsulatum*). Le test Platelia ™ *Aspergillus* EIA développé par la société BIO RAD qui recherche l'antigène galactomannane d'*Aspergillus* sp. et qui a fait l'objet de réactions croisées décrites avec *H. capsulatum* (90-93). Le test de détection d'antigène (1,3)-Beta-D-Glucan, composant important du mur fongique, qui pourrait être une option diagnostique de l'histoplasmose mais très peu investiguée (94). Le partenaire était le laboratoire de parasitologie et mycologie; service de microbiologie; CHU Paris - Hôpital Necker-Enfants Malades qui a réalisé l'ensemble des analyses sur fonds propres avec la participation d'une technicienne de laboratoire de Cayenne envoyée sur site.
- Prévalence de la cryptococcose dans le sérum des participants avec une nouvelle méthode LFA, basée sur une détection monoclonale d'antigène de *Cryptococcus* sp., récemment recommandée par l'OMS pour le diagnostic de la cryptococcose chez les patients infectés par le VIH (95). Ce test a été obtenu dans le cadre d'une collaboration scientifique portant sur une cession gratuite des tests sans aucune contrepartie. Le partenaire était la société IMMY (Norman, Oklahoma, USA) évoquée précédemment.

Si la totalité des analyses de laboratoire de ces projets ancillaires ont été réalisées, les résultats doivent faire l'objet d'analyses plus fines à l'aide de l'ensemble des données recueillies dans les cahiers d'observations de l'étude ANRS 12260 EDIRAPHIS. Nous ne présenterons pas les résultats dans ce manuscrit.

2. Activité de coopération : le projet OPS-OMS

Comme nous l'avons évoqué précédemment, les collaborations initiées et les financements de recherche obtenus ont été le moteur de la coopération régionale, légitimant de fait cette dernière. L'activité de recherche a tiré dans son sillage une coopération transfrontalière en santé à propos des infections fongiques autrement impossible à financer.

Dans un premier temps, un financement conjoint a été obtenu en 2012, auprès de l'Agence Régionale de Santé de Guyane (ARS Guyane) et de l'Agence Française de Développement (AFD) permettant d'initier un programme d'harmonisation des pratiques pour le diagnostic mycologique standard intitulé « *Histoplasma capsulatum* in the Guiana Shield: Harmonizing the diagnosis practices of deep fungal infections ».

Dans un deuxième temps, fin 2013, un financement a été obtenu auprès de l'Organisation Panaméricaine de la Santé - bureau régional de l'Organisation Mondiale de la Santé (OPS-OMS) pour la mise en œuvre d'une coopération régionale élargie dans le cadre des financements Technical Cooperation among Countries (TCC) et intitulée « Control of Histoplasmosis on HIV-infected patients in the Guiana Shield ».

La coopération transfrontalière se voyait étendue au territoire du Guyana où la situation de la coinfection VIH et histoplasmose était à priori encore plus problématique qu'au Suriname. Le pays était deux fois plus peuplé avec un produit intérieur brut encore inférieur, parmi les plus bas d'Amérique Latine. La prévalence du VIH était importante avec une très importante mortalité hospitalière des patients infectés par le VIH au stade SIDA. L'histoplasmose y était totalement inconnue et les antifongiques efficaces sur les infections fongiques invasives non disponibles à la pharmacie nationale.

Pour satisfaire aux objectifs des financements TCC de l'OPS-OMS, nous avons proposé un programme de coopération intégrant l'ensemble des actions initiées alors (recherche et coopération avec le Suriname en partenariat avec les CDC-MDB) et leurs financements. L'OPS-OMS a validé le cadre de travail présenté ci-dessous dont la finalité visait à la réduction de la mortalité liée au SIDA sur le plateau des Guyanes. A notre connaissance, pour la première fois, l'OPS-OMS finançait une action dont le thème central était la problématique de l'histoplasmose au cours de l'infection par le VIH.

GOAL

Burden of AIDS-related deaths reduced in the Guiana shield

PURPOSE

Mortality and morbidity due to American Histoplasmosis in the Guiana Shield reduced

EXPECTED RESULTS

1. Awareness of the problem of American Histoplasmosis in HIV-positive patients increased in Guiana Shield,
2. Diagnostic capacity building for American Histoplasmosis improved in the Guiana Shield,
3. Clinical practice in HIV patients in the Guiana Shield improved,
4. Strategy for Histoplasmosis control developed,
5. Operational network of trained health professionals reinforced in the Guiana Shield.

ACTIVITIES

1. Laboratory training
2. Clinical training
3. Situation analysis
4. Workshop in Paramaribo, Suriname
5. Organization of health professional network for Histoplasmosis control.
6. Evaluation and monitoring

Un rapport détaillé des réalisations dans le cadre des activités 1 à 6 de ce programme de TCC est présenté à l'Annexe 4. Un rappel des indicateurs évaluant ces activités est présenté au Tableau 2.

Tableau 2 : Indicateurs de réalisation des activités du programme de TCC « Control of Histoplasmosis on HIV infected patients in the Guiana Shield », 2013-2015.

		FRENCH GUIANA				SURINAME				GUYANA			
Activities	**Indicators**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
#1. Laboratory training	✓ Number of participants trained	2	0	2	0	10	7	3	0	2	0	2	0
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#2. Clinical training	✓ Number of participants trained (at least 10/country)	60	0	15 + 45	0	120	60	60	0	60	0	15	45
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#3. Situation analysis	✓ Document produced	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#4. Workshop in Paramaribo	✓ Workshop held					2	1	-	1				
	✓ Number of participants (30 expected)					130	30	-	100				
	✓ Number of countries attending (at least 10 countries)					21	7	-	14				
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#5. Network of Health professionals	✓ Network operational (at least 2 health professionals from Brazil, French Guiana, Guyana and Suriname)	YES	-	-	-	YES	-	-	-	YES	-	-	-
	✓ Evaluation of the TCC completed.	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			

Au plan des « pratiques de laboratoire » (Activité n°1), sur la base de missions exploratoires d'évaluation conjointe des besoins, nous avons mis en œuvre les actions suivantes : formations de personnels de laboratoire à la paillasse pour le diagnostic des infections fongiques profondes, développement et validation de protocoles de laboratoire bilingues pour l'examen direct et la culture fongique des échantillons de moelle osseuse, renseignement d'une base de données sous EPIDATA, achat de tout l'équipement requis, procédure de contrôle qualité des pratiques mises en œuvre avec la venue d'experts sur chaque site et, validation d'une procédure pérenne de demande d'expertise via un formulaire et iconographies envoyés par courriel au laboratoire hospitalo-universitaire de parasitologie-mycologie du centre hospitalier de Cayenne, centre de référence pour le diagnostic standard des infections fongiques dans nos différents projets. La mise en œuvre du test ELISA de détection antigénique des CDC-MDB faisait partie de l'activité 1. Ce dernier, mis en place en Guyane et au Suriname avec les résultats présentés précédemment, n'a pas été mis en place au Guyana par manque de financements.

Au plan des « pratiques médicales » (Activité n°2), sur la base de missions exploratoires d'évaluation conjointe des besoins, nous avons mis en œuvre les actions suivantes : formations des cliniciens et étudiants en médecine dans le cadre de petits séminaires d'échanges à propos des pratiques cliniques pour le diagnostic des infections fongiques invasives chez le patient infecté par le VIH, formation des médecins à la pratique du myélogramme au lit du malade avec réalisation de lames

d'examen direct au laboratoire, achat de tout l'équipement requis pour la pratique d'un myélogramme, développement sans validation de protocoles en anglais pour la prise en charge des infections fongiques invasives au cours de l'infection par le VIH et, partage d'expérience avec une communauté médicale plus élargie sur chaque site dans le cadre de sessions locales de formation médicale continue.

Les réalisations des activités 3 à 5 seront présentées dans la troisième partie de ce manuscrit. L'idée étant de respecter le cheminement chronologique qui a vu se développer une initiative (activités n°4 et n°5), dont l'argumentaire était basée sur une analyse progressive de la situation (activité n°3).

L'évaluation des objectifs et des retombées attendues, dont les indicateurs sont présentés respectivement au Tableau 3 et au Tableau 4, montre l'important travail de formation et de sensibilisation des professionnels de santé. Si leur impact était difficilement évaluable au plan de la mortalité imputable, on retrouvait tout de même des tendances intéressantes au Suriname. L'augmentation significative de la proportion de patients hospitalisés qui bénéficiaient d'une recherche d'infections fongiques invasives était accompagnée d'une augmentation significative du nombre de diagnostic et de traitement de l'histoplasmose chez les patients infectés par le VIH.

Tableau 3 : Indicateurs de réalisation des objectifs du programme de TCC « Control of Histoplasmosis on HIV infected patients in the Guiana Shield », 2013-2015.

		FRENCH GUIANA				SURINAME				GUYANA			
Goal	**Indicator**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
Burden of AIDS-related deaths reduced in the Guiana shield	Annual number of AIDS-related deaths by country	48	24	12	12	"Not yet available"	89	"Not yet available"	"Not yet available"				
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
Purpose	**Indicators**	**Total**	**2013**	**2014**	**2015**	**Total**	**2013**	**2014**	**2015**	**Total**	**2013**	**2014**	**2015**
Mortality and morbidity due to Histoplasmosis in the Guiana Shield reduced.	Annual number of HIV-infected patients deaths due to disseminated histoplasmosis	2 of 31	2 of 16	0 of 12	0 of 3 (incomplete)	"Not yet available"	"Not yet available"	"Not yet available"	"Not yet available"				
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
	Annual % of HIV patients diagnosed for histoplasmosis and dying at 1 month after initiation of antifungal therapy against histoplasmosis.	13%	0%	13%	NA	"Not yet available"	"Not yet available"	"Not yet available"	"Not yet available"	33%	-	-	33%
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
	✓ Annual % of HIV patients diagnosed with disseminated histoplasmosis	28 cases	13 cases	15 cases	NA	"Not yet available"	29%	31%	NA	3 cases	-	-	3 cases
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			

Tableau 4 : Indicateurs de réalisation des retombées attendues du programme de TCC « Control of Histoplasmosis on HIV infected patients in the Guiana Shield », 2013-2015.

		FRENCH GUIANA				SURINAME				GUYANA			
Expected results	**Indicators**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
#1. Awareness of the problem of American Histoplasmosis in HIV-positive patients increased in Guiana Shield,	✓ Number of trained professionals												
	- Laboratory	2		2		10	7	3		2		2	
	- Clinicians and others	60		15 + 45		120	60	60		60		15	45
	✓ Number of meetings	3	1	2	0	8	3	3	2	5	1	2	2
	✓ Number of focal points	4	4	4	4	4	4	4	4	3	3	3	3
	✓ Number of Information Education and Communication (IEC) materials developed and disseminated	3	3	3	3	3	3	3	3	2	2	2	2
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#2. Diagnostic capacity for American Histoplasmosis improved in the Guiana Shield,	✓ Number of mycologic examinations for histoplasmosis in HIV-infected patients hospitalized (direct examination, cultures, pathology)	121	Not yet available	Not yet available	Not yet available	352	51	301	Not yet available	12	0	0	12
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
	✓ Number of ELISA tests performed for histoplasmosis in HIV-infected patients hospitalized (urines + serum)	341	Not yet available	Not yet available	Not yet available	633	Not yet available	Not yet available	Not yet available				
	Data source:	Research project				Research project				Project Not Performed			
#3. Clinical practice in HIV patients in the Guiana Shield improved,	✓ Proportion of hospitalized HIV-infected patients screened for histoplasmosis (Fungal culture and ELISA test)	Not yet available	Not yet available	Not yet available	Not yet available	Not yet available	50-60%	>90%	Not yet available				
	✓ Number of HIV-infected patients hospitalized and treated for histoplasmosis	Not yet available	Not yet available	Not yet available	Not yet available	Not yet available	80 (AZP)	116 (AZP)	Not yet available				
	Data source:	Trans border cooperation & research project				Trans border cooperation & research project				TCC project			
#4. Strategy for Histoplasmosis control developed	✓ Document elaborated	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#5. Operational network of trained health professionals reinforced in the Guiana Shield.	✓ Network operational (at least 10 health professionals)	YES	1	1	1	YES	1	1	1	YES	1	1	1
	✓ Number of communications and publications on the topic.	9	3	4	2	5	3	1	1	3	2	0	1
	✓ Data Base information system specifically related to histoplasmosis and hiv wil be developed	YES	1	1	1	YES	1	1	1	YES	0	0	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			

Au moment de l'évaluation du programme, en comparaison avec les données du Suriname, il était plus difficile de rendre compte de l'impact des activités au Guyana. La mise en œuvre du projet a été beaucoup plus laborieuse, notamment du fait d'un turnover permanent des personnels, et de difficultés administratives importantes pour l'obtention des fonds OPS-OMS, l'achat et la livraison des équipements sur site (matériel de laboratoire et aiguilles de myélogramme).

Parmi les principales retombées de ce programme, l'étape majeure fut la démonstration de la présence de l'histoplasmose, avec les premières identifications d'*H. capsulatum* dans la moelle osseuse de patients infectés par le VIH au Suriname (Figure 16) et au Guyana (Figure 17). Plus la recherche de l'agent pathogène était systématisée, plus le nombre de diagnostic augmentait. L'hypothèse de départ, selon laquelle la coinfection histoplasmose et VIH sur le plateau des Guyanes serait d'une ampleur équivalente à celle observée en Guyane française, mais actuellement méconnue, semblait être confortée par les faits observés.

Figure 16 : Première identification d'*H. capsulatum* sur prélèvement de moelle osseuse chez un patient infecté par le VIH, 2013, Hôpital AZP, Paramaribo, Suriname.

Figure 17 : Première identification d'*H. capsulatum* sur prélèvement de moelle osseuse chez un patient infecté par le VIH, 2015, National Public Health Institute, Georgetown, Guyana.

Pour mémoire, depuis le début de l'année 2012, sous l'égide du groupe santé de la commission transfrontalière de coopération entre la Guyane française et le Brésil, animé par l'ARS Guyane, nous avons œuvré au développement de partenariats avec le Laboratoire Central de Santé Publique de l'état de l'Amapa à Macapa (LACEN AP) et l'Institut Evandro Chagas de l'état du Para à Belém. L'idée d'étendre la mise en place du projet de recherche ANRS 12260 et son transfert de technologie diagnostique de l'histoplasmose par les CDC-MDB fut approuvée par le Ministère de la Santé du Brésil fin 2013. Malgré de nombreux efforts pour son financement, seul un financement partiel sur la réserve parlementaire du Gouverneur de l'Etat de l'Amapa a été obtenu mi-2014. Depuis, malgré l'avis favorable du comité national d'éthique brésilien pour démarrer le recrutement de participants, l'obtention d'un financement complémentaire se fait toujours attendre. Dans l'intervalle, des formations au diagnostic mycologique standard des personnels de laboratoire de Macapa ont été réalisées par le laboratoire de mycologie de Belém. Au plan des pratiques médicales, les médecins de l'hôpital public de Macapa participent à l'initiative régionale sur la coinfection histoplasmose et VIH.

Après l'évaluation très favorable de notre programme de coopération par l'OPS-OMS et la confirmation de nos hypothèses de travail sur le poids important de la problématique de la coinfection histoplasmose et VIH dans la région, l'enjeu était de maintenir la dynamique visant à la réduction de la mortalité des patients au stade SIDA de l'infection par le VIH.

Ainsi, dans la troisième partie de ce manuscrit, nous exposerons les actions dont la finalité était d'intégrer l'objectif de la prise en charge de l'histoplasmose chez les patients infectés par le VIH dans les recommandations internationales et nationales. Ce qui permettrait l'obtention de financements pour lutter contre cette maladie curable, trop souvent mortelle dans la région. Également, cela permettrait de justifier l'intégration définitive de l'histoplasmose dans les algorithmes de prise en charge des patients infectés par le VIH en Amérique Latine.

TROISIEME PARTIE : DEVELOPPEMENT D'UNE INITIATIVE INTERNATIONALE

Dans le cadre de ce travail de thèse, nous avons été amené à réaliser de nombreux déplacements dans des centres de référence pour le diagnostic et la prise en charge de l'infection par le VIH ou des centres de références pour le diagnostic des infections fongiques. L'idée était de convaincre nos interlocuteurs de développer un réseau de collaborateurs autour de la problématique des infections fongiques invasives au cours de l'infection par le VIH. Infections qui, en l'absence de diagnostic, ou confondues avec la tuberculose dans le cas de l'histoplasmose, sont pourvoyeuses d'une mortalité importante mais évitable lorsque les moyens du diagnostic et de la prise en charge sont développés (ARTICLE 1).

Au cours du temps nous avons, par ordre chronologique, visités des partenaires dans les pays suivants : USA, Suriname, Guyana, Brésil (Macapa, Belem, Rio de Janeiro, Brazilia, Manaus Belo Horizonte, Fortaleza et Porto Alegre), Trinidad & Tobago, Colombie, Guatemala et Pérou.

Si l'objectif de partenariat scientifique était toujours au premier plan avec les cliniciens, chercheurs ou personnels de laboratoire, chaque mission s'envisageait également comme une activité de plaidoyer pour la prise en compte de la problématique de l'histoplasmose chez le patient infecté par le VIH (prise de contact avec les ambassades de France et représentants de l'OMS ou ONUSIDA ou autres représentants locaux des ministères de la Santé, le cas échéant).

Par ailleurs, la mise en œuvre d'un réseau de professionnels de santé autour de l'histoplasmose chez le patient infecté par le VIH constituait un objectif du programme de coopération régionale TCC, financé par l'OPS-OMS et présenté précédemment (activité n°5).

Afin de réunir physiquement tous les acteurs rencontrés au cours des deux premières années, issus du plateau des Guyanes et de nos collaborations scientifiques naissantes (USA et Colombie), nous avons organisé une première manifestation fin 2013. Intitulée « 1st Histoplamosis International Network meeting (HiNET) », elle a réuni des partenaires (institutions gouvernementales ou internationales, professionnels de santé pour le diagnostic et la prise en charge de l'histoplasmose et autres étudiants en santé) issus de sept pays (Brésil, France, Suriname, Guyana, Trinidad & Tobago, Colombie, USA) à Paramaribo, Suriname (Figure 18).

Sur la base d'une présentation standardisée, il a été demandé à chaque participant de rendre compte des aspects épidémiologiques, des pratiques médicales et des outils pour le diagnostic des infections fongiques invasives ainsi que des molécules antifongiques disponibles dans chaque contexte. L'objectif était de rendre compte d'une analyse détaillée de la problématique de l'histoplasmose

chez le patient infecté par le VIH (élargie aux infections fongiques invasives pour certains centres experts en mycologie) dans chacun des pays (voire états pour les partenaires brésiliens en provenance des états de l'Amapa, du Para et de Rio de Janeiro).

Les acteurs réunis ont affirmé le caractère « négligé » de l'histoplamose chez le patient infecté par le VIH sur le plateau des Guyanes, en Amazonie et plus largement en Amérique Latine. De la même manière, ils ont convenu que l'histoplasmose occupait probablement une des premières places dans la liste des événements opportunistes inauguraux du stade SIDA en Amérique du Sud, à l'origine d'un grand nombre de décès évitables.

Synthèse des discussions, un éditorial fondateur statuait pour la première fois sur les causes et les conséquences de l'absence ou du manque d'intérêt pour l'histoplasmose dans la région (ARTICLE 12).

Figure 18 : Principaux partenaires du "1st Histoplasmosis International NETwork (HiNET) meeting", 17-18 octobre 2013, Paramaribo, Suriname.

ARTICLE 12 : Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage

Author: Nacher, M., **Adenis, A**., Mc Donald, S., Do Socorro Mendonca Gomes, M., Singh, S., Lopes Lima, I., Malcher Leite, R., Hermelijn, S., Wongsokarijo, M., Van Eer, M., Marques Da Silva, S., Mesquita Da Costa, M., Silva, M., Calvacante, M., do Menino Jesus Silva Leitao, T., Gomez, B. L., Restrepo, A., Tobon, A., Canteros, C. E., Aznar, C., Blanchet, D., Vantilcke, V., Vautrin, C., Boukhari, R., Chiller, T., Scheel, C., Ahlquist, A., Roy, M., Lortholary, O., Carme, B., Couppie, P. and Vreden, S.

Year: 2013 **Journal:** PLoS Negl Trop Dis

ISSN: 1935-2735 (Electronic) **DOI:** 10.1371/journal.pntd.0002319PNTD-D-12-01557 [pii]

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24278485

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Editorial

Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage

Mathieu Nacher[1,2]*, Antoine Adenis[1,2], Sigrid Mc Donald[3], Margarete Do Socorro Mendonca Gomes[4], Shanti Singh[5], Ivina Lopes Lima[4], Rosilene Malcher Leite[4], Sandra Hermelijn[3], Merril Wongsokarijo[6], Marja Van Eer[7], Silvia Marques Da Silva[8], Maurimelia Mesquita Da Costa[8], Marizette Silva[9], Maria Calvacante[9], Terezinha do Menino Jesus Silva Leitao[10], Beatriz L. Gómez[11], Angela Restrepo[11], Angela Tobon[11], Cristina E. Canteros[12], Christine Aznar[2], Denis Blanchet[2], Vincent Vantilcke[13], Cyrille Vautrin[13], Rachida Boukhari[13], Tom Chiller[14], Christina Scheel[14], Angela Ahlquist[14], Monika Roy[14], Olivier Lortholary[15,16], Bernard Carme[1,2], Pierre Couppié[2], Stephen Vreden[3]

1 Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane (Inserm/DGOS CIE 802), Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, **2** Epidemiologie Parasitoses et Mycoses Tropicales, EA 3593, Université Antilles Guyane, Cayenne, French Guiana, **3** Academisch Ziekenhuis Paramaribo Hospital, Paramaribo, Suriname, **4** Laboratório Central de Saúde Pública do Amapá, Macapa, Brazil, **5** National AIDS Program, Georgetown, Guyana, **6** Public Health Central Laboratory of Suriname, Paramaribo, Suriname, **7** Diakonessenhuis Hospital, Paramaribo, Suriname, **8** Instituto Evandro Chagas, Belém, Brazil, **9** Hospital de Clinicas Dr. Alberto Lima, Macapa, Brazil, **10** Universidade Federal do Ceara, Faculdade de Medicina, Departamento de Saude Comunitaria, Fortaleza, Ceara, Brazil, **11** Corporación para Investigaciones Biológicas, Medellín, Colombia, **12** INEI-ANLIS "Dr. Carlos G. Malbrán," Buenos Aires, Argentina, **13** Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, French Guiana, France, **14** Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **15** Institut Pasteur, National Reference Center for Mycoses and Antifungals, Molecular Mycology Unit, Paris, France, **16** CNRS URA3012, Paris, France

HIV/AIDS is not a neglected disease. Histoplasmosis is not considered a neglected disease in North America. However, in South America, it should be. It often affects neglected populations and represents a lethal blind spot of the HIV/AIDS data collection systems. Counts of new AIDS cases and AIDS-related deaths are useful to follow the epidemic; however, they overlook the exact cause of death. In the context of the South American pathogen ecology, the systemic mycosis due to *Histoplasma capsulatum var. capsulatum* is probably on the top of the list of AIDS-defining illnesses and AIDS-related deaths [1], yet it is mostly undiagnosed and is not even on the diagnostic algorithm used by a significant proportion of clinicians facing a febrile, severely immunodepressed patient in the region.

The Invisible Burden

Studies performed in the 1950s and 1960s on the histoplasmin skin test positivity in South America showed positivity rates around 30% from Trinidad and Tobago in the North, to Uruguay and Argentina in the South. The pathogen is there [2]. Despite this, expertise and awareness of this disease is limited to mycologists and some clinical teams scattered throughout the South American continent [2–16]. But those with expertise are the exception rather than the rule. Imported cases in Europe occurring in HIV-infected residents or travellers from South America, notably in France, Spain, and Italy, are starting to be recognized, but often late in the course of the disease because clinicians are not familiar with this "endemic" disease [17–19].

For too long, the absence of a simple, reliable, and affordable diagnostic test has made it difficult to determine the burden of this disease in HIV-infected patients in much of South America. The gold standard for diagnosis relies, so far, on the culture of fluid and tissue samples [20]. This requires invasive investigations by clinicians (bone marrow aspirates; biopsies of the liver, lymph node, and intestine; etc.). From the lab perspective, direct examination may accelerate diagnosis, but culture may take weeks and require a BSL 2 laboratory. Detection of specific antigens in serum or urine samples is not available for diagnosis in most of South America, and galactomannan detection (cross reactivity during histoplasmosis) is not being used as a standard of care. Contact with clinicians from various countries suggests that, although severe histoplasmosis often kills in a few days, most clinicians are not very aggressive in their investigations. Moreover, presumptive antifungal therapy is rare. Biopsies are usually immersed in formalin by surgeons rather than sent for culture. Most often, clinicians do not take proper samples for mycological diagnosis, creating a vicious cycle that diminishes the capacity of mycological laboratories and perpetuates underfunding and the absence of diagnosis and, thus, the "nonexistence" of the very disease that is killing numerous patients.

In French Guiana, there has been a mycology laboratory since 1997 with a BSL 3 laboratory. The virtuous cycle between laboratory and clinicians has been fruitful: clinicians are well informed about histoplasmosis and are quite aggressive in looking for the infection, while the mycology laboratory has the capacity to appropriately process and identify *Histoplasma* in clinical specimens [21]. Although published maps [2] show no histoplasmosis in most of French Guiana, the recent

Citation: Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, et al. (2013) Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage. PLoS Negl Trop Dis 7(11): e2319. doi:10.1371/journal.pntd.0002319

Published November 21, 2013

Funding: No funding was received for this work.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mathieu.nacher@ch-cayenne.fr

figures that have emerged are striking. With 1.5 cases per 100 patient-years, histoplasmosis is the most common cause of AIDS-defining illness. Interestingly, despite awareness of this disease and availability of liposomal amphotericin B, it has also been the leading cause of AIDS-related death for decades. A recent 2-year study of all HIV patients admitted in Saint Laurent du Maroni hospital showed that 41% of admitted patients with CD4 counts below 200 had disseminated histoplasmosis, and 85% of admitted patients with CD4 counts below 50 and isolated fever had disseminated histoplasmosis. This is a clear message for physicians when admitting a severely immunodepressed HIV patient in the region: "*Don't miss histoplasmosis!*"

No Data = No Existence. Meanwhile, Patients Continue Dying from a Treatable Disease...

The high prevalence of HIV–*Histoplasma* coinfections on the South American continent is not a trivial problem. The scarcity of the published research on this topic reflects the tragic fact that this problem is evolving under the radar of health care systems and is truly a neglected disease. Generations of young doctors will learn to look for tuberculosis, pneumocystosis, and bacterial pneumonia when confronted with a febrile patient with respiratory signs, but not for histoplasmosis. Similarly, important clinical clues, such as cytopenia (ascribed to bone marrow involvement with or without haemophagocytic syndrome) and liver enzyme abnormalities will often not lead to the suspicion of disseminated histoplasmosis. A big danger is that a smear negative, treatment resistant, "tuberculosis-like" syndrome may often be labelled drug-resistant tuberculosis, when it was never tuberculosis in the first place. It is thus of paramount importance to fill this knowledge gap and revise the diagnostic and therapeutic algorithms in the region. The "*Know your epidemic, know your response*" UNAIDS slogan should also be applied to histoplasmosis.

We Need Research and We Need to Act Now

The HIV/AIDS epidemic is still active in countries in the Amazon basin. Guyana, Suriname, French Guiana, and the Brazilian state of Amapa all have HIV prevalence rates over 1% of the population. Although the AIDS incidence has steadily declined in the southern states of Brazil, the situation in the northern (Amazonas, Roraima, and Amapa) and the northeastern states of Brazil is still concerning. A very coarse calculation based on 600,000 HIV patients and an annual histoplasmosis incidence rate of 1.5% would estimate the annual number of cases to be in the thousands. Unfortunately, histoplasmosis thus still has a future in HIV patients. Although there is a need for epidemiologic research to measure the true burden of disease in various regions of South America, actions can, and should, be taken to diagnose and treat patients now. We need to develop standard mycological practices in the area that emphasize early and aggressive clinical diagnosis, and we need to develop new rapid diagnostic tools and advocate for affordable treatment. New affordable diagnostic assays (CDC, Immy) are presently being tested in Brazil, French Guiana, Suriname, and Colombia. We hope they will allow us to improve our knowledge of local epidemiologies and reduce patient mortality through early diagnosis and increased awareness. Although amphotericin B is available, it has potential significant renal side effects. Liposomal amphotericin B is the treatment of choice of the most severe cases of HIV-associated histoplasmosis, but its cost exceeds 800 US dollars per day. Gilead Sciences, Inc. has committed to the procurement of HIV drugs at affordable prices. Extending this policy to the problem of treating HIV-associated histoplasmosis, a neglected disease, would be an important step in improving the health of HIV/AIDS patients in the region. In the near future, DNDi (Drugs for Neglected Diseases initiative) should provide a low-cost, heat-stable alternative to liposomal amphotericin B that could be valuable for histoplasmosis treatment. Our focus has been HIV-associated disseminated histoplasmosis; however, it should be emphasized that the problem of missing the diagnosis of histoplasmosis in South America also extends to some immunocompetent patients or patients with causes of immunodepression other than HIV [22].

We need tests; we need treatments; but first of all, we physicians need to integrate our South American epidemiology in our diagnostic algorithms. Looking for malaria in febrile patients in malaria-endemic areas is automatic; looking for histoplasmosis in febrile, immunosuppressed, HIV-infected patients in South America, Central America, and perhaps way beyond [23,24], is not. The sooner we do it, the better for our patients.

References

1. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, et al. (2011) What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. Am J Trop Med Hyg 84: 239–240.
2. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M (2011) Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol 49: 785–798.
3. Arango M, Castaneda E, Agudelo CI, De Bedout C, Agudelo CA, et al. (2012) Histoplasmosis: results of the Colombian national survey, 1992–2008. Biomedica 31: 344–356.
4. Bava AJ (1995) Histoplasmosis in the Muniz Hospital of Buenos Aires. Rev Inst Med Trop Sao Paulo 37: 531–535.
5. Brilhante RS, Fechine MA, Mesquita JR, Cordeiro RA, Rocha MF, et al. (2012) Histoplasmosis in HIV-positive patients in Ceara, Brazil: clinical-laboratory aspects and in vitro antifungal susceptibility of Histoplasma capsulatum isolates. Trans R Soc Trop Med Hyg 106: 484–488.
6. Cermeno JR, Hernandez I, Godoy G, Cabello I, Cermeno JJ, et al. (2005) [Mycoses at Hospital Universitario "Ruiz y Paez," Ciudad Bolivar, Venezuela, 2002]. Invest Clin 46: 37–42.
7. Daher EF, Silva GB, Jr., Barros FA, Takeda CF, Mota RM, et al. (2007) Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil. Trop Med Int Health 12: 1108–1115.
8. de Francesco Daher E, de Sousa Barros FA, da Silva Junior GB, Takeda CF, Mota RM, et al. (2006) Risk factors for death in acquired immunodeficiency syndrome-associated disseminated histoplasmosis. Am J Trop Med Hyg 74: 600–603.
9. Eza D, Cerrillo G, Moore DA, Castro C, Ticona E, et al. (2006) Postmortem findings and opportunistic infections in HIV-positive patients from a public hospital in Peru. Pathol Res Pract 202: 767–775.
10. Guimaraes LC, Silva AC, Micheletti AM, Moura EN, Silva-Vergara ML, et al. (2012) Morphological changes in the digestive system of 93 human immunodeficiency virus positive patients: an autopsy study. Rev Inst Med Trop Sao Paulo 54: 89–93.
11. Mora DJ, dos Santos CT, Silva-Vergara ML (2008) Disseminated histoplasmosis in acquired immunodeficiency syndrome patients in Uberaba, MG, Brazil. Mycoses 51: 136–140.
12. Pietrobon D, Negro-Marquinez L, Kilstein J, Galindez J, Greca A, et al. (2004) Disseminated histoplasmosis and AIDS in an Argentine hospital: clinical manifestations, diagnosis and treatment. Enferm Infecc Microbiol Clin 22: 156–159.
13. Pontes LB, Leitao Tdo M, Lima GG, Gerhard ES, Fernandes TA (2010) Clinical and evolutionary characteristics of 134 patients with disseminated histoplasmosis associated with AIDS in the State of Ceara. Rev Soc Bras Med Trop 43: 27–31.
14. Davel G, Canteros CE (2007) Epidemiological status of mycoses in the Argentine Republic. Rev Argent Microbiol 39: 28–33.
15. Tobon AM, Agudelo CA, Rosero DS, Ochoa JE, De Bedout C, et al. (2005) Disseminated histoplasmosis: a comparative study between patients with acquired immunodeficiency syndrome and non-human immunodeficiency virus-infected individuals. Am J Trop Med Hyg 73: 576–582.

16. Tobón AM MA, Orozco L, Restrepo C, Molina D, de Bedout C (2011) Histoplasmosis diseminada en una cohorte de pacientes coinfectados con el VIH. Acta Med Colombiana. Acta Med Colombiana 36: 63–67.
17. Peigne V, Dromer F, Elie C, Lidove O, Lortholary O (2011) Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. Am J Trop Med Hyg 85: 934–941.
18. Antinori S, Magni C, Nebuloni M, Parravicini C, Corbellino M, et al. (2006) Histoplasmosis among human immunodeficiency virus-infected people in Europe: report of 4 cases and review of the literature. Medicine (Baltimore) 85: 22–36.
19. Buitrago MJ, Gomez-Lopez A, Monzon A, Rodriguez-Tudela JL, Cuenca-Estrella M (2007) [Assessment of a quantitative PCR method for clinical diagnosis of imported histoplasmosis]. Enferm Infecc Microbiol Clin 25: 16–22.
20. Couppie P, Aznar C, Carme B, Nacher M (2006) American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis 19: 443–449.
21. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, et al. (2008) AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. AIDS 22: 1047–1053.
22. Assi MA, Sandid MS, Baddour LM, Roberts GD, Walker RC (2007) Systemic histoplasmosis: a 15-year retrospective institutional review of 111 patients. Medicine (Baltimore) 86: 162–169.
23. Gutierrez ME, Canton A, Sosa N, Puga E, Talavera L (2005) Disseminated histoplasmosis in patients with AIDS in Panama: a review of 104 cases. Clin Infect Dis 40: 1199–1202.
24. Bulmer AC, Bulmer GS (2001) Incidence of histoplasmin hypersensitivity in the Philippines. Mycopathologia 149: 69–71.

Ainsi, le constat des cliniciens guyanais établit au début des années 2000, suspectant la probable non prise en compte ou difficile prise en charge de l'histoplasmose au cours de l'infection par le VIH dans la majorité des pays d'Amérique du Sud et de la Caraïbe, était réél et confirmé par les acteurs de terrain dans chacun des pays.

Dans le sillage de cette première conclusion, un éditorial un peu provocateur s'envisageait comme une quantification simple et rapide de l'incidence et de la mortalité annuellement associées à l'histoplasmose chez le patient infecté par le VIH au sein du biome Amazonien (ARTICLE 13). En appliquant les taux d'incidence et de mortalité observés en Guyane française à la population amazonienne, on donnait un ordre de grandeur à l'ampleur du phénomène histoplasmose dans la région. Et, si l'on mettait en perspective le nombre de décès calculés pour une année sur trente ans d'épidémie d'infection par le VIH, les chiffres étaient considérés intolérables pour une infection fongique qui, prise en charge précocement, aurait été le plus souvent résolutive.

ARTICLE 13 : How many have died from undiagnosed Human Immunodeficiency virus-associated histoplasmosis, a treatable disease? Time to act.

Author: Nacher, M., **Adenis, A**., Aznar, C., Blanchet, D., Vantilcke, V., Demar, M., Carme, B. and Couppie, P.

Year: 2013 **Journal:** Am J Trop Med Hyg

ISSN: 1476-1645 (Electronic) **DOI:** ajtmh.13-0226 [pii]10.4269/ajtmh.13-0226

Abstract: Human immunodeficiency virus (HIV)-associated disseminated Histoplasma capsulatum capsulatum infection often mimics tuberculosis. This disease is well know in the United States but is dramatically underdiagnosed in Central and South America. In the Amazon region, given the available incidence data and the regional HIV prevalence, it is expected that, every year, 1,500 cases of histoplasmosis affect HIV patients in that region alone. Given the mortality in undiagnosed patients, at least 600 patients would be expected to die from an undiagnosed but treatable disease. The lack of a simple diagnostic tool and the lack of awareness by clinicians spiral in a vicious cycle and made a major problem invisible for 30 years. The HIV/acquired immunodeficiency syndrome community should tackle this problem now to prevent numerous avoidable deaths from HIV-associated histoplasmosis in the region and elsewhere.

URL:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=24277783

Am. J. Trop. Med. Hyg., 90(2), 2014, pp. 193–194
doi:10.4269/ajtmh.13-0226

Perspective Piece

How Many Have Died from Undiagnosed Human Immunodeficiency Virus–Associated Histoplasmosis, A Treatable Disease? Time to Act

Mathieu Nacher,* Antoine Adenis, Christine Aznar, Denis Blanchet, Vincent Vantilcke, Magalie Demar, Bernard Carme, and Pierre Couppié

Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane CIC-EC CIE 802, Cayenne General Hospital, Cayenne, French Guiana; Coordination Regionale de la Lutte Contre le VIH de Guyane, Laboratoire Hospitalo-Universitaire de Parasitologie Mycologie, Unité des Maladies Infectieuses et Tropicale, et Service de Dermatologie Vénéréologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana; Equipe EPaT EA3593 Epidemiologie des Parasitoses et Mycoses Tropicales, Université Antilles Guyane, Cayenne, French Guiana

Abstract. Human immunodeficiency virus (HIV)–associated disseminated *Histoplasma capsulatum capsulatum* infection often mimics tuberculosis. This disease is well know in the United States but is dramatically underdiagnosed in Central and South America. In the Amazon region, given the available incidence data and the regional HIV prevalence, it is expected that, every year, 1,500 cases of histoplasmosis affect HIV patients in that region alone. Given the mortality in undiagnosed patients, at least 600 patients would be expected to die from an undiagnosed but treatable disease. The lack of a simple diagnostic tool and the lack of awareness by clinicians spiral in a vicious cycle and made a major problem invisible for 30 years. The HIV/acquired immunodeficiency syndrome community should tackle this problem now to prevent numerous avoidable deaths from HIV-associated histoplasmosis in the region and elsewhere.

In the Guianas and the Amazon Basin, the prevalence of human immunodeficiency virus (HIV) is approximately 1%. There have been some decreases in incidence in some states in this region, but recent increases in incidence in northern states of Brazil have been reported.[1–3] The population of the Amazon basin is estimated to be approximately 10 million persons,[4] which would imply that approximately 100,000 persons are HIV positive in the Amazon Basin.

The Amazonian environment is suitable for the growth of *Histoplasma capsulatum*.[5] For immunocompetent patients, this organism causes mostly benign infections, but in severely immunodepressed HIV-infected patients, infection with this organism leads to a fatal disease in the absence of diagnosis and treatment. There lies the problem. Clinical symptoms are unspecific and often mimic those of tuberculosis.[6,7] Diagnosis is difficult and requires invasive procedures (biopsies, bone marrow smears), and trained staff to detect *H. capsulatum*, often after weeks of culture.[8] Severe infections are often fatal within days.[9] However, death often occurs after long delays in which patients are unsuccessfully treated for unconfirmed tuberculosis. Patients die because they are not treated for a treatable disease and because there is no diagnosis test. With no diagnosis, this possibility is not included in the diagnostic and treatment algorithms of clinicians who, despite unknowingly encountering this disease on a regular basis, have never seen a case because it was never diagnosed. In this context, then why give presumptive treatment of a disease that is not present?

It is tragic but it makes total sense. It is even frighteningly tragic when one crunches the numbers to estimate what it means that after 30 years of the HIV epidemic, one of the leading causes of acquired immunodeficiency syndrome (AIDS) in the Amazon[10] still goes largely unrecognized and evolves under the radar of national plans and international funding efforts.

The only incidence data available for this region suggests that the incidence of histoplasmosis during the highly active antiretroviral therapy era was 1.5 cases/100 person-years.[10] The historical mortality rate of disseminated histoplasmosis was > 30% despite mycology expertise.[7,8] This finding indicates that for 100,000 HIV patients, there would be 1,500 cases of histoplasmosis/year and 600 deaths/year, and probably more if undiagnosed. This finding also indicates that for more than 30 years the cumulated death rate in the region must have been huge, in the tens of thousands.

A rational sceptic could rightly doubt this claim from the generalization of data from the smallest South American territory to the entire Amazon and elsewhere. However, when one reviews the literature, it becomes evident that histoplasmosis is present throughout the region, this fact has been known for decades, and that we should have been paying more attention.[5] The high prevalence of histoplasmin test reactivity in the region was known even before AIDS was identified in 1981.[11] Histoplasmosis has been an AIDS-defining illness since 1993. We should have connected the dots earlier.

How could something so huge escape the attention of the HIV/AIDS community in the region? One explanation for this dramatic blind spot is that in the region, the diagnostic capacity for mycology has been insufficient. It has been long argued that medical mycology is a neglected area of biology, and that the often low incidence of mycoses is caused by a lack of medical mycologists rather than the absence of the mycoses.[12] Another explanation is that the standard conceptualization of HIV/AIDS, the usual indicators, and the Joint United Nations Programme on HIV/AIDS terminology and framework did not explicitly entail disseminated histoplasmosis or the regional AIDS-defining illnesses. The anesthetic effect of the familiarity of vertical concepts and vertical programs can make it difficult to reframe the problem and see what was always there.

For better diagnostic and treatment, we should know what AIDS is to direct diagnostic hypotheses when caring for individual patients. Misdiagnosing histoplasmosis as tuberculosis, not only delays a life-saving treatment of the individual patient, but it can confound tuberculosis statistics (incidence, resistance, mortality) and make it difficult to evaluate tuberculosis program results.

The current financial difficulties should not stand in the way of building the diagnostic capacity for detection of histoplasmosis.

*Address correspondence to Mathieu Nacher, Centre Hospitalier de Cayenne, Rue des Famboyants, Cayenne 97300, French Guiana. E-mail: mathieu.nacher@ch-cayenne.fr

It does not necessarily cost much to do the diagnosis. Treatment relies on amphotericin B for severe forms and itraconazole for non-severe forms and prophylaxis.[7] Both drugs are generic drugs that are perfectly affordable. The toxicity of amphotericin B leads industrialized countries to use the costly liposomal version of the drug. However, The Drugs for Neglected Disease Initiative is releasing a cheap alternative that was developed for treatment of cryptococcosis.[13] This is an opportunity for resource-limited countries in disease-endemic areas for treatment of histoplasmosis. We should not wait any longer. Every year wasted to build capacity for diagnosis and treatment of histoplasmosis in the Amazon Basin and elsewhere leads to hundreds of deaths that could have been avoided. This is not acceptable.

Received April 27, 2013. Accepted for publication June 5, 2013.

Published online November 25, 2013.

Authors' addresses: Mathieu Nacher, Centre Hospitalier de Cayenne, Rue des Flamboyants, Cayenne 97300, French Guiana, E-mail: mathieu.nacher@ch-cayenne.fr. Antoine Adenis, Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane, INSERM DGOS CIE 802, Cayenne General Hospital, Avenue des Flamboyants, Cayenne 97306, French Guiana, E-mail: antoine.adenis@ch-cayenne.fr. Christine Aznar, Service Hospitalo Universitaire de Parasitologie Mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mail: christine.aznar@ch-cayenne.fr. Denis Blanchet, Laboratoire Hospitalo Universitaire de Parasitologie Mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mail: denis.blanchet@ch-cayenne.fr. Vincent Vantilcke, Service de Médecine, Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, French Guiana, E-mail: v.vantilcke@ch-ouestguyane.fr. Magalie Demar, Department of Infectious and Tropical Disease, Centre Hospitalier Andrée Rosemon, Cayenne, French Guiana, E-mail: magalie.demar@ch-cayenne.fr. Bernard Carme, Centre d'Investigation Clinique Épidémiologie Clinique Antilles Guyane, CIE INSERM 802, Pole de Guyane, Centre Hospitalier de Cayenne, Cayenne, French Guiana, E-mail: bernard.carme@ch-cayenne.fr. Pierre Couppié, Equipe EA 3593, Epidemiologie des Parasitoses et Mycoses Tropicales, Universite des Antilles et de la Guyane, Cayenne, French Guiana, E-mail: couppie.pierre@voila.fr.

REFERENCES

1. Cohen J, 2006. HIV/AIDS: Latin America and the Caribbean, South America. *Science 313:* 484.
2. Nacher M, Vantilcke V, Parriault MC, Van Melle A, Hanf M, Labadie G, Romeo M, Adriouch L, Carles G, Couppie P, 2010. What is driving the HIV epidemic in French Guiana? *Int J STD AIDS 21:* 359–361.
3. Brazil MoHo, 2012. *Boletim Epidemiológico Aids e DST, 2012 Report.*
4. OAS States, 2005. *Amazon River Basin.*
5. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M, 2011. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol 49:* 785–798.
6. Krug ES, Glenn HR, 1948. Comparative studies in tuberculosis and histoplasmosis among 2000 students entering Pennsylvania State College, September, 1947. *J Lancet 68:* 206–208.
7. Couppie P, Aznar C, Carme B, Nacher M, 2006. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. *Curr Opin Infect Dis 19:* 443–449.
8. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, Vaz T, Vantilcke V, Mahamat A, Magnien C, Chauvet E, Carme B, Couppie P, 2008. AIDS-related *Histoplasma capsulatum* var. *capsulatum* infection: 25 years experience of French Guiana. *AIDS 22:* 1047–1053.
9. Couppie P, Sobesky M, Aznar C, Bichat S, Clyti E, Bissuel F, El Guedj M, Alvarez F, Demar M, Louvel D, Pradinaud R, Carme B, 2004. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. *Clin Infect Dis 38:* 134–138.
10. Nacher M, Adenis A, Adriouch L, Dufour J, Papot E, Hanf M, Vantilcke V, Calvez M, Aznar C, Carme B, Couppie P, 2011. What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. *Am J Trop Med Hyg 84:* 239–240.
11. Hay RJ, White HS, Fields PE, Quamina DB, Dan M, Jones TR, 1981. Histoplasmosis in the eastern Caribbean: a preliminary survey of the incidence of the infection. *J Trop Med Hyg 84:* 9–12.
12. Ajello L, 1971. The medical mycological iceberg. *HSMHA Health Rep 86:* 437–448.
13. Initiative DND, 2012. *Alternative Formulations of Amphotericin B.*

De 2013 à 2016, nous avons poursuivis nos déplacements et notre activité de plaidoyer en Amérique Latine et participé à des symposiums dans des congrès nationaux ou internationaux.

En Guyane française, nous avons organisé, du 14 au 16 janvier 2014, en partenariat avec l'Institut Pasteur de Guyane, un séminaire dans le cadre du financement européen du consortium de recherches transdisciplinaires sur les maladies infectieuses et émergentes « STRonGer pour Strengthening Transdisciplinary Research on Infectious and Emerging Diseases in French Guiana: linking fieldwork, benchside and bedside ». Le séminaire s'intitulait « Les principales infections fongiques invasives chez l'homme : épidémiologie et impact sur la santé humaine », en présence notamment du Pr O. Lortholary (directeur du département des maladies infectieuses et tropicales de l'Hôpital Necker -Enfants Malades à Paris mais également directeur adjoint du Centre National de Référence des Mycoses Invasives et Antifongiques). L'objectif était de sensibiliser les acteurs locaux de la recherche à la problématique des infections fongiques invasives (Annexe 5), de faire une analyse critique des projets en cours et des perspectives de développement pertinentes à mettre en œuvre à propos des infections fongiques invasives et plus particulièrement de l'histoplasmose.

A l'international, nous avons participé annuellement au congrès national d'immunologie et d'infectiologie du Suriname avec des présentations sur l'histoplasmose et l'infection par le VIH afin de sensibiliser chaque année un peu plus de professionnels de santé.
Hors participation à différents séminaires internationaux, notamment au Brésil, nous avons principalement participé à l'organisation et aux présentations de deux symposiums au sein du congrès annuel de l'American Society of Tropical Medicine and Hygiene (ASTMH).
Le premier symposium s'intitulait "Histoplasmosis and HIV in the Americas: the fight against a neglected fungal disease" (Symposium 139) à l'occasion du 64ème congrès annuel, 25 au 29 octobre 2015, Philadelphie, USA (Annexe 6). L'objectif était de présenter un état des connaissances du poids de la maladie dans les Amériques et de présenter les avancées récentes, notamment au plan des méthodes diagnostiques. L'initiative en cours et le développement du réseau de partenaire ont également était présentés.
Le second symposium s'intitulait "If you neglect it, it will grow: addressing fungal infections in advanced HIV care" (Symposium 65) à l'occasion du 65ème congrès annuel, 13 au 17 novembre 2016, Atlanta, USA (Annexe 7). L'objectif était de sensibiliser les acteurs du monde de la médecine tropicale à la problématique des infections fongiques invasives chez les patients infectés par le VIH. Des estimations d'incidence et de mortalité pour les principales infections fongiques invasives survenant au cours de l'infection par le VIH ont été présentées. Avec pour idée centrale qu'en l'absence de

diagnostic et de traitement précoces, elles étaient probablement pourvoyeuses de plus de 50% des décès au stade SIDA en zone d'endémie pour les pathogènes concernés.

Fin 2015, à l'occasion de l'évaluation de notre programme de coopération régionale TCC, financé par l'OPS-OMS et présenté précédemment, nous avons organisé une deuxième manifestation dans le cadre du réseau de partenaire en développement. Intitulé « Histoplasmosis in the Americas and the Caribbean: 1st meeting », elle a regroupé des partenaires (institutions gouvernementales ou internationales, professionnels de santé pour le diagnostic et la prise en charge de l'histoplasmose) issus de 13 pays (Argentine, Brésil, Colombie, France, Guatemala, Guyana, Mexique, Panama, Royaume-Uni, Suriname, Trinidad & Tobago, USA et Venezuela) et 6 états pour les participants brésiliens (Amazonas, Amapa, Ceara, Para, Rio de Janeiro, Rio Grande do Sul) (Figure 19).
En plus d'un état des lieux actualisé de la situation de chaque pays par rapport à 2013, de la présentation de nouvelles méthodes diagnostiques par des industriels et de propositions aux participants d'intégrer leurs équipes à des essais cliniques académiques, des tables rondes thématiques (Données épidémiologiques et surveillance, accès au diagnostic, accès au traitement, inclusion de la coinfection histoplasmose et VIH dans les plans stratégiques des Amériques – méthodes pour le plaidoyer et la mise en réseau) ont réunis les acteurs.
La synthèse des discussions est décrite à l'ARTICLE 14. Cette manifestation est l'acte fondateur d'une initiative décidée par les participants et intitulée « 80by20 ». Son objectif est de mettre à disposition d'ici 2020, dans 80% des hôpitaux de référence pour la prise en charge des patients infectés le VIH, des tests diagnostics rapides de l'histoplasmose ainsi que les théapeutiques antifongiques efficaces.

Figure 19 : Principaux partenaires du "Histoplasmosis in the Americas and the Caribbean, 1st meeting", 4-6 décembre 2015, Paramaribo, Suriname.

ARTICLE 14 : Proceedings of First Histoplasmosis in the Americas and the Caribbean Meeting, Paramaribo, Suriname, December 4–6, 2015

Author: Mathieu, Nacher and **Antoine, Adenis**

Year: 2016 **Journal:** Emerging Infectious Disease journal

ISSN: 1080-6059 **DOI:** 10.3201/eid2209.160408

URL: http://wwwnc.cdc.gov/eid/article/22/9/16-0408

N.B : Cette publication est seulement disponible en tant que page internet au format HTML. Une version éditée par mes soins est proposée dans ce manuscrit afin de visualiser son contenu.

Volume 22, Number 9—September 2016

Conference Summary

Proceedings of First Histoplasmosis in the Americas and the Caribbean Meeting, Paramaribo, Suriname, December 4–6, 2015

Mathieu Nacher✉ and Antoine Adenis✉
Author affiliations: Centre d'Investigation Clinique Antilles Guyane, INSERM 1424, Centre Hospitalier Andrée Rosemon, Cayenne, French Guiana; EA3593 Ecosystèmes Amazoniens et Pathologie Tropicale, Université de Guyane, Cayenne

Suggested citation for this article

Histoplasmosis is not defined as a neglected disease, but in Latin America and the Caribbean, it is suspected that nearly 10,000 HIV-positive patients die from histoplasmosis disseminated beyond the respiratory tract yearly, placing this AIDS-defining illness among the main causes of death of HIV patients on a similar level as HIV-associated tuberculosis (1,2). During December 4–6, 2015, researchers and health professionals from 13 countries (Argentina, Brazil, Colombia, French Guiana, Guatemala, Guyana, Mexico, Panama, Surinam, Trinidad and Tobago, the United Kingdom, the United States, and Venezuela), representing organizations including the Pan American Health Organization, the US Centers for Disease Control and Prevention, the Foundation for Scientific Research of Suriname, and the Institut National de la Santé et de la Recherche Médicale, met in Paramaribo, Suriname, to define the next steps necessary to fight disseminated histoplasmosis in HIV patients (Figure).

The result of shared analysis of the situation surrounding disseminated histoplasmosis was that this disease is still rarely recognized in much of Latin America, notably among clinicians who care for HIV patients. Disseminated histoplasmosis is still often mistaken for tuberculosis and probably confounds tuberculosis statistics regarding treatment failure and mortality rates (3). All country representatives who attended the meeting concurred that this situation stems from the complexity and length of the diagnostic process of standard mycology and the absence of rapid, affordable, easy-to-use rapid diagnostic tests. Effects of the missed diagnoses are compounded by the lack of availability of liposomal amphotericin, the best treatment for disseminated histoplasmosis, for which attendees agreed the expense is much too high for most of Latin America (2). Furthermore, amphotericin B, deoxycholate, or itraconazole were sometimes unavailable in some countries.

Disseminated histoplasmosis is often diagnosed in patients in whom HIV is diagnosed very late in the course of the HIV infection (2). This finding suggests that, despite improved access to antiretroviral drugs, a large number of persons with HIV remain are undiagnosed and are at risk for disseminated histoplasmosis.

Recognizing these determinants of the current situation, the researchers and health professionals who attended the meeting agreed to launch the 80 by 20 Initiative. This initiative aims to provide rapid diagnostic tests and effective treatments for disseminated

histoplasmosis to 80% of the reference hospitals in Latin America and the Caribbean by the year 2020.

New rapid antigen tests are soon to be released on the market, including in Latin America and the Caribbean areas. These new tests require urine or serum samples instead of samples requiring more invasive procedures, such as bone marrow, lymph node, and liver biopsies. The tests are based on ELISA methods or lateral flow devices and thus do not require very advanced laboratory methods and are relatively inexpensive. Additionally, the meeting participants recognized that regional distributors of these tests often considerably increase the cost and that the distribution network should be chosen with care to keep prices affordable.

Access to inexpensive diagnostic tests and encouragement for screening of immunocompromised HIV-infected patients who have signs of infection is expected to generate a large increase of data related to this problem and rapidly lead to updated clinical and therapeutic algorithms for patients with advanced HIV disease, as well as an adaptation of country-specific HIV strategic plans. Of greatest consequence, a rapid diagnostic test will lead to a reduction in mortality rates, as was shown in the conference presentations by representatives of Colombia, Guatemala, and Brazil, where Histoplasma antigen detection in urine samples led to rapid gains in patient survival. The question of negotiating low-priced liposomal amphotericin B is in need of rapid consideration because this would affect the most severely ill patients (4) and is already the standard recommendation in Europe and the United States.

The 80 by 20 Initiative stems from the coalescence of shared analyses of a widespread problem through a bottom-up mechanism. However, the implementation of this initiative also requires a top-down input by national public health agencies and international health organizations. The ever-increasing circle of concerned professionals has now reached a critical mass to make this specific goal a realistic one that would save numerous lives in a cost-effective way.

Dr. Nacher is a professor of epidemiology at Université de Guyane, director of the Centre d'Investigation Clinique Antilles Guyane (INSERM 1424), and the chairman for the HIV/AIDS Programme of French Guiana. His main research interest is epidemiology of disseminated histoplasmosis. Dr. Adenis is Deputy Chief of INSERM CIC1414 and is a

Dr. Adenis is senior researcher at the Centre d'Investigation Clinique Antilles Guyane (INSERM CIC1424), and is enrolled in the PhD program for Clinical Epidemiology at Université de Guyane. His main research interests are HIV and fungal diseases.

Acknowledgment

Funding for the conference was provided by Agence Nationale de la Recherche sur le Sida et les Hépatites, Agence Régionale de la Santé de Guyane, Centre Hospitalier de Cayenne, Fonds Européens de Développement Régional (Presage 31362), Foundation For Scientific Research Suriname, Global Action Fund for Fungal Infections, IMMY, Institut National de la Santé et de la Recherche Médicale, Laboratoire d'Excellence Centre d'Etudes de la Biodiversité Amazonienne, Ministry of Health Suriname, Université de Guyane.

References

1. Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, Lopes Lima I, Disseminated histoplasmosis in HIV-infected patients in South America: a neglected killer continues on its rampage. PLoS Negl Trop Dis. 2013;7:e2319. DOIPubMed
2. Neglected Histoplasmosis in Latin America Group. Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations. AIDS. 2016;30:167–70. DOIPubMed
3. Adenis A, Nacher M, Hanf M, Basurko C, Dufour J, Huber F, Tuberculosis and histoplasmosis among human immunodeficiency virus-infected patients: a comparative study. Am J Trop Med Hyg. 2014;90:216–23. DOIPubMed
4. Johnson PC, Wheat LJ, Cloud GA, Goldman M, Lancaster D, Bamberger DM, ; U.S. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. Ann Intern Med. 2002;137:105–9. DOIPubMed

Suggested citation for this article: Nacher M, Adenis A. Proceedings of first Histoplasmosis in the Americas and the Caribbean meeting, Paramaribo, Suriname, December 4–6, 2015. Emerg Infect Dis. 2016 Sep [date cited]. http://dx.doi.org/10.3201/eid2209.160408

DOI: 10.3201/eid2209.160408

Table of Contents – Volume 22, Number 9—September 2016

Comments

Please use the form below to submit correspondence to the authors or contact them at the following address:

Addresses for correspondence: Mathieu Nacher; ; Antoine Adenis; ; Centre d'Investigation Clinique Antilles Guyane, Inserm CIC1424, Centre Hospitalier de Cayenne, Av. des flamboyants, BP 6006, 97306 Cayenne CEDEX

Dans la perspective de cette manifestation inédite dans la région par le nombre de participants et d'institutions représentées, un éditorial visait à fixer l'argumentaire et les lignes directrices de l'initiative à mettre en œuvre lors de cette manifestation (ARTICLE 15). Toutefois, si le manuscrit a été accepté en octobre 2015, il ne sera publié que début 2016, soit juste après la manifestation.

Parmi les éléments importants de cet article, on retrouvait l'estimation simple et brute de la mortalité imputable à l'histoplasmose chez les patients infectés par le VIH à l'échelle de l'Amérique Latine (Amérique Centrale et Amérique du Sud, hors Caraïbe). Celle-ci était comparée graphiquement avec la mortalité imputable au SIDA, à la tuberculose et au paludisme, pour lesquels il existe des mécanismes de surveillance et de financement nationaux et internationaux.

Ce type de publication permettait de donner du corps au réseau de partenaires internationaux et à son choix de porter l'initiative 80by20.

ARTICLE 15 : Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations The neglected histoplasmosis in Latin America Group

Author: Nacher, Mathieu, **Adenis, Antoine**, Arathoon, Eduardo, Samayoa, Blanca, Lau-Bonilla, Dalia, Gomez, Beatriz L, Tobon, Angela, Caceres, Diego, da Silva, Silvia Marques and da Costa, Maurimelia Mesquita and the neglected histoplasmosis in Latin America, Group

Year: 2016 **Journal:** AIDS

ISSN: 1473-5571 (Electronic) **DOI:** 10.1097/QAD.0000000000000961

URL: http://www.ncbi.nlm.nih.gov/pubmed/26684816

OPINION

Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations

The neglected histoplasmosis in Latin America Group

AIDS 2016, **30**:167–170

Keywords: burden, disseminated histoplasmosis, HIV, international organizations, mortality

Nowhere to be seen

Histoplasma capsulatum is endemic in the Americas [1,2]. It has been an AIDS-defining infection since 1987 [3]. In the USA, it is a well known pathogen that can be promptly diagnosed and treated. In South and Central America, and may be the Caribbean, it is another story. Since the onset of the HIV epidemic, there have been a number of convergent reports that suggest that disseminated histoplasmosis is one of the major AIDS-defining infections and a major killer of HIV-infected patients [4–8]. However, most hospitals still have no way of diagnosing the disease and often lack the best treatments for the disease. There is thus a double tragedy, with clinicians failing to diagnose what is killing their patients, and public health authorities failing to tackle one of the major burdens of disease. Mycologic diagnosis rests on direct examination and culture of tissue samples that is often invasive and may take weeks to reveal *H. capsulatum* [9]. Molecular biology is not commercially available and thus not available in most hospitals. The detection of *H. capsulatum* antigens in urine or serum by enzyme immune assays remains a simple, noninvasive, sensitive method, with an increasing number of alternatives that are being evaluated but are still distributed on a small scale in Latin America [9]. The future diagnostic tests that could radically change the picture should be ASSURED, that is affordable, sensitive, specific, user friendly, rapid, equipment free, and delivered to those who need it [10].

Connecting the dots

Histoplasmin skin test studies show how widespread histoplasmosis is [1,2]. Mycologists have long been aware that the disease is there, but they often do not receive samples from clinicians in charge of HIV patients. Therefore, reports mention that a large proportion of histoplasmoses are HIV positive. However, this perspective is not likely to enhance histoplasmosis in HIV programmes. A more fruitful question, and one whose answer should get clinicians, public health authorities, and international authorities to act is how does histoplasmosis rank compared with other opportunistic infections? or what proportion of AIDS cases are in fact histoplasmosis cases? To answer requires sustained collaboration between mycologists and HIV clinics. A few teams have answered this question – in Panama, 7.65% of patients with HIV infection had culture-positive *H. capsulatum* [6]; in Guatemala, histoplamosis is the second opportunistic infection just after tuberculosis, but with a greater mortality [8]; in Venezuela, before the highly active antiretroviral therapy era, among 200

Centre d'Investigation Clinique Antilles Guyane CIC INSERM 1424, Centre Hospitalier de Cayenne, Université de Guyane, Guyane Française.

Correspondence to Mathieu Nacher, MD, PhD, Centre d'Investigation Clinique Antilles Guyane CIC INSERM 1424, Centre Hospitalier de Cayenne; EA3593 Université de Guyane, Guyane Française.

E-mail: mathieu.nacher@ch-cayenne.fr

Received: 28 July 2015; revised: 30 September 2015; accepted: 5 October 2015.

DOI:10.1097/QAD.0000000000000961

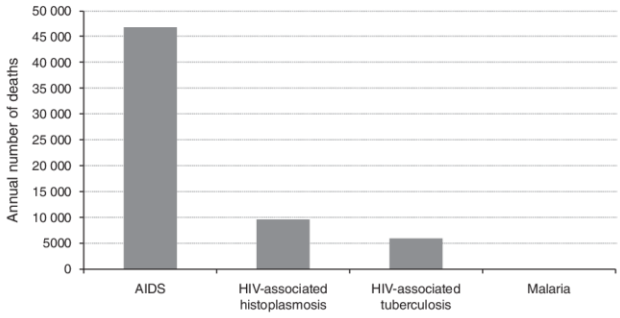


Fig. 1. Estimated number of deaths per year for different major infectious diseases in Latin America.

patients with AIDS, histoplasmosis was diagnosed in 43 (21.5%) [7], and in one study it was documented in 29 of 66 (44%) autopsies performed [1]; in Fortaleza Brazil, in 378 consecutively admitted HIV patients, 164 (43%) had disseminated histoplasmosis [5]; in French Guiana, histoplasmosis is the first AIDS-defining infection and has long been the first cause of AIDS-related death [4,11]; and a recent study showed that 42% of HIV patients admitted with $CD4^+$ cell counts less than 200 and 85% of those with $CD4^+$ cell counts less than 50 had disseminated histoplasmosis [12]. One may argue that these are focal hotspots of histoplasmosis; however, this does not fit with the spread of endemic regions for histoplasmosis. On the contrary, if one connects the dots, the picture is staggering.

Histoplasmosis inflating tuberculosis statistics

Reducing tuberculosis deaths, and notably HIV-associated tuberculosis, is a major objective for the Joint United Nations programme on HIV/AIDS (UNAIDS), the Pan American Health Organization (PAHO), and numerous AIDS programmes. Recently, data from Latin America showed a 60% increase in culture-negative relative to culture-positive tuberculosis [13]. The conclusion was that culture-negative cases were possibly because of other causes. Presumably, a large proportion of these deadly cases were disseminated histoplasmosis misdiagnosed as tuberculosis [14,15].

A staggering invisible burden

There are an estimated 1 600 000 HIV patients in the Americas. If we apply the incidence rate of 1.5 per 100 person-years measured in French Guiana [4], this suggests that there are 24 000 histoplasmosis cases in the Americas per year. The historical death rate of 40% [16] of deaths in histoplasmosis would mean there are 9600 deaths per year. In addition, it is arguable that the incidence rate is a low estimate of that of other regions in the Americas. Indeed in this French territory, 30% of patients have less than 200 $CD4^+$ cell counts when tested and over 85% of the patients are on treatment, a situation that is probably more favourable than in other countries where up to 60% of patients are at stage C, where treatment initiation is still delayed and a large proportion of the HIV population is not yet on antiretroviral treatment [17]. In addition, for undiagnosed histoplasmosis, mortality is likely to be far greater than 40%. For the Americas, the estimated annual number of malaria deaths for 2013 was 84 [18]; HIV–tuberculosis annual deaths are estimated 6000 [19], and AIDS, without specification, is estimated 47 000 annual deaths [17]. So the number of deaths from tuberculosis in AIDS patients is at a similar level than HIV-associated histoplasmosis deaths (Fig. 1). However, one is a strategic objective of UNAIDS/PAHO/the CDC/GATES foundation's HIV/AIDS strategic plans, and the other is nowhere to be seen.

How could 9600 deaths a year – approximately the equivalent number of deaths as 70 Boeing 737 plane crashes a year – have gone unnoticed? The extrapolation of this to the 34 years since the AIDS epidemic was recognized puts the cumulated number of deaths in Latin America well over 100 000. We concede that such estimates are gross approximations, that histoplasmosis incidence may be more heterogeneous than we portray, but the order of magnitude is plausible.

Correcting the lethal blind spot

The fact that a network of independent HIV care and mycology specialists reaches the same conclusion should

be taken seriously. So far, the scattered scientific publications do not seem to have percolated up towards the upper spheres of public health decision making. The expanding and earlier access to antiretroviral treatments should have a substantial impact on the mortality of histoplasmosis [20,21]. However, despite efforts to promote early HIV testing and to put greater numbers of persons on antiretroviral treatment, histoplasmosis still has a bright future because late testing, adherence and follow-up difficulties are still common.

The international public health system and numerous HIV programmes have a blind spot; we believe a huge global health tragedy has been overlooked, and apathy to change when the stakes are so high would be unacceptable. Mycology is a neglected specialty [22], often struggling to keep functioning; innovation is hampered by financial anaemia. Recently, PAHO has funded technical cooperation on histoplasmosis at a level of 50 000 dollars, a good start but it represents less than one dollar per estimated histoplasmosis death.

We need simple diagnostic tests, and we need to lobby for affordable liposomal amphotericin B in endemic countries, teach clinicians to adapt diagnostic and treatment algorithms and get histoplasmosis on board of the HIV/AIDS strategic plans. Surely, major funders have the power to make things right, by funding and inciting national AIDS programmes to correct this gap and avoid more unnecessary deaths.

Acknowledgements

Writing committee: Mathieu Nacher[a,b], Antoine Adenis[a,b], Eduardo Arathoon[c], Blanca Samayoa[c], Dalia Lau-Bonilla[c], Beatriz L. Gomez[d], Angela Tobon[d], Diego Caceres[d], Silvia Marques da Silva[c], Maurimelia Mesquita da Costa[e], Rosely Zancope[f], Terezinha Silva Leitão[g], Margarete Do Socorro Mendonca Gomes[h], Ivina Lopes Lima[h], Rosilene Malcher Leite[h], Stephen Vreden[i], Marja Van Eer[j], Sigrid Mac Donald[i], Sandra Hermelin[i], Magalie Demar[b,k], Denis Blanchet[b,k], Felix Djossou[b,l], Vincent Vantilcke[m], Maria Mercedez Panizo[n], Maribel Dolande[n], Christina Canteros[o], Marcus Lacerda[p,q], Pierre Couppié[br], Angela Restrepo[d].

[a]Centre d'Investigation Clinique Antilles Guyane, CIC INSERM 1424, Centre Hospitalier de Cayenne, Cayenne, French Guiana. [b]EA3593 Ecosystèmes Amazoniens et Pathologie Tropicale, Université de Guyane, Cayenne, French Guiana. [c]Clínica Familiar 'Luis Angel García', Asociación de Salud Integral, Guatemala city, Guatemala. [d]School of Medicine and Health Sciences, Universidad Rosario, Bogotá and Corporacion para Investigationnes Biologicas, Medellin, Colombia. [e]Instituto Evandro Chagas – IEC/SVS/MS Seção de Bacteriologia e Micologia – SABMI Laboratório de Micologia, Belem, Brazil. [f]Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, Brazil. [g]Federal University of Ceará, Fortaleza, Brazil. [h]Laboratório Central de Saude Publica do Amapá, Macapa, Brazil. [i]Academic Hospital Paramaribo, Suriname. [j]Diakonessenhuis, Paramaribo, Suriname. [k]Laboratoire Hospitalo-Universitaire de parasitologie mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana. [l]Service des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne, Cayenne, French Guiana. [m]Service de Médecine, Centre Hospitalier de l'Ouest Guyanais,Saint Laurent du Maroni, French Guiana. [n]Departamento de Micología, Instituto Nacional de Higiene Rafael Rangel, Caracas, Venezuela. [o]INEI-ANLIS 'Dr Carlos G. Malbran,' Buenos Aires, Argentina. [p]Instituto Leônidas e Maria Deane (Fiocruz – Amazônia). [q]Fundação de Medicina Tropical Dr Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil. [r]Service de Dermatologie-Vénéréologie, Centre Hospitalier de Cayenne, French Guiana.

Authors' contributions: M.N. wrote the first draft, and all authors amended and endorsed the final version.

In memory: We wish to dedicate this article to the memory of Maurimelia Mesquita da Costa, a wonderful person and a great mycologist.

Funding: This work has benefited from an 'Investissement d'Avenir' grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01).

Conflicts of interest

There are no conflicts of interest.

References

1. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M. **Epidemiology of endemic systemic fungal infections in Latin America.** *Med Mycol* 2011; **49**:785–798.
2. Guimaraes AJ, Nosanchuk JD, Zancope-Oliveira RM. **Diagnosis of histoplasmosis.** *Braz J Microbiol* 2006; **37**:1–13.
3. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, Kauffman CA. **Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America.** *Clin Infect Dis* 2007; **45**:807–825.
4. 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**:239–240.
5. Daher EF, Silva GB, Barros FA, Takeda CF, Mota RM, Ferreira MT, *et al*. **Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil.** *Trop Med Int Health* 2007; **12**:1108–1115.
6. Gutierrez ME, Canton A, Sosa N, Puga E, Talavera L. **Disseminated histoplasmosis in patients with AIDS in Panama: a review of 104 cases.** *Clin Infect Dis* 2005; **40**:1199–1202.

7. Redondo MC. **Disseminated histoplasmosis in Venezuelan AIDS patients.** *Infect Dis Clin Pract* 1995; **4**:300–303.
8. Samoya B, Mercado E, Argueta D, Scheel C, Guzman B, Amado I, et al. Disseminated histoplasmosis (DH) before and after the implementation of urine antigen detection ELISA (UADE) in an HIV clinic in Guatemala. ICCAC, San Francisco, 9 September 2012.
9. Adenis AA, Aznar C, Couppie P. **Histoplasmosis in HIV-infected patients: a review of new developments and remaining gaps.** *Curr Trop Med Rep* 2014; **1**:119–128.
10. Mabey D, Peeling RW, Ustianowski A, Perkins MD. **Diagnostics for the developing world.** *Nat Rev Microbiol* 2004; **2**:231–240.
11. Lewden C, Sobesky M, Cabié A, Couppié P, Boulard F, Bissuel F, *et al*. **Causes of death among HIV infected adults in French Guyana and the French West Indies in the era of highly active antiretroviral therapy (HAART).** *Med Mal Infect* 2004; **34**:286–292.
12. Vantilcke V, Sobesky M, Cabié A, Couppié P, Boulard F, Bissuel F. **Fever in hospitalized HIV-infected patients in Western French Guiana: first think histoplasmosis.** *Int J STD AIDS* 2014; **25**:656–661.
13. Sterling T. Culture negative TB is associated with increased mortality in HIV-infected persons. In CROI. 2015; Seattle, Washington, USA.
14. Nacher M, Adenis A, Sambourg E, Huber F, Abboud P, Epelboin L, *et al*. **Histoplasmosis or tuberculosis in HIV-infected patients in the amazon: what should be treated first?** *PLoS Negl Trop Dis* 2014; **8**:e3290.
15. Adenis A, Nacher M, Hanf M, Basurko C, Dufour J, Huber F, Aznar C. **Tuberculosis and histoplasmosis among human immunodeficiency virus-infected patients: a comparative study.** *Am J Trop Med Hyg* 2014; **90**:216–223.
16. Adenis A, Nacher M, Hanf M, Vantilcke V, Boukhari R, Blanchet D, Demar M. **HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority.** *PLoS Negl Trop Dis* 2014; **8**:e3100.
17. UNAIDS. *Global Statistics. 2015. Fact sheet.* Geneva, Switzerland: UNAIDS.
18. PAHO. Malaria. 2015. http://www.paho.org/hq/index.php?option=com _content&view=article&id=2573:general-information-malaria&Itemid=2060&lang=fr.
19. WHO. Global tuberculosis report 2014. 2014. http://www.who.int/tb/publications/global_report/en/.
20. Nacher M, Adenis A, Blanchet D, Vantilcke V, Demar M, Basurko C, Gaubert-Marechal E. **Risk factors for disseminated histoplasmosis in a cohort of HIV-infected patients in French Guiana.** *PLoS Negl Trop Dis* 2014; **8**:e2638.
21. Hajjeh RA, Pappas PG, Henderson H, Lancaster D, Bamberger DM, Skahan KJ, Phelan MA. **Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons.** *Clin Infect Dis* 2001; **32**:1215–1220.
22. Ajello L. **The medical mycological iceberg.** *HSMHA Health Rep* 1971; **86**:437–448.

Au titre des dernières réalisations utiles à l'argumentation dans le cadre du plaidoyer pour la réduction de la mortalité imputable à l'histoplasmose chez le patient infecté par le VIH et, faisant suite aux premières tentatives de quantification du problème (ARTICLE 13 et 15), nous avons réalisé une estimation comparée de l'incidence et de la mortalité de l'histoplasmose et de la tuberculose chez les patients infectés par le VIH en Amérique Latine (ARTICLE 16).

Pour la première fois, ces estimations étaient basées sur les données disponibles dans la littérature et autorisaient une quantification fine et détaillée pays par pays ainsi qu'à l'échelle de la région Amérique Latine. La prévalence en population générale, l'incidence et la mortalité au cours de l'infection par le VIH, étaient ainsi quantifiées et cartographiées à l'échelle des pays, autorisant chacun à prendre conscience de la magnitude du problème dans son pays.

De plus, pour la première fois également, une comparaison pays par pays avec la tuberculose, bien connue des acteurs de la lutte contre le VIH et principal diagnostic différentiel de l'histoplasmose dans la région, incitera probalement les pays les plus concernés et l'OPS-OMS à décliner rapidement de nouvelles stratégies nationales et internationales.

Pour mémoire, les premiers résultats de cet article ont fait l'objet d'une présentation orale au 65ème congrès annuel de l'ASTMH, 13 au 17 novembre 2016, Atlanta, USA (Annexe 6). En marge de ce congrés, ces résultats ont été présentés lors d'une réunion de travail sur le thème « Advanced HIV disease » réunissant les responsables américains suivants : direction du President's Emergency Plan for AIDS Relief (PEPFAR) et directeurs des branches infection par le VIH, tuberculose et maladies fongiques des Centers for Diseases control and prevention (CDC).

Cet article aurait pu intégrer la première partie de ce manuscrit, sur les travaux scientifiques, mais son insertion à la fin de ce manuscrit semblait plus pertinente. Ce travail est une forme d'aboutissement des reflexions et constats partagés avec nos nombreux partenaires, ouvrant une nouvelle ère pour l'argumentation chiffrée du poids de la coinfection histoplasmose et VIH dans chaque pays et à l'échelle de l'Amérique Latine.

ARTICLE 16 : Burden of disease of HIV-associated histoplasmosis in Latin America: a country by country analysis in comparison with tuberculosis.

Author: A. ADENIS, A. VALDES, C. CROPET, O.Z. Mc COTTER, G. DERADO, P. COUPPIE, T. CHILLER, M. NACHER

Year: 2017 **Journal:** *In submission*

ISSN: **DOI:**

Abstract: Background-Fungal infections remain a major contributor to the opportunistic infections that affect people living with HIV (PLHIV). Among them, histoplasmosis is considered neglected, misdiagnosed as tuberculosis and responsible for numerous deaths in Latin America. The objective of this study was to estimate the burden of HIV-associated histoplasmosis in comparison with tuberculosis in Latin American countries. Methods-Based on historical histoplasmin skin test studies, we estimated histoplasmosis prevalence in the general population, HIV-associated histoplasmosis annual incidence and number of deaths for the year 2012 in Latin American countries. Data on HIV-associated tuberculosis and HIV/AIDS infection were extracted from the World Health Organization reports for the year 2012. We systematically propagated uncertainty throughout all the steps of the estimation process. Ethical approval was not required. Findings-For the year 2012, we estimated 22,637 [18,934-26,225] cases of HIV-associated histoplasmosis, in comparison to the 26,204 [25,150-27,996] reported cases of HIV-associated tuberculosis, in Latin America. Hotspot areas for histoplasmosis prevalence (>30%) and incidence (>1.5p100 PLHIV) were Central America, the northern tip of South America and Argentina. According to a conservative scenario, the estimated annual number of histoplasmosis-related deaths ranged from 1,118 [947-1,311] to 4,473 [3,787-5,245] in comparison to the 5,062 [3,777-6,405] tuberculosis-related deaths reported in Latin America. **I**nterpretation-Our estimates of histoplasmosis incidence and deaths are high and consistent with published data. For the first time, the burden of histoplasmosis is estimated to be equivalent in incidence and even higher in deaths when compared with tuberculosis among PLHIV in Latin America. Funding-None

URL:

Elsevier Editorial System(tm) for The Lancet Infectious Diseases

Manuscript Draft

Manuscript Number:

Title: Burden of disease of HIV-associated histoplasmosis in Latin America: a country by country analysis in comparison with tuberculosis

Article Type: Article (Original Research)

Keywords: HIV; AIDS; Histoplasma capsulatum; Histoplasmosis; Tuberculosis; Prevalence; Incidence; Death; Latin America; Central America; South America

Corresponding Author: Dr. antoine adenis, M.D., MPH, DTM&H

Corresponding Author's Institution: Centre Hospitalier de Cayenne

First Author: ANTOINE ADENIS

Order of Authors: ANTOINE ADENIS; audrey valdes; claire cropet; Orion Z. Mc Cotter; gordana Derado; pierre couppie; tom chiller; mathieu nacher

Manuscript Region of Origin: FRENCH GUIANA

Abstract: Background
Fungal infections remain a major contributor to the opportunistic infections that affect people living with HIV (PLHIV). Among them, histoplasmosis is considered neglected, misdiagnosed as tuberculosis and responsible for numerous deaths in Latin America. The objective of this study was to estimate the burden of HIV-associated histoplasmosis in comparison with tuberculosis in Latin American countries.
Methods
Based on historical histoplasmin skin test studies, we estimated histoplasmosis prevalence in the general population, HIV-associated histoplasmosis annual incidence and number of deaths for the year 2012 in Latin American countries. Data on HIV-associated tuberculosis and HIV/AIDS infection were extracted from the World Health Organization reports for the year 2012. We systematically propagated uncertainty throughout all the steps of the estimation process. Ethical approval was not required.
Findings
For the year 2012, we estimated 22,637 [18,934-26,225] cases of HIV-associated histoplasmosis, in comparison to the 26,204 [25,150-27,996] reported cases of HIV-associated tuberculosis, in Latin America. Hotspot areas for histoplasmosis prevalence (>30%) and incidence (>1.5p100 PLHIV) were Central America, the northern tip of South America and Argentina. According to a conservative scenario, the estimated annual number of histoplasmosis-related deaths ranged from 1,118 [947-1,311] to 4,473 [3,787-5,245] in comparison to the 5,062 [3,777-6,405] tuberculosis-related deaths reported in Latin America.
Interpretation
Our estimates of histoplasmosis incidence and deaths are high and consistent with published data. For the first time, the burden of

histoplasmosis is estimated to be equivalent in incidence and even higher in deaths when compared with tuberculosis among PLHIV in Latin America.

Funding
None

Title page

Burden of disease of HIV-associated histoplasmosis in Latin America: a country by country analysis in comparison with tuberculosis.

Antoine A. ADENIS [1,2], Audrey VALDES [1], Claire CROPET [1], Orion Z. Mc COTTER [3], Gordana DERADO [4], Pierre COUPPIE [2,5], Tom CHILLER [3], Mathieu NACHER [1,2],

[1] Centre d'Investigation Clinique Antilles Guyane, Inserm CIC 1424, Centre Hospitalier de Cayenne, Cayenne.

[2] Equipe EA3593, Ecosystèmes Amazoniens et Pathologie Tropicale, Université des Antilles et de la Guyane, Cayenne.

3 Mycotic Diseases Branch, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia.

[4] Biostatistics and Information Management Office, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia.

[5] Service de Dermatologie Vénérologie, Centre Hospitalier de Cayenne, Cayenne.

Running title: Burden of HIV-associated histoplasmosis in Latin America

Total number of words: 4 574

Corresponding author: Antoine Adenis, M.D., MPH, DTM&H

Address: Centre d'Investigation Clinique Antilles-Guyane, Inserm CIC 1424, Centre Hospitalier de Cayenne, avenue des flamboyants, BP 6006, 97 300 Cayenne, France.

Phone: +594 594 395008

Fax: +594 594 394872

E-mail: antoine.adenis@gmail.com

Research in context

Evidence before this study

Progress in care and treatment of HIV infection, notably antiretroviral therapy, had a great impact on the reduction of AIDS incidence and AIDS-related deaths in high income countries. In low and middle-income countries the incidence of AIDS and AIDS-related deaths remain challenging with fungal opportunistic infections being significant and often unsuspected contributors. Histoplasmosis is suspected to be the main AIDS-defining condition and AIDS-related cause of death in Latin America. Misdiagnosed as tuberculosis, histoplasmosis is not in WHO or UNAIDS strategic plans, perpetuating the lack of funds to tackle the issue. Data published on histoplasmosis are mainly histoplasmin skin test studies, case reports or series with a few seroprevalence studies conducted in different populations and, are represented as points or areas of endemicity on maps. For people living with HIV, histoplasmosis data are mainly case reports or series, with a few recent burden studies at the country scale. Despite this knowledge, HIV-associated histoplasmosis is considered a neglected, unsuspected, undiagnosed and untreated major killer across Latin America.

Added value of this study

To the best of our knowledge, this is the first study to estimate with a clear and accurate method (following the GATHER statement) the burden of HIV-associated histoplasmosis at the country scale and at the Latin American region scale. Similarly, for the first time, estimates at the country level have been represented on maps instead of the historical representation with points and areas at risk. For the first time HIV-associated histoplasmosis estimates per country have been compared with HIV-associated tuberculosis country data reported by WHO for the year 2012. The study findings showed that incidence and deaths of histoplasmosis and tuberculosis in people living with HIV were comparable with numerous overlapping hotspots for the two diseases in Latin America. Histoplasmosis, which is

underdiagnosed in most countries, is suspected to be among the top AIDS-defining conditions and AIDS-related cause of deaths in Latin America.

Implications of all the available evidence

Estimates on the histoplasmosis burden in comparison with tuberculosis are now available for the first time to policy makers. The country level estimates are strong incentives for countries to revise strategic plans, to upgrade fungal diagnosis and treatment, to directly measure the burden of histoplasmosis in their country and to reduce histoplasmosis incidence and mortality. These data show that HIV-associated histoplasmosis is a major problem for the Latin American region and that international organizations should address it more forcefully. Finally, this is a problem for people living with HIV, but other immunocompromising conditions (transplant recipients, hematological malignancies, patients treated with tumor necrosis factor (TNF) antagonists or corticosteroids, etc.) may also be greatly impacted by histoplasmosis in Latin America.

Number of words: 441

Abstract

Background

Fungal infections remain a major contributor to the opportunistic infections that affect people living with HIV (PLHIV). Among them, histoplasmosis is considered neglected, misdiagnosed as tuberculosis and responsible for numerous deaths in Latin America. The objective of this study was to estimate the burden of HIV-associated histoplasmosis in comparison with tuberculosis in Latin American countries.

Methods

Based on historical histoplasmin skin test studies, we estimated histoplasmosis prevalence in the general population, HIV-associated histoplasmosis annual incidence and number of deaths for the year 2012 in Latin American countries. Data on HIV-associated tuberculosis and HIV/AIDS infection were extracted from the World Health Organization reports for the year 2012. We systematically propagated uncertainty throughout all the steps of the estimation process. Ethical approval was not required.

Findings

For the year 2012, we estimated 22,637 [18,934-26,225] cases of HIV-associated histoplasmosis, in comparison to the 26,204 [25,150-27,996] reported cases of HIV-associated tuberculosis, in Latin America. Hotspot areas for histoplasmosis prevalence (>30%) and incidence (>1.5p100 PLHIV) were Central America, the northern tip of South America and Argentina. According to a conservative scenario, the estimated annual number of histoplasmosis-related deaths ranged from 1,118 [947-1,311] to 4,473 [3,787-5,245] in comparison to the 5,062 [3,777-6,405] tuberculosis-related deaths reported in Latin America.

Interpretation

Our estimates of histoplasmosis incidence and deaths are high and consistent with published data. For the first time, the burden of histoplasmosis is estimated to be equivalent in incidence and even higher in deaths when compared with tuberculosis among PLHIV in Latin America.

Funding

None

Abstract total number of words: 249

Keywords: HIV; AIDS; Histoplasma capsulatum; Histoplasmosis; Tuberculosis; Prevalence; Incidence; Death; Latin America; Central America; South America

Text

Introduction

Fungal infections remain a major contributor to the opportunistic infections that affect people living with HIV (PLHIV), notably in low and middle income countries (1). Histoplasma capsulatum is a fungus endemic in Latin America (2). It causes histoplasmosis which can be asymptomatic or self-limited, but also invasive and life-threatening in those that are immunocompromised (3).

Prevalence in the general population, based on histoplasmin skin test studies detecting previous exposure to Histoplasma capsulatum, varies from 0.8% to 89% depending on the territory (2, 4, 5). In PLHIV, almost all undiagnosed and/or untreated histoplasmosis cases will lead to death (3).

With the spread of HIV, disseminated histoplasmosis became an increasing threat with significant fatality rates ranging from 10% to 53% among culture-positive cases (6-8). Despite sharp decline in fatality rates observed in a small number of reference centers, fatality rates remain high in almost all centers where the disease is known (6, 7). Moreover, fatality is suspected to be much higher in countries with a high HIV prevalence and where histoplasmosis is endemic, especially when histoplasmosis is unreported or rarely described in PLHIV. Hence, histoplasmosis-related deaths may be greatly underestimated (9). Lack of awareness, lack of diagnostic tools and facilities, lack of mycological expertise are explanations for this (3). Similarly, since histoplasmosis is often mistaken for tuberculosis and falsely labeled as 'drug resistant tuberculosis', part of the unknown burden of histoplasmosis-related deaths in PLHIV probably accounts for numerous cases among the HIV-associated tuberculosis incidence and mortality statistics (9).

According to regional data compiled by the World Health Organization (WHO), tuberculosis is also endemic in Latin America, with a yearly incidence rate around 28 cases per100,000 HIV-infected individuals in Central America and 41 to 63 cases per100,000 HIV-infected individuals in South America in 2013. Additionally, an estimated 6,100 (4,600–8,000) tuberculosis deaths among HIV-infected people occurred across the Americas in 2013 (including the Caribbean, North, Central and South America) (10).

In Latin America, the estimated number of PLHIV is approximately 1.5 million (11). Histoplasmosis has been identified as a neglected disease with a yearly number of deaths recently suspected as equivalent or superior to tuberculosis in PLHIV (9, 12). In contrast with tuberculosis, there are no histoplasmosis statistics in Latin American countries out of academic publications reporting case series and a few recent burden studies at the country level. Intuitively, regions with high histoplasmin positivity rates and high HIV prevalence should have more deaths due to histoplasmosis. However, this is very a crude way to estimate the burden of histoplasmosis in PLHIV. Improved knowledge of the disease burden is thus essential to plan future public health programs.

In order to improve awareness and to promote future interventions, we aimed to estimate HIV-associated histoplasmosis incident cases and deaths in each Latin American country and at the Latin American region scale. A secondary objective was to compare histoplasmosis estimates to tuberculosis cases reported in PLHIV.

Methods

Ethics Statement

Ethical approval was not required in this study since it was conducted with aggregated level and anonymous published data and estimations based on open access secondary sources.

Literature review:

We searched for histoplasmin prevalence in Latin America using MEDLINE, Scielo, Lilacs and Google Scholar. Latin America was defined as all South and Central American countries with the exception of the Caribbean region according to the United Nations geoscheme definitions. The search terms were « histoplasmin» or « histoplasmosis prevalence » associated with « South America », « Central America », « Latin America » or any country name among the following: Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, Paraguay, El Salvador, Suriname, Uruguay and Venezuela. Relevant data were: name of the country and region (part of Central or South America), absolute number of persons tested and number of persons with a positive histoplasmin skin test, dilution level of histoplasmin antigen used for skin testing (>1/10), type of population tested (only general populations considered to ensure the estimates' representativeness). Hence, if these data were available we considered that the study inclusion criteria to compute the estimates were met. Still, when histoplasmin skin test studies available in one country were only performed on a specific population subgroup we kept them to compute country's estimates.

All types of published articles were considered, in any languages, and without time limitations since we made the assumption that, in endemic areas, the prevalence of this telluric fungal pathogen remained stable over time.

Among 1,310 records identified on June 1st, 2015, 174 records were considered relevant for the study based on abstracts. Only 77 records were considered relevant based on the availability of the above information required to calculate histoplasmosis prevalence. At the end of the selection process, 24 articles were included for the study (31 records were excluded because they did not meet the inclusion criteria and another 22 records were excluded because

information was already available elsewhere in atlases or reviews).The bibliography of selected references is available in technical appendix 1.

Histoplasmosis data and calculation of estimates:

Histoplasmosis prevalence in the general population

We selected histoplasmin positive rates in skin testing studies performed in the general population with a specified antigen dilution rate over 1/10 (range of dilution kept between 1/100 and 1/1,000) to avoid false positives due to cross-reactivity.

For Guatemala, Guyana and Suriname, since no other studies were available, prevalence was estimated using the data from the studies performed on hospitalized patients of varying ages. For Chile, since the environmental conditions vary a lot, and since histoplasmin skin test studies were mainly performed in children, the mean of all available studies was used. Costa Rica, Nicaragua and El Salvador had no general population estimations available, so histoplasmin skin test prevalence was approximated with the mean of the bordering countries' estimates, assuming that since environmental conditions are similar, the geographical distribution of histoplasmosis prevalence would be similar as well. Estimates of the low and high bounds for the latter countries corresponded to the lowest and highest bounds of the bordering countries' estimates.

As several histoplasmin skin test studies have been performed in most Latin American countries, for a given country, the mean prevalence was calculated using the total number of positive patients identified divided by the total number of persons tested. The mean prevalence at the Latin American scale was estimated in the same way. A 95% confidence interval (95%CI) was calculated using the relation $\hat{p} \pm 1.96\sqrt{\hat{p}(1-\hat{p})/n}$ where $\hat{p}$ is the

estimated prevalence, $\hat{p}(1-\hat{p})/n$ is the corresponding estimated variance and n is the total number of persons tested in all histoplasmin skin test studies performed in one country of interest and at the regional scale.

As detailed hereafter, we accounted for uncertainty around each estimate. We first estimated the mean prevalence, and the corresponding 95%CI. From this distribution, we drew a random sample built of 100,000 iterations of the prevalence values using Monte Carlo simulations. Then, we estimated, for each country, as well as the whole Latin American region, an expected prevalence together with a 95% confidence interval based on the median and the 2.5th and 97.5th percentile of the Monte Carlo distribution generated, respectively. Furthermore, these values allowed us to proceed with further computations to estimate histoplasmosis incidence and deaths.

Histoplasmosis incidence in PLHIV

General assumptions

Because incidence data is scarce, we used the following assumptions to estimate histoplasmosis incidence.

First, an estimated disease duration was calculated using the prevalence population relation $P_p = \frac{I_d E_i(D)}{1 + I_d E_i(D)}$ where P_p denotes the prevalence, I_d incidence density, and $E_i(D)$ expected value of duration of histoplasmosis obtained from an incidence (i) case series. It was assumed that the duration of histoplasmosis was constant and that the population was in a steady-state (13). Secondly, for incidence densities (I_d) smaller than 0.10, cumulative incidence (I_c) is a good approximation of the incidence density. As an example, for I_d=0.1, using the relation $I_c = 1 - e^{-Id}$, I_c=0.095 (13), which for practical purposes could be rounded to 0.1, this allowed us to proceed with further computations to estimate the burden of histoplasmosis.

Only two histoplasmosis incidence calculations are based on prospective cohorts of PLHIV in the literature. In the French Guiana PLHIV cohort, the duration of histoplasmosis was estimated at 0.321 years considering an annual histoplasmosis incidence density of 1.5 p100 HIV-infected person-years and a histoplasmosis prevalence in the general population of 32.5% (14, 15). In a North American PLHIV cohort, the duration of histoplasmosis was similar at 0.395 years considering an annual histoplasmosis incidence of 2.64 p100 HIV-infected patients and a histoplasmosis prevalence of 51.1% among non-anergic PLHIV (16). In order to perform calculations in the general population subgroup represented by PLHIV, we made the assumption that histoplasmosis prevalence in PLHIV was similar to estimates obtained from histoplasmin skin test prevalence studies performed in the general population.

Incidence rate estimates

We estimated the annual incidence rate of histoplasmosis per 100 HIV-infected patients for each Latin American country separately, and for the whole Latin American region, using the relation $I_d = \frac{P_p}{E_i(D)\times(1-P_p)}$ and previously estimated histoplasmosis prevalence for each of the countries and the whole Latin American region. Disease duration was set to 0.321 years. Hence, for each country and the Latin American region we reported an estimated incidence rate, a lower and upper bound of a 95%CI corresponding to the median, 2.5th and 97.5th percentile of the computed distribution of incidence values, respectively.

Incident number of cases estimates (total number and number of symptomatic cases only)

To obtain the estimated annual number of incident HIV-associated histoplasmosis cases (symptomatic and asymptomatic) along with the 95%CI, for each country and the Latin American region, we computed in the same way each value of the HIV-associated

histoplasmosis incidence rate estimates with the number of PLHIV (all ages) estimated by the Joint United Nations Programme on HIV/AIDS (UNAIDS) for the year 2012 (11).

Then, to estimate the annual number of symptomatic incident HIV-associated histoplasmosis cases, in each country and the Latin American region, we applied three scenarios to each value of the estimated annual number of incident HIV-associated histoplasmosis cases (symptomatic and asymptomatic): N[30], N[50], N[70] scenarios corresponding to 30%, 50%, 70% of the estimated annual number of incident HIV-associated histoplasmosis cases with a CD4 count below 200/mm3, respectively. Indeed, accuracy in histoplasmosis incident cases estimates among PLHIV required to take into account that histoplasmosis is mainly an asymptomatic and spontaneously self-limited infection in "immunocompetent individuals" and, is classically reported to be primarily symptomatic and fatal without appropriate antifungal therapy in PLHIV with a CD4 count below 200/mm3 (3). As no estimates were available for the number of PLHIV with a CD4 count below 200/mm3 in Latin America, we therefore approximated the annual number of incident HIV-associated histoplasmosis symptomatic cases. Several studies based on cohort data have reported the large proportion of patients with a CD4 count below 200/mm3 at the time of antiretroviral therapy initiation ranging from 30% (observed in European settings like in French Guiana (17)) to 70% in countries across Latin America (18-22). Patients included in these studies were late diagnosed for HIV (i.e. CD4 count<200/mm3 or with a history of an AIDS-defining illness), due to late testing or late presentation and subsequently late antiretroviral therapy initiated.

For each scenario, in each country and at the regional scale, we reported an estimated number of symptomatic incident HIV-associated histoplasmosis cases, a lower and upper bound of a 95%CI, corresponding to the median, 2.5^{th} and 97.5^{th} percentile of the computed incidence scenarios, respectively.

Histoplasmosis mortality in PLHIV

To obtain the annual number of histoplasmosis-related deaths we computed each value of the three scenario estimates of the annual number of symptomatic incident HIV-associated histoplasmosis cases using three fatality scenarios, for each country separately and the whole Latin American region: F[10], F[20], F[40] scenarios corresponding to 10%, 20%, 40% of histoplasmosis-related deaths in each incidence scenarios, respectively. Three scenarios were chosen because fatality rates may vary according to country conditions such as access to antiretroviral therapy (ART) and effective antifungal therapy, availability of fungal diagnostic methods and the level of awareness of physicians (23, 24). For each fatality scenario (F[10]N[30], F[10]N[50], F[10]N[70], F[20]N[30], F[20]N[50], F[20]N[70] and F[40]N[30], F[40]N[50], F[40]N[70]) in each country and Latin America we estimated a number of deaths, a lower and upper bound of a 95%CI, corresponding to the median, 2.5^{th} and 97.5^{th} percentile of the computed values of the number of deaths, respectively.

Tuberculosis data and calculation of estimates:

The number of incident tuberculosis cases and deaths in PLHIV, for each country and the Latin American region, were extracted from the WHO notifications and outcomes tables for the year 2012 (available online at http://apps.who.int/gho/data/view.main.TBHIVWHOREG). Incidence rate calculations of incident tuberculosis cases per 100 HIV-infected patients were performed with the WHO case notification data as the numerator and the UNAIDS estimates for the number of people living with HIV (all ages) as the denominator. In order to account for uncertainty of both the numerator and the denominator, we modeled the two parameters in the same way as histoplasmosis prevalence. Hence, for each country and the Latin American region, we reported an estimated tuberculosis incidence, a lower and upper bound of a 95%CI,

corresponding to the median, 2.5th and 97.5th percentile of the computed incidence values, respectively.

As no estimate was available for French Guiana, we used the published estimate of the number of tuberculosis-related deaths among PLHIV (25). The number of incident tuberculosis cases was estimated from the published incidence density of 0.77 p100 HIV-infected patients-year (95%CI: 0.45-1.09) according to estimates of the number of PLHIV (14). The two latter parameters were modeled in the same way as histoplasmosis incidence density in order to express an expected tuberculosis incidence number along with a 95%CI.

HIV data and calculation of estimates:

The 2012 estimates of the number of people living with HIV (all ages) by country and for Latin America, were extracted from the 2013 UNAIDS Global report (11). For Brazil, the estimated number of PLHIV was calculated as the mean of the low and high UNAIDS estimates in Brazil. For French Guiana, as the number of people living with HIV (all ages) was not available, we assumed a scenario based on a heterosexual HIV transmission and approximate prevalence with the published prevalence of 1.2% in pregnant women for the year 2012 (25). As HIV prevalence is quite stable over time, low and high values for HIV prevalence were set at 0.8% and 1.4%, respectively (6). The estimated number of PLHIV (all ages) in French Guiana, low and high estimates, were calculated according to the last census reporting 241,922 inhabitants in 2013 (26).

Hence, for each country and for the Latin American region, an estimated number of PLHIV (all ages) was available together with a low and high estimate.

Uncertainty analysis

In order to enhance the robustness of estimates, we systematically accounted for uncertainty at each step of the estimation process.

Since it was briefly explained previously, in the following paragraph we first describe the estimation process for histoplasmosis prevalence, incidences and deaths, then for tuberculosis incidence; and then we also describe how we accounted for uncertainty around UNAIDS HIV data. Finally, the modeling strategy is described in more detail.

For histoplasmosis estimates, the prevalence mean, low and high bounds of the 95%CI were used to generate 100,000 values of the prevalence in each country and the whole Latin American region. To propagate uncertainty across the estimation process, these values were set as starting data for the subsequent estimations of incidences (rate and numbers) and number of deaths.

For tuberculosis estimates, the number of incident cases, low and high WHO estimates were used to generate 100,000 values of the incident cases in each country and the entire Latin American region. These values were set as starting data for the subsequent incidence rate estimations.

To account for uncertainty of UNAIDS HIV data, we generated 100,000 values of the number of PLHIV in each country and the whole Latin American region. These values were combined with those generated throughout the estimation process of histoplasmosis and tuberculosis incidences.

Tuberculosis deaths numbers were not computed and only presented as reported by WHO for each country and the Latin American region.

We used the beta - PERT distribution as our basic descriptive distribution since it allowed us to specify a minimum, maximum, and modal value, as well a fourth parameter that controls the spread (variance) of the distribution (27). This family of distributions is widely used and is an attractive choice for problems in which many estimates and sources of uncertainty need to

be combined, due to intuitive nature of its parameters (27). The following four parameters were specified: the most likely value (value of the quantity of interest computed), the lowest and highest estimates (using the low and high value of the quantity of interest computed or the low and high bounds of the calculated 95%CI when available) and the shape or scale value (default values set at 4). The outputs of our models were summarized using posterior distributions calculated by Monte Carlo simulation, with 100,000 replicates. Final results were expressed with the median, the 2.5^{th} and 97.5^{th} percentile being respectively the lowest and highest estimates, then expressed as 95%CI.

Monte Carlo simulation and uncertainty analysis were performed using SAS software V9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA).

Cartography:

The mapping software used was MapInfo Pro v11.0. Base maps were downloaded from http://www.arcgis.com.

Results

Histoplasmosis prevalence estimates in the general population (Table 1 and Figure 1)

The overall estimated prevalence for Latin America was 32.2%, but ranged between a minimum of 0.1% in Chile to a maximum of 57.2% in Guatemala. Prevalence was over 20% in most countries except Chile, Paraguay, Peru and Uruguay. Areas with the highest prevalence (>30%) were the Guiana Shield (French Guiana, Guyana, Suriname), Venezuela, Argentina and Central America (except Mexico). These areas represented 57% (12/21) of the Latin American countries.

Histoplasmosis and tuberculosis incidence rate estimates in PLHIV for the year 2012 (Table 1 and Figure 2)

The estimated incidence rates of histoplasmosis cases (symptomatic and asymptomatic) ranged between a minimum of 0.003 p100 PLHIV in Chile to a maximum of 4.16 p100 PLHIV in Guatemala. Areas with the highest incidence (≥1.5 p100 PLHIV) were the Guiana Shield, Central America (except Mexico), Argentina and Venezuela. At the regional scale, the incidence rate was estimated at 1.48 (%95CI [1.47-1.49]) p100 PLHIV in Latin America.

The estimated incidence rates of tuberculosis cases ranged between minimum of 0.15 p100 PLHIV in Chile to a maximum of 2.84 p100 PLHIV in Guyana. Countries with the highest incidence (≥1.5 p100 PLHIV) were located within the Andean region (Ecuador, Peru, Bolivia), the Guiana Shield (Guyana and Suriname), Central America (Honduras, Mexico and Panama), Brazil and Paraguay. At the regional scale, the incidence rate was estimated at 1.74 (%95CI [1.48-2.06]) p100 PLHIV in Latin America.

Countries where estimated incidence rates of HIV-associated histoplasmosis and HIV-associated tuberculosis cases are similar were: Colombia, Guyana, Honduras and Panama.

Histoplasmosis (estimates) and tuberculosis (reported by WHO) annual number of cases in PLHIV for the year 2012 (Table 1 and Figure 3)

In Latin America, the estimated total number of HIV-associated histoplasmosis cases (symptomatic and asymptomatic) represented 22,637 [18,934-26,225] cases. According to three scenarios where 30%, 50% or 70% of these PLHIV having a CD4 count below 200/mm3 when they experienced histoplasmosis, the estimated total number of symptomatic histoplasmosis cases ranged between 6,710 cases, 11,183 cases and 15,657 cases, respectively. In comparison, the total number of symptomatic HIV-associated tuberculosis cases reported by WHO, in the same year, was 26,204 [25,150-27,996].

The estimated total number of HIV-associated histoplasmosis cases (symptomatic and asymptomatic) corresponded to 46% of the combined number of histoplasmosis and tuberculosis cases in PLHIV. Similarly, in Figure 3, the N[30], N[50], N[70] scenarios of symptomatic histoplasmosis cases represented 20%, 30% and 37% of the total combined number of symptomatic histoplasmosis and tuberculosis cases in PLHIV across Latin America, respectively.

Areas where the estimated number of HIV-associated histoplasmosis cases (symptomatic and asymptomatic) was higher or nearly equivalent to the reported number of HIV-associated tuberculosis cases were: Central America (excluding Mexico), the Guiana Shield, Colombia, Venezuela and Argentina. This represented 62% (13/21) of the Latin American countries. Similarly, in Figure 3, considering the N[30], N[50], N[70] scenarios for the estimated number of symptomatic HIV-associated histoplasmosis cases compared with the reported number of HIV-associated tuberculosis cases, we found 29% (6/21), 43% (9/21) and 62% (13/21) of the Latin American countries with a higher or nearly equivalent number of histoplasmosis cases.

Histoplasmosis (estimates) and tuberculosis (reported by WHO) annual number of deaths in PLHIV for the year 2012 (Table 2 and Figure 3)

In table 2, estimations of the annual number of histoplasmosis-related deaths in PLHIV were expressed as a proportion (10%, 20% and 40% fatality rate) of the estimated number of symptomatic HIV-associated histoplasmosis cases. Considering the median scenario (N[50]) for the number of symptomatic histoplasmosis cases, estimates for the total number of histoplasmosis-related deaths among Latin American PLHIV were 1,118 [947-1,311], 2,237 [1,893-2,622] and 4,473 [3,787-5,245], according to the 10%, 20% and 40% fatality scenarios

respectively. For the same year, 5,062 [3,777-6,405] tuberculosis-related deaths were reported among PLHIV in Latin America. Considering the worst combined scenario of 70% (N[70]) symptomatic histoplasmosis cases and a 40% fatality rate, the total number of histoplasmosis-related deaths in PLHIV across Latin America was estimated at 6,263 [5,302-7,343]. In the worst scenario, countries with more than 400 deaths per year resulting from symptomatic HIV-associated histoplasmosis cases were in descending order: Brazil, Venezuela, Guatemala, Argentina, Mexico, and Colombia. For the same year, countries with more than 400 deaths reported among HIV-associated tuberculosis cases were in descending order: Brazil, Peru, Mexico and Guatemala.

In figure 3, considering the median scenario (N[50]) for the number of symptomatic histoplasmosis cases, the three fatality rate scenarios (10%, 20% and 40%) of the estimated number of histoplasmosis-related deaths are graphically compared to the number of tuberculosis-related deaths observed in PLHIV. According to the 40% lethality scenario (F[40]N[50]) histoplasmosis and tuberculosis represented 9,535 deaths in 2012, with 4,473 (47%) and 5,062 (53%) deaths respectively. Areas with a higher or nearly equivalent number of deaths from histoplasmosis compared to tuberculosis deaths were Central America, the Guiana Shield, Colombia, Venezuela and Argentina. Those represented 67% (14/21) of Latin American countries. Regardless of the fatality rate scenarios, five countries had a greater or similar number of deaths from histoplasmosis relative to tuberculosis: Argentina, Belize, Costa Rica, El Salvador and French Guiana.

Discussion:

Histoplasmosis prevalence estimations in Latin America are high with more than one third of the general population having been exposed to H. capsulatum. With the exception of Chile, where the disease is scarcely reported (28), histoplasmosis is widespread and should be

considered as a very common disease by public health authorities. Specific guidance and requirements regarding histoplasmosis care and treatment in the general population should be made available, notably for people with constitutive or acquired immuncompromising conditions and at risk for severe histoplasmosis (29).

Among Latin American PLHIV, the estimated histoplasmosis incidence is high, 1.48 p100 PLHIV, which amounts to over 22,000 cases per year. Countries with the highest histoplasmosis incidence ($\geq$ 1.5 p100 PLHIV) follow the same geographic distribution as histoplasmosis prevalence hotspots in the general population: Central America, Argentina and the Northern tip of South America. Nevertheless, despite lower incidence densities, countries with large populations (Brazil, Colombia and Mexico) had a large number of estimated incident cases of histoplasmosis, (>1,500 cases/year).

Incidence estimates computed in this study are concordant with incidence rates reported in retrospective PLHIV cohorts or in recent burden studies at the country level (6, 14, 30-34).

At the Latin American region scale, the estimated HIV-associated histoplasmosis incidence was similar to tuberculosis incidence, which was estimated at 1.74 p100 PLHIV. Hence, the total number of incident histoplasmosis cases was estimated to be of similar magnitude, around 25,000 cases per year, than the total number of HIV-associated tuberculosis cases reported in Latin America for the year 2012. At the country scale, the number of histoplasmosis cases was similar or higher than tuberculosis cases in most of Latin American countries (62%). Areas where the incidence magnitudes for both diseases overlap were Chile for low burdens of disease and Central America (Honduras and Panama) and the Northern tip of South America (Colombia and Guyana) for high burdens of disease.

With a number of symptomatic histoplasmosis cases in PLHIV ranging from 6,710 to 15,657 each year in Latin America, histoplasmosis is likely among the main AIDS-defining condition.

In hotspots described in this study, it might be the first AIDS-defining condition as shown in French Guiana (14) or suspected in Guatemala and Argentina (35, 36). The median scenario (N[50]) of symptomatic infections is realistic and probably even conservative. Indeed, apart from French Guiana where 30% of PLHIV are diagnosed with advanced disease, this proportion remains much higher (50-70%) across Latin America (20).

The order of magnitude of HIV-associated histoplasmosis deaths across Latin America was recently crudely estimated as similar to HIV-associated tuberculosis deaths, 9,600 versus 6,000 deaths for the year 2013, respectively (9).

With our country by country approach based on historical data and a median scenario (F[40]N[50]), we hereby confirmed that estimates of HIV-associated histoplasmosis deaths (4,473 [3,787-5,245]) and reported HIV-associated tuberculosis deaths (5,062 [3,777-6,405]) were comparable. Hence, together histoplasmosis and tuberculosis would have accounted for a quarter of the 52,000 AIDS-related deaths estimated by UNAIDS in Latin America for the year 2012 (11), with histoplasmosis ranking among the top AIDS-related cause of deaths (36). This fatality scenario of histoplasmosis-related deaths is conservative because the disease is widely undiagnosed and untreated across Latin America (9, 12). The true burden of deaths might be closer to the worst scenario with 6,263 deaths per year. This represents at least 62% (14/21) of Latin American countries with more histoplasmosis-related than tuberculosis-related deaths.

The burden of histoplasmosis-related deaths is a problem that can be improved with increased awareness, early diagnosis and appropriate therapy which has been shown to lead to a marked reduction of mortality (6). Enzyme Immunoassay urinary antigen tests and health care providers training have resulted in an increase in the diagnosis of histoplasmosis (23) and a

reduction of mortality (24). New rapid and simple diagnostic tools in development and improvements in antifungals available on the WHO essential medicines list could speed up the goal of reducing histoplasmosis mortality and AIDS-related deaths by 2020 (37-39).

The present study has limitations. The estimated mean of histoplasmosis prevalence in a country is a crude approximation of in country situation, since prevalence may vary widely within one country, which may have led to under- or over-estimations of incidence and deaths. Moreover, the different scenarios may not always accurately reflect the reality in all countries. However, in the absence of relevant and reliable data in most countries, the appropriate computations were performed using realistic estimations from the available literature.

Conclusion:

Despite ART scale-up, histoplasmosis remains an important cause of morbidity and mortality. These estimations emphasize the urgency of consideration of histoplasmosis in Latin America, where far too many PLHIV die from a treatable infection, in the absence of empiric antifungal therapy and rapid diagnostic tools (39, 40). To our knowledge, this is the first study aiming at quantifying and mapping the burden of this neglected public health problem. This study is aimed to help Latin American countries and international institutions prioritize future interventions and research. Prospective studies are now needed to directly measure the true burden of histoplasmosis among PLHIV in endemic countries.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Author's contribution:

A.Adenis: literature search, study design, data collection, data analysis, data interpretation, cartography, writing, figures and tables.

A. Valdes: literature search, data collection, data analysis, cartography, writing, figures and tables.

C. Cropet: data analysis, data interpretation, figures and tables.

O. Mc Cotter: study design, data interpretation, writing.

G. Derado: study design, data analysis, data interpretation.

P. Couppié: study design, data interpretation, writing

T. Chiller: study design, data interpretation, writing.

M. Nacher: study design, data interpretation, writing.

Sources of funding:

None

Conflicts of Interest Statements:

AA gave one talk for Janssen Pharmaceutical Companies of Johnson & Johnson (09/30/2016) and received diagnostic tests for research from the CDC Mycotic Diseases Branch and from IMMY (ImmunoMycologics, Inc.) between 2013 and 2015.

Acknowledgements:

AA acknowledge an Investissement d'Avenir grant of the Agence Nationale de la Recherche (CEBA: ANR-10-LABX-25-01). AA is supported by the Agence Nationale de Recherche sur le SIDA et les hépatites virales - Agence autonome de l'Institut National de la Santé et de la

Recherche Médicale (ANRS-Inserm n°12 260) and by the European Union (PO FEDER 2007-2013, N° Présage : 31 362, Guyane française).

Authors acknowledge L. Valmy and V. Dufit who participated in the study on the statistics and cartography, respectively.

Keywords:

HIV; AIDS; Histoplasma capsulatum; Histoplasmosis; Tuberculosis; Prevalence; Incidence; Death; Latin America; Central America; South America

Figures legends:

Figure 1: Histoplasmosis prevalence estimates (%) in the general population of Latin American countries

Prevalence was estimated using histoplasmin skin test studies available in the literature and performed in the general population with a histoplasmin dilution level (>1/10) to avoid false positives due to cross-reactivity. For a given country calculations used the total number of positive patients identified and divided by the total number of persons tested.

Weighted average of the bordering countries was used to estimate the prevalence for Costa Rica, El Salvador and Nicaragua since no histoplasmin skin test studies were found.

Literature review only found histoplasmin skin test studies performed in specific population not corresponding to the general population for Guatemala, Guyana and Suriname.

Figure 2: Annual incidence estimates of histoplasmosis (a) and tuberculosis (b) cases per 100 HIV-infected patients, in Latin America, for the year 2012

HIV: Human Immunodeficiency Virus

Histoplasmosis incidence rate was estimated in People Living with HIV (PLHIV) from histoplasmosis prevalence estimates in the general population using the relation $I_d = \frac{P_p}{E_i(D)\times(1-P_p)}$ with I_d: incidence, P_p: prevalence, $E_i(D)$: disease duration set at 0,321 years. All data were estimated for the year 2012.

Tuberculosis incidence rate was estimated in PLHIV with the available online WHO case notification data as the numerator (N) and UNAIDS estimates of PLHIV (all ages) as the denominator. All data were estimated for the year 2012.

Figure 3: Comparison of the number of incident cases (a.) and the number of deaths (b.) estimated for symptomatic histoplasmosis and reported for tuberculosis in people living with HIV, in Latin America, for the year 2012.

a. The x axis represented the ratio (%) of the estimated number of cases associated with symptomatic histoplasmosis cases among the number of reported tuberculosis cases combined with the estimated number of symptomatic histoplasmosis cases according to three scenarios of 30% (N[30]), 50% (N[50]) and 70% (N[70])

of the estimated annual number of histoplamsosis cases (asymptomatic and symptomatic) occurring in people living with HIV and having a CD4 count below 200/mm3.

b. The x axis represented the ratio (%) of the estimated number of deaths associated with symptomatic histoplasmosis cases among the number of AIDS-related tuberculosis deaths combined with the estimated number of histoplasmosis-related deaths in people living with HIV. Estimates of histoplasmosis number of deaths were computed in a scenario N[50] of symptomatic histoplasmosis cases based on a median estimate of 50% of the annual number of HIV-associated histoplasmosis cases (symptomatic and asymptomatic) having a CD4 count below 200/mm3. Three deaths estimates in this subgroup are presented: 10%, 20%, 40% fatality rates corresponding to F[10]N[50], F[20]N[50],and F[40]N[50].

HIV-associated tuberculosis cases (a.) and deaths (b.) were extracted for all countries from the WHO notification and outcome tables of the year 2012. For French Guiana, these data were given by the Public Health Surveillance authority.

Tables legends:

Table 1: Estimated histoplasmosis prevalence in the general population, annual incidence of tuberculosis and histoplasmosis (all cases and only symptomatic cases) in people living with HIV, in Latin America for the year 2012.

95%CI: 95% Monte Carlo confidence interval

* Histoplasmosis prevalence is reported as a proportion (%) of the general population (including PLHIV) and was estimated using histoplasmin skin test studies available in the literature and performed in the general population with a histoplasmin dilution level >1/10. For a given country calculations used the total number of positive patients identified and divided by the total number of persons tested.

† PLHIV: People Living with HIV (all ages) estimated by UNAIDS for the year 2012 and available in the UNAIDS Global report for the year 2013. Estimate for Brazil was the mean of the low and high estimates available in the UNAIDS global report. Estimate for French Guiana was obtained from the published HIV prevalence (1.2% [95%CI: 0.8-1.4]) in a population of 241,922 according to the last census.

‡ Histoplasmosis incidence rate was estimated in PLHIV from the prevalence estimates in the general population using the relation $I_d = \frac{P_p}{E_i(D)\times(1-P_p)}$, with I_d: incidence, P_p: prevalence, $E_i(D)$: disease duration of 0,321 years. The incident number of histoplasmosis cases in PLHIV was estimated from the incidence rate combined with the number of PLHIV extracted from UNAIDS estimates. The incident number of symptomatic only histoplasmosis cases in PLHIV was estimated from the number of incident histoplasmosis cases in PLHIV with three scenarios low N[30], median N[50], high N[70] corresponding to 30%, 50%, 70% of incident histoplasmosis cases in PLHIV with a CD4 count below 200/mm3, respectively. All data were estimated for the year 2012.

§ Tuberculosis incidence rate was estimated in PLHIV with the available online WHO case notification data as the numerator and UNAIDS estimates of PLHIV (all ages) as the denominator. For French Guiana, the number of HIV-associated tuberculosis cases was estimated from the published incidence rate combined with the number of PLHIV. All data were estimated for the year 2012.

Table 2: Annual number of deaths estimated for symptomatic histoplasmosis cases and reported for tuberculosis cases in people living with HIV, in Latin America, for the year 2012.

PLHIV: People living with HIV; 95%CI: 95% Monte Carlo confidence interval; NA: Not available

* Histoplasmosis-related deaths were estimated as a proportion of the estimated annual number of symptomatic HIV-associated histoplasmosis cases for the year 2012. Three fatality scenarios (F[10], F[20], F[40] corresponding to 10%, 20%, 40% lethality, respectively) were combined with three scenarios of the estimated number of symptomatic HIV-associated histoplasmosis cases (N[30], N[50], N[70] corresponding to 30%, 50%, 70% of the estimated annual number of HIV-associated histoplasmosis cases, symptomatic and asymptomatic, having a CD4 count below 200/mm3). All data were estimated for the year 2012.

† Tuberculosis-related deaths were extracted from the World Health Organization outcomes tables of the year 2012, available online at http://apps.who.int/gho/data/view.main.TBHIVWHOREG. For French Guiana, information was given by the Public Health Surveillance authorities.

Figure 1

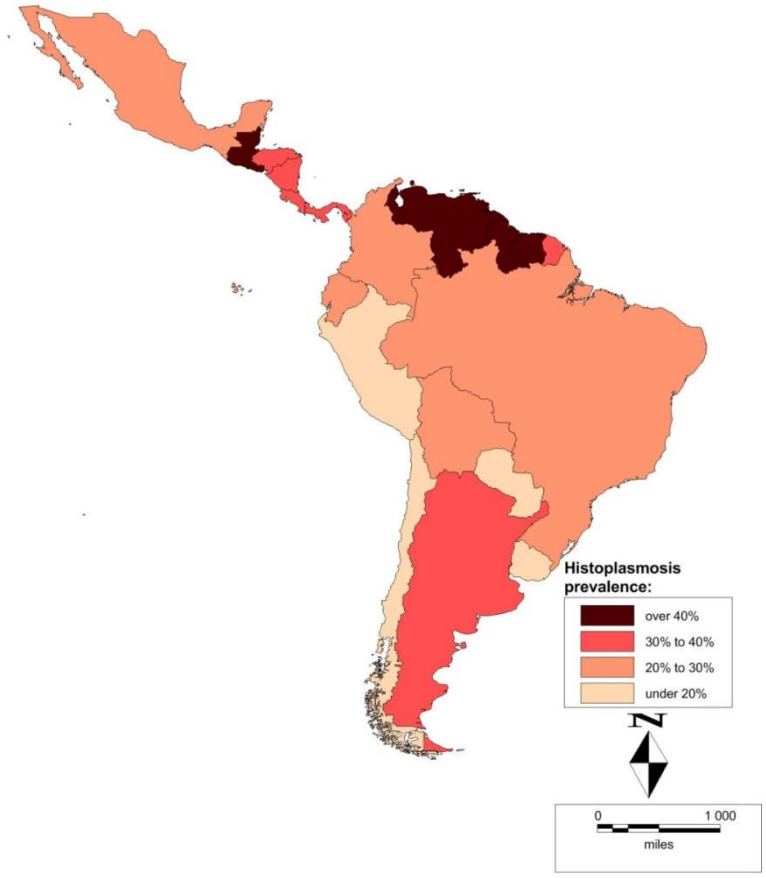

Histoplasmosis prevalence:
over 40%
30% to 40%
20% to 30%
under 20%
N
0
1 000
miles

Figure 2

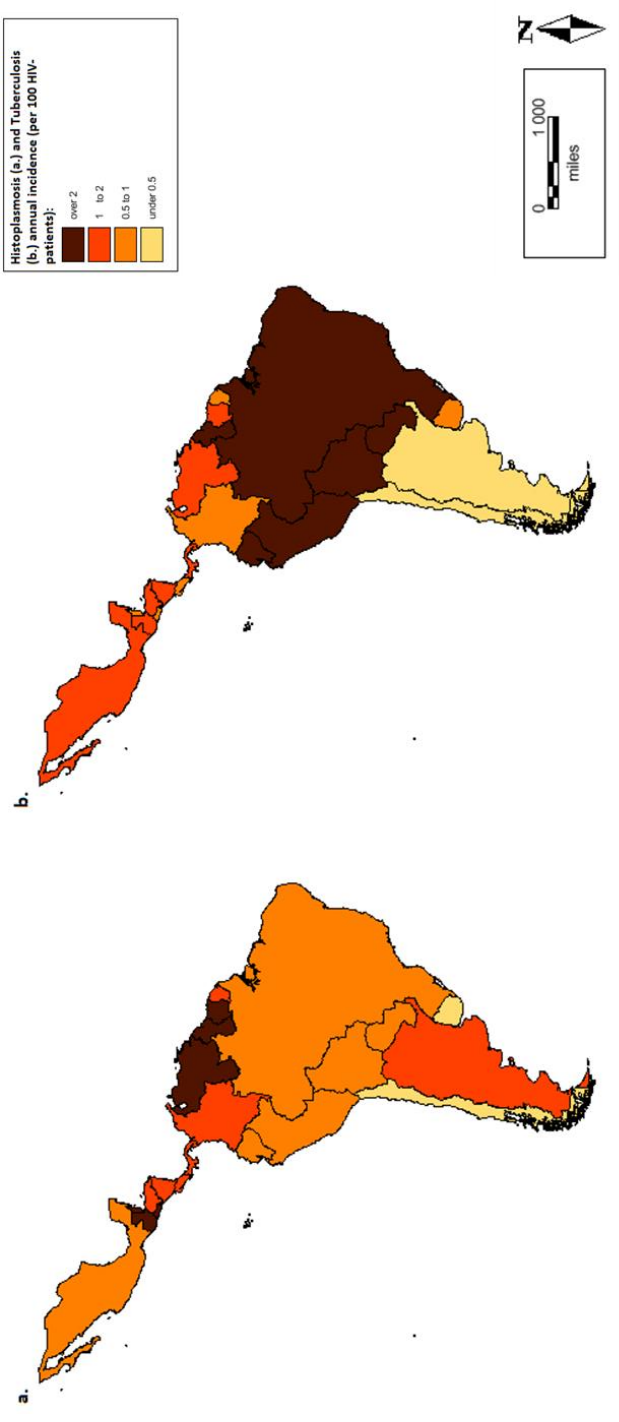
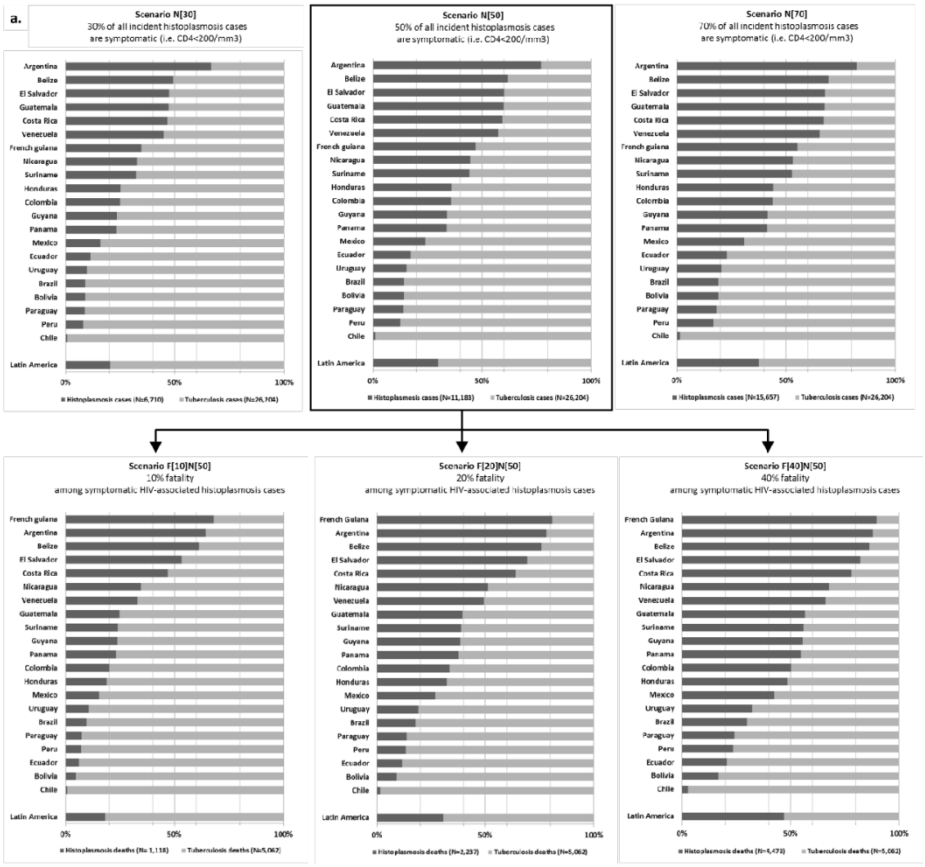

Histoplasmosis (a.) and Tuberculosis (b.) annual incidence (per 100 HIV-patients):
over 2
1 to 2
0.5 to 1
under 0.5
N
0
1,000
miles
b.
a.

Figure 3


a.
Scenario N[30]
30% of all incident histoplasmosis cases are symptomatic (i.e. CD4<200/mm3)
Scenario N[50]
50% of all incident histoplasmosis cases are symptomatic (i.e. CD4<200/mm3)
Scenario N[70]
70% of all incident histoplasmosis cases are symptomatic (i.e. CD4<200/mm3)
Scenario F[10]N[50]
10% fatality
among symptomatic HIV-associated histoplasmosis cases
Scenario F[20]N[50]
20% fatality
among symptomatic HIV-associated histoplasmosis cases
Scenario F[40]N[50]
40% fatality
among symptomatic HIV-associated histoplasmosis cases
Argentina
Belize
El Salvador
Guatemala
Costa Rica
Venezuela
French guiana
Nicaragua
Suriname
Honduras
Colombia
Guyana
Panama
Mexico
Ecuador
Uruguay
Brazil
Bolivia
Paraguay
Peru
Chile
Latin America
0%
50%
100%

1

Table 1

	Histoplasmosis prevalence*	People living with HIV (PLHIV)†	Histoplasmosis annual incidence in PLHIV‡		Symptomatic histoplasmosis annual incidence in PLHIV‡			Tuberculosis annual incidence in PLHIV§	
	% [95%CI]	N [Low-High]	p100 PLHIV [95%CI]	N [95%CI]	N[30] [95%CI]	N[50] [95%CI]	N[70] [95%CI]	N [Low-High]	p100 PLHIV [95%CI]
Argentina	37.8 [35.3-40.3]	98,000 [80,000-120,000]	1.89 [1.70-2.10]	1,864 [1,546-2,232]	559 [464-670]	932 [773-1,116]	1,305 [1,082-1,562]	280 [270-630]	0.33 [0.26-0.49]
Belize	49.4 [41.7-57.1]	3,100 [2,800-3,400]	3.04 [2.22-4.15]	94 [68-130]	28 [21-39]	47 [34-65]	66 [48-91]	29 [26-31]	0.93 [0.84-1.02]
Bolivia	22.0 [20.9-23.1]	16,000 [8,500-24,000]	0.88 [0.82-0.94]	141 [93-191]	42 [28-57]	70 [47-96]	99 [65-134]	420 [390-560]	2.73 [1.96-4.21]
Brazil	20.3 [19.7-20.8]	595,000 [530,000-660,000]	0.79 [0.77-0.82]	4,714 [4,321-5,120]	1,414 [1,296-1,536]	2,357 [2,161-2,560]	3,300 [3,025-3,584]	14,000 [13,990-,14,010]	2.35 [2.18-2.55]
Chile	0.1 [0.03-0.17]	39,000 [25,000-61,000]	0.003 [0.001-0.005]	1.22 [0.35-2.36]	0.37 [0.10-0.71]	0.61 [0.17-1.18]	0.85 [0.24-1.65]	51 [48-120]	0.15 [0.10-0.25]
Colombia	25.1 [24.4-25.7]	150,000 [110,000-190,000]	1.04 [1.01-1.08]	1,565 [1,264-1,867]	469 [379-560]	782 [632-933]	1,095 [885-1,307]	1,400 [1,200-1,700]	0.94 [0.76-1.21]
Costa Rica	36.7 [34.5-38.5]	9,800 [8,800-11,000]	1.81 [1.64-1.95]	177 [156-199]	53 [47-60]	89 [78-100]	124 [109-140]	61 [55-68]	0.62 [0.56-0.70]
Ecuador	22.6 [20.0-25.2]	52,000 [36,000-99,000]	0.91 [0.78-1.05]	508 [346-762]	153 [104-228]	254 [173-381]	356 [242-533]	1,200 [1,100-1,400]	2.17 [1.48-3.12]
El Salvador	44.5 [35.9- 55.0]	25,000 [16,000-45,000]	2.50 [1.75-3.80]	660 [383-1,166]	198 [115-350]	330 [191-583]	462 [268-816]	220 [210-220]	0.83 [0.58-1.21]
French Guiana	32.5 [29.3-35.7]	2,900 [1,935-3,390]	1.50 [1.29-1.73]	42 [32-53]	13 [10-16]	21 [16-26]	30 [23-37]	22 [14-30]	0.77 [0.45-1.09]
Guatemala	57.2 [54.8-59.6]	58,000 [36,000-130,000]	4.16 [3.78-4.60]	2,676 [1,665-4,322]	803 [500-1,297]	1,338 [833-2,161]	1,873 [1,166-3,025]	900 [810-1,000]	1.40 [0.87-2.24]
Guyana	47.0 [37.1-56.9]	7,200 [4,300-12,000]	2.76 [1.84-4.12]	204 [116-353]	61 [35-106]	102 [58-177]	143 [81-247]	200 [200-270]	2.84 [2.01-4.25]

32

Honduras	38.8 [38.5-39.0]	26,000 [21,000-33,000]	1.97 [1.95-1.99]	518 [441-607]	155 [132-182]	259 [220-304]	363 [308-425]	460 [360-560]	1.75 [1.38-2.19]
Mexico	22.8 [22.1-23.6]	170,000 [150,000-210,000]	0.92 [0.88-0.96]	1,589 [1,411-1,818]	477 [423-545]	794 [706-909]	1,112 [988-1,273]	2,500 [2,500-2,600]	1.46 [1.28-1.63]
Nicaragua	36.7 [34.5-38.6]	9,600 [6,600-15,000]	1.81 [1.64-1.95]	179 [130-241]	54 [39-72]	89 [65-120]	125 [91-168]	110 [100-120]	1.11 [0.83-1.51]
Panama	34.9 [33.8-36.0]	17,000 [12,000-22,000]	1.67 [1.59-1.75]	284 [224-346]	85 [67-104]	142 [112-173]	198 [157-242]	280 [250-310]	1.65 [1.34-2.10]
Paraguay	17.5 [16.4-18.6]	13,000 [7,400-24,000]	0.66 [0.61-0.71]	90 [57-134]	27 [17-40]	45 [29-67]	63 [40-94]	280 [260-300]	2.04 [1.38-3.21]
Peru	19.7 [18.4-21.0]	76,000 [36,000-230,000]	0.76 [0.70-0.83]	693 [334-1,302]	208 [100-391]	346 [167-651]	485 [234-912]	2,400 [2,200-2,700]	2.66 [1.42-5.51]
Suriname	43.1 [40.7-45.5]	4,000 [3,600-4,400]	2.36 [2.14-2.60]	94 [83-107]	28 [25-32]	47 [42-53]	66 [58-75]	59 [51-67]	1.47 [1.30-1.67]
Uruguay	10.2 [10.0-10.5]	13,000 [9,800-19,000]	0.35 [0.35-0.36]	47 [37-60]	14 [11-18]	24 [19-30]	33 [26-42]	130 [120-140]	0.97 [0.76-1.24]
Venezuela	48.2 [47.8-48.6]	110,000 [74,000-160,000]	2.90 [2.85-2.95]	3,243 [2,422-4,178]	973 [727-1,253]	1,622 [1,211-2,089]	2,270 [1,695-2,925]	1,200 [1,000-1,200]	1.04 [0.81-1.40]
Total Latin America	32.2 [32.0-32.4]	1,500,000 [1,200,000-1,900,000]	1.48 [1.47-1.49]	22,367 [18,934-26,225]	6,710 [5,680-7,867]	11,183 [9,467-13,112]	15,657 [13,254-18,357]	26,204 [25,150-27,996]	1.74 [1.48-2.06]

1

2

3

4

5

33

Table 2

	Histoplasmosis-related deaths annual incidence in people living with HIV (PLHIV)*									Tuberculosis-related deaths annual incidence in PLHIV†
	10% fatality			20% fatality			40% fatality			
	N[30] [95%CI]	N[50] [95%CI]	N[70] [95%CI]	N[30] [95%CI]	N[50] [95%CI]	N[70] [95%CI]	N[30] [95%CI]	N[50] [95%CI]	N[70] [95%CI]	N [Low-High]
Argentina	56 [46-67]	93 [77-112]	130 [108-156]	112 [93-134]	186 [155-223]	261 [216-312]	224 [186-268]	373 [309-446]	522 [433-625]	52 [16-110]
Belize	3 [2-4]	5 [3-6]	7 [5-9]	6 [4-8]	9 [7-13]	13 [10-18]	11 [8-16]	19 [14-26]	26 [19-36]	3 [2-4]
Bolivia	4 [3-6]	7 [5-10]	10 [7-13]	8 [6-11]	14 [9-19]	20 [13-27]	17 [11-23]	28 [19-38]	39 [26-54]	140 [100-170]
Brazil	141 [130-154]	236 [216-256]	330 [302-358]	283 [259-307]	471 [432-512]	660 [605-717]	566 [519-614]	943 [864-1,024]	1,320 [1,210-1,434]	2,200 [1,600-2,800]
Chile	0.04 [0.01-0.07]	0.06 [0.02-0.12]	0.09 [0.02-0.17]	0.07 [0.02-0.14]	0.12 [0.03-0.24]	0.17 [0.05-0.33]	0.15 [0.04-0.28]	0.24 [0.07-0.47]	0.34 [0.10-0.66]	8 [4-14]
Colombia	47 [38-56]	78 [63-93]	110 [89-131]	94 [76-112]	156 [126-187]	219 [177-261]	188 [152-224]	313 [253-373]	438 [354-523]	310 [230-400]
Costa Rica	5 [5-6]	9 [8-10]	12 [11-14]	11 [9-12]	18 [16-20]	25 [22-28]	21 [19-24]	35 [31-40]	50 [44-56]	10 [8-13]
Ecuador	15 [10-23]	25 [17-38]	36 [24-53]	31 [21-46]	51 [35-76]	71 [48-107]	61 [41-91]	102 [69-152]	142 [97-213]	390 [320-460]
El Salvador	20 [11-35]	33 [19-58]	46 [27-82]	40 [23-70]	66 [38-117]	92 [54-163]	79 [46-140]	132 [77-233]	185 [107-327]	29 [20-39]
French Guiana	1 [1-2]	2 [2-3]	3 [2-4]	3 [2-3]	4 [3-5]	6 [5-7]	5 [4-6]	8 [6-11]	12 [9-15]	1 [NA-NA]
Guatemala	80 [50-130]	134 [83-216]	187 [117-303]	161 [100-259]	268 [167-432]	375 [233-605]	321 [200-519]	535 [333-864]	749 [466-1,210]	410 [340-480]
Guyana	6 [3-11]	10 [6-18]	14 [8-25]	12 [7-21]	20 [12-35]	29 [16-49]	25 [14-42]	41 [23-71]	57 [32-99]	33 [21-48]
Honduras	16 [13-18]	26 [22-30]	36 [31-43]	31 [26-36]	52 [44-61]	73 [62-85]	62 [53-73]	104 [88-121]	145 [123-170]	110 [76-150]
Mexico	48 [42-55]	79 [71-91]	111 [99-127]	95 [85-109]	159 [141-182]	222 [198-255]	191 [169-218]	318 [282-364]	445 [395-509]	430 [330-540]
Nicaragua	5 [4-7]	9 [7-12]	13 [9-17]	11 [8-14]	18 [13-24]	25 [18-34]	21 [16-29]	36 [26-48]	50 [36-67]	17 [13-22]
Panama	9 [7-10]	14 [11-17]	20 [16-24]	17 [13-21]	28 [22-35]	40 [31-48]	34 [27-41]	57 [45-69]	79 [63-97]	47 [35-61]
Paraguay	3 [2-4]	5 [3-7]	6 [4-9]	5 [3-8]	9 [6-13]	13 [8-19]	11 [7-16]	18 [11-27]	25 [16-38]	57 [46-69]
Peru	21 [10-39]	35 [17-65]	48 [23-91]	42 [20-78]	69 [33-130]	97 [47-182]	83 [40-156]	139 [67-260]	194 [94-365]	450 [340-570]
Suriname	3 [3-3]	5 [4-5]	7 [6-7]	6 [5-6]	9 [8-11]	13 [12-15]	11 [10-13]	19 [17-21]	26 [23-30]	15 [11-19]
Uruguay	1 [1-2]	2 [2-3]	3 [3-4]	3 [2-4]	5 [4-6]	7 [5-8]	6 [5-7]	9 [7-12]	13 [10-17]	20 [15-26]
Venezuela	97 [73-125]	162 [121-209]	227 [170-292]	195 [145-251]	324 [242-418]	454 [339-585]	389 [291-501]	649 [484-836]	908 [678-1,170]	330 [250-410]
Total Latin America	671 [568-787]	1,118 [947-1,311]	1,566 [1,325-1,836]	1,342 [1,136-1,573]	2,237 [1,893-2,622]	3,131 [2,651-3,671]	2,684 [2,272-3,147]	4,473 [3,787-5,245]	6,263 [5,302-7,343]	5,062 [3,777-6,405]

References

1. Limper AH, Adenis A, Le T, Harrison TS. Fungal infections in HIV/AIDS. The Lancet Infectious Diseases. 2017.
2. Colombo AL, Tobon A, Restrepo A, Queiroz-Telles F, Nucci M. Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol. 2011;49(8):785-98.
3. Adenis AA, Aznar C, Couppie P. Histoplasmosis in HIV-Infected Patients: A Review of New Developments and Remaining Gaps. Current tropical medicine reports. 2014;1:119-28.
4. Comstock GW, Vicens CN, Goodman NL, Collins S. Differences in the distribution of sensitivity to histoplasmin and isolations of Histoplasma capsulatum. Am J Epidemiol. 1968;88(2):195-209.
5. Principe A, Convit J, Pifano F. [Results of the epidemiological investigations on histoplasmosis, coccidioidomycosis and tuberculosis carried out in various regions of Venezuela]. Mycopathologia. 1961;15:11-52.
6. Adenis A, Nacher M, Hanf M, Vantilcke V, Boukhari R, Blachet D, et al. HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority. PLoS Negl Trop Dis. 2014;8(8):e3100.
7. Peigne V, Dromer F, Elie C, Lidove O, Lortholary O. Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. Am J Trop Med Hyg. 2011;85(5):934-41.
8. Homei A, Worboys M. Fungal Disease in Britain and the United States 1850-2000 Mycoses and Modernity. 2013/11/15 ed. Centre for the History of Science TaM, University of Manchester, England (http://chstm.manchester.ac.uk), editor. Manchester, England: Palgrave Macmillan; 2013. 225 p.
9. Nacher M, Adenis A, Arathoon E, Samayoa B, Lau-Bonilla D, Gomez BL, et al. Disseminated histoplasmosis in Central and South America, the invisible elephant: the lethal blind spot of international health organizations The neglected histoplasmosis in Latin America Group. AIDS. 2016;30(2):167-70.
10. Del Granado M, Lopez R, Volz A, Marx F, Mujica O, Munaico C, et al. Tuberculosis in the Americas - Regional Report 2014 - Epidemiology, Control, and Financing. PAHO/WHO, 2015.
11. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2013. Geneva: UNAIDS, 2013 Contract No.: UNAIDS / JC2502/1/E.
12. Nacher M, Adenis A, Mc Donald S, Do Socorro Mendonca Gomes M, Singh S, Lopes Lima I, et al. Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage. PLoS Negl Trop Dis. 2013;7(11):e2319.
13. Freeman J, Hutchison GB. Prevalence, incidence and duration. Am J Epidemiol. 1980;112(5):707-23.
14. 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(2):239-40.
15. Floch H. Revue critique des investigations et de la littérature mycologiques pour les annés 1946–1956 en Guyane Française. Mycopathologia et mycologia applicata. 1957;8(3):194-205.

16. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, Brewer J, et al. Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. Clin Infect Dis. 1997;24(6):1195-203.
17. 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. 2013;90(2):193-4.
18. Crabtree-Ramírez B, Caro-Vega Y, Shepherd BE, Wehbe F, Cesar C, Cortés C, et al. Cross-Sectional Analysis of Late HAART Initiation in Latin America and the Caribbean: Late Testers and Late Presenters. PLoS ONE. 2011;6(5):e20272.
19. Bonjour MA, Montagne M, Zambrano M, Molina G, Lippuner C, Wadskier FG, et al. Determinants of late disease-stage presentation at diagnosis of HIV infection in Venezuela: A case-case comparison. AIDS Research and Therapy. 2008;5(1):1-12.
20. Piñeirúa A, Sierra-Madero J, Cahn P, Guevara Palmero RN, Martínez Buitrago E, Young B, et al. The HIV care continuum in Latin America: challenges and opportunities. The Lancet Infectious Diseases. 2015;15(7):833-9.
21. Vidal JE, Penalva de Oliveira AC, Dauar RF, Boulware DR. Strategies to reduce mortality and morbidity due to AIDS-related cryptococcal meningitis in Latin America. The Brazilian Journal of Infectious Diseases. 2013;17(3):353-62.
22. García JI, Samayoa B, Sabidó M, Prieto LA, Nikiforov M, Pinzón R, et al. The MANGUA Project: A Population-Based HIV Cohort in Guatemala. AIDS Research and Treatment. 2015;2015:372816.
23. Caceres DH, Zuluaga A, Arango-Bustamante K, de Bedout C, Tobon AM, Restrepo A, et al. Implementation of a Training Course Increased the Diagnosis of Histoplasmosis in Colombia. Am J Trop Med Hyg. 2015;93(3):662-7.
24. Samayoa B, Mercado D, Argueta E, editors. Disseminated histoplasmosis (DH) before and after the implementation of urine antigen detection ELISA (UADE) in an HIV clinic in Guatemala. Interscience Conf on Antimicrobial Agents and Chemotherapy; 2012.
25. Nacher M. Rapport d'activités 2012 COREVIH Guyane. Cayenne: COREVIH, 2013.
26. Journal-Officiel-de-la-République-Française. Décret n° 2014-1611 du 24 décembre 2014 authentifiant les chiffres des populations de métropole, des départements d'outre-mer de la Guadeloupe, de la Guyane, de la Martinique et de La Réunion, de Saint-Barthélemy, de Saint-Martin et de Saint-Pierre-et-Miquelon. Paris2014.
27. Vose D. Risk Analysis: A Quantitative Guide, 2nd edition. Chichester (UK): John Wiley & Sons; 2000.
28. Cruz R, Opazo H, Barthel E, Campos S, Piontelli E. Disseminated allocthonous histoplasmosis in a acquired immunodeficience syndrome patient. Boletín Micológico. 2006;21:77-84.
29. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. Infect Dis Clin North Am. 2016;30(1):207-27.
30. Bava AJ. Histoplasmosis in the Muniz Hospital of Buenos Aires. Rev Inst Med Trop Sao Paulo. 1995;37(6):5.
31. Unis G, Oliveira Fde M, Severo LC. [Disseminated histoplasmosis in Rio Grande do Sul]. Rev Soc Bras Med Trop. 2004;37(6):463-8.
32. Velásquez Uribe G, Rueda ZV, Vélez LA, Aguirre DC, Gómez-Arias RD. Histoplasmosis in AIDS patients: A cohort study in Medellín, Colombia. Infectio. 2010;14:s99-s106.
33. Corzo‐León D, Armstrong‐James D, Denning D. Burden of serious fungal infections in Mexico. Mycoses. 2015;58(S5):34-44.

34. Medina N, Samayoa B, Lau-Bonilla D, Denning DW, Herrera R, Mercado D, et al. Burden of serious fungal infections in Guatemala. European Journal of Clinical Microbiology & Infectious Diseases. 2017:1-5.
35. López Daneri AG, Arechavala A, Iovannitti CA, Mujica MT. Histoplasmosis diseminada en pacientes HIV/Sida. Buenos Aires, 2009-2014. Medicina (BAires). 2016;76(6):6.
36. Debroy P, Medina N, Herrera R, Mercado D, Perez JC, Samayoa B, et al., editors. Opportunistic infections as a mayor cause of mortality during the first year of HIV diagnosis in a national reference clinic in Guatemala - PUB007. 20th International AIDS Conference; 2014 July 20-25, 2014; Melbourne, Australia.
37. Mathieu N, Antoine A. Proceedings of First Histoplasmosis in the Americas and the Caribbean Meeting, Paramaribo, Suriname, December 4–6, 2015. Emerging Infectious Disease journal. 2016;22(9).
38. Denning DW. Minimizing fungal disease deaths will allow the UNAIDS target of reducing annual AIDS deaths below 500 000 by 2020 to be realized. Philosophical Transactions of the Royal Society B: Biological Sciences. 2016;371(1709):20150468.
39. Samayoa B, Roy M, Cleveland AA, Medina N, Lau-Bonilla D, Scheel CM, et al. High Mortality and Coinfection in a Prospective Cohort of Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome Patients with Histoplasmosis in Guatemala. The American Journal of Tropical Medicine and Hygiene. 2017:-.
40. Nacher M, Adenis A, Sambourg E, Huber F, Abboud P, Epelboin L, et al. Histoplasmosis or Tuberculosis in HIV-Infected Patients in the Amazon: What Should Be Treated First? PLoS neglected tropical diseases. 2014;8(12):e3290.

CONCLUSION

Les infections fongiques invasives représentent un problème de santé publique méconnu, négligé et pourvoyeur d'un grand nombre de décès évitables. Parmi elles, l'histoplasmose chez les patients infectés par le VIH représente un problème de santé publique méconnu, négligé et pourvoyeur d'un grand nombre de décès évitables sur le plateau des Guyanes et en Amérique Latine. Ce travail de thèse décrit l'importance de la problématique de l'histoplasmose chez les patients infectés par le VIH en Guyane française : problème bien connu des professionnels de santé locaux depuis plus de 20 ans. Les tendances récentes montrent une incidence stable et une mortalité très largement abaissée. Cette expérience démontre que la mortalité liée à l'histoplasmose n'est pas une fatalité au cours de l'infection par le VIH. De la même manière, des arguments supplémentaires ont été apportés pour aider à l'analyse du contexte (franche saisonnalité et nouvelle infection prédominante), au diagnostic clinique (diagnostic différentiel entre histoplasmose et tuberculose), au diagnostic paraclinique (test de détection d'antigène dans le sérum) et à la prise en charge médicale (définition de la sévérité et pertinence d'une stratégie empirique antifongique).

Si la qualité de la prise en charge de l'infection par le VIH en Guyane française joue un rôle important dans ces évolutions, le développement d'une expertise mycologique et ses compétences diagnostiques, combinées à un haut niveau de compétence médicale pour le diagnostic et la prise en charge thérapeutique des cas d'histoplasmose, permettent d'atteindre l'objectif de réduction de la mortalité. Il paraît trivial de rapporter ce constat mais l'expertise en mycologie médicale ne semble pas être une priorité de santé publique dans de nombreux pays de la région.

Si la mortalité baisse, le nombre de cas d'histoplasmose observés chez les patients infectés par le VIH en Guyane française est stable, dans un contexte où le taux de dépistage tardif des patients infectés par le VIH ($CD4<200/mm^3$) est stable autour de 30% depuis plus de dix ans. Si l'épidémie d'infection par le VIH venait à marquer le pas à l'avenir, les autres causes d'immunodépression pourraient prendre le relais et maintenir à un niveau élevé l'incidence globale de l'histoplasmose comme le rapporte l'expérience américaine. Ainsi, les efforts pour l'amélioration des connaissances sur l'histoplasmose doivent être poursuivis et amplifiés.

Au titre des pistes de développements en Guyane française, la mise à disposition de méthodes diagnostiques rapides et performantes autorisant un diagnostic simple et précoce de l'histoplasmose, constitue une priorité pour espérer réduire encore un peu plus la mortalité au stade SIDA. Comme nous l'avons décrit, des collaborations sont en cours pour comparer différentes méthodes diagnostiques rapides avec l'espoir de pouvoir proposer une solution intéressante aux

cliniciens d'ici peu. Au plan thérapeutique, les stratégies empiriques antifongiques ou la prévention primaire antifongique sont régulièrement discutées, sans jamais faire l'objet d'un consensus local. Des études complémentaires doivent être menées sur ces thématiques. Pour éviter d'exposer les patients à des SRIS, la tuberculose et la cryptococcose (si CD4<100/mm^3) sont systématiquement recherchées avant la mise en route des trithérapies antirétrovirales. De la même manière, lors de la première consultation de tout patient infecté par le VIH, résidant ou ayant résidé en zone d'endémie connue, l'histoplasmose devrait être systématiquement recherchée, dans des conditions à définir (CD4<150/mm^3). Le problème réside là encore dans l'absence d'outil de diagnostic adapté au contexte. Toutefois, certaines équipes travaillent au développement de méthodes intéressantes de type Interferon-Gamma Release Assays (IGRAs) pour l'histoplasmose (96), méthodes très utilisées et recommandées dans le diagnostic des tuberculoses latentes (97).

Il reste encore beaucoup à faire en Guyane française pour améliorer et maintenir une prise en charge de qualité de l'histoplasmose chez les patients infectés par le VIH. Cet intérêt pour la problématique et l'expertise développée localement étaient les moteurs d'une dynamique de réflexions à l'origine des travaux scientifiques et projets de coopération réalisés dans le cadre de ce travail de thèse.

Si les résultats doivent encore être affinés, nous avons tout de même fait le constat d'une prévalence importante de l'histoplasmose chez les patients infectés par le VIH hospitalisés au Suriname. Dans un pays où l'histoplasmose était pratiquement inconnue au début de nos travaux, une stratégie nationale et des recommandations de bonnes pratiques ont été développées pour la prise en charge de l'histoplasmose chez les patients infectés par le VIH.

Au Guyana, la situation économique est très difficile et ne facilite pas la pérennité d'activités non financées par les organisations internationales, ce qui est le cas de l'histoplasmose. Si la preuve du concept a été faite avec des méthodes simples, la mise en exergue de la problématique de l'histoplasmose devrait peut-être s'appuyer sur le programme national tuberculose et son réseau de « clinics ». Structure pérenne et bien organisée, elle associe une collecte de données systématique en sus de la prise en charge médicale des patients. Un grand nombre de patients au stade SIDA décèdent sous antituberculeux au Guyana. L'idée serait de mettre en regard l'histoplasmose par rapport à la tuberculose, comme nous le faisons souvent dans nos publications, afin de générer une prise de conscience des acteurs. Dans ce pays où l'endémie tuberculeuse est une des plus importantes des Amériques, l'histoplasmose est probablement pourvoyeuse d'un grand nombre de cas de tuberculose non prouvée (examen direct négatif et culture non réalisable au Guyana) ou « résistante » (recherche de résistances peu ou non réalisée).

L'hypothèse des cliniciens guyanais formulée en 2006 s'est vue confirmée et précisée dans le cadre d'un consensus d'acteurs et d'experts issus de la majorité des pays d'Amérique Latine. L'histoplasmose chez les patients infectés par le VIH en Amérique Latine est probablement une des principales infections opportunistes au stade SIDA. Méconnue et négligée, elle est probablement une des principales causes de décès au stade SIDA dans de nombreux pays. Pour la première fois, nous avons estimé qu'en dépit d'une incidence annuelle inférieure, la mortalité liée à l'histoplasmose était équivalente voire supérieure en comparaison à la tuberculose. Cette tendance est lourde en termes de vies humaines perdues et de négligences de la part des pays concernés. La méconnaissance de l'histoplasmose ne semble pas être nécessairement indexée sur le niveau de revenus des pays d'Amérique Latine. En effet, le constat fait au Guyana, avec ces cas de tuberculose qui n'en sont pas, pourvoyeurs d'une importante mortalité au stade SIDA, est également fait par des institutions reconnues dans les grands pays d'Amérique du Sud, en zone d'endémie pour l'histoplasmose. Ces institutions rapportaient que les patients infectés par le VIH avec une tuberculose, dont la culture était négative, avaient plus de risque de décéder que ceux dont la culture était positive (98). Sans proposer de diagnostic différentiel, les auteurs concluaient qu'il devait y avoir une autre maladie que la tuberculose responsable des décès.

De plus, malgré des progrès considérables dans la prise en charge du VIH et l'utilisation à grande échelle des trithérapies antirétrovirales dans la majorité des pays d'Amérique Latine, la proportion de personnes dépistées tardivement pour l'infection par le VIH est toujours trop importante (50% à 70% des patients avec CD4<200/mm3). Ce nombre important de patients au stade avancé de l'infection par le VIH, à risque de développer une histoplasmose symptomatique, et la méconnaissance de l'histoplasmose en font un problème majeur de santé publique à l'échelle de l'Amérique Latine.

Ce constat motive la poursuite des activités de plaidoyer pour la prise en compte du problème et l'élargissement du réseau de collaborateurs initié dans le cadre de ce travail de thèse. L'initiative « 80by20 » doit s'accompagner d'une intégration plus forte de la problématique de l'histoplasmose dans les plans stratégiques nationaux et internationaux. La mention à ajouter pourrait être : « Réduire la mortalité imputable à l'histoplasmose chez les patients infectés par le VIH ». Cette initiative devra également se donner les moyens de mettre à disposition des molécules antifongiques efficaces, parfois trop onéreuses comme dans le cas de l'amphotéricine B liposomale recommandée dans le traitement des formes sévères d'histoplasmose. Toute initiative peut être accompagnée d'un mantra pour atteindre son objectif, dans le cas présent nous répétons aux acteurs « *First think Histoplasmosis* ».

CONCLUSION PERSONNELLE

Arrivé au centre hospitalier de Cayenne fin 2005 dans le cadre de mon internat de Médecine Générale, je voulais être médecin urgentiste, plus particulièrement au sein des équipes de SAMU-SMUR. Puis, en mai 2006, je découvre un peu par hasard la prise en charge de l'infection par le VIH en zone tropicale et son cortège d'infections en "-ose" qui, dans mon Limousin natal, n'étaient que peu représentées. C'est à l'occasion de ce semestre de stage, dans le service de Dermatologie-Vénérologie du Pr. P. Couppié, avec le Dr F. Huber comme chef de clinique en salle, que je me découvre un vif intérêt pour la fascinante prise en charge des maladies infectieuses et surtout tropicales.

C'est là, un peu à la manière d'un célèbre personnage de bande dessinée, que je suis tombé dans la problématique (pour ne pas dire la "marmite") passionnante de la coinfection histoplasmose et VIH. Terminée la médecine d'urgence, je décide d'orienter l'ensemble de mes choix professionnels vers la prise en charge de l'infection par le VIH et des maladies tropicales. Après quelques diplômes d'Université et un aller-retour d'un an aux Antilles, je passe à nouveau dans le service du Pr P. Couppié, hors internat, dans le cadre d'une disponibilité, avec le Dr J. Dufour comme chef de clinique en salle. Tout en poursuivant mon apprentissage au lit du malade, je développe progressivement un intérêt pour l'épidémiologie et la santé publique. Je voulais mettre en œuvre des méthodes objectives pour répondre aux questions de routine clinique, récurrentes et potentiellement péjoratives pour la survie des patients. Parmi ces problématiques, la coinfection histoplasmose et VIH était déjà en tête de liste de mes sujets d'intérêt.

Après quelques mois au sein du Centre d'Investigation Clinique Antilles Guyane (CIC AG, Inserm CIC1424), alors dirigé par le Pr B. Carme avec M. Nacher comme médecin délégué, j'ai suivi un mastère spécialisé de santé publique spécialité maladies infectieuses au sein de l'école Pasteur-Cnam de santé publique à Paris. De retour mi-2011 au CIC AG, sur le site du centre hospitalier de Cayenne, je complète des travaux initiés en 2009 et développe de nouveaux projets à l'échelle régionale, pour la plupart décrits dans ce manuscrit. En parallèle, je poursuis mon cursus universitaire avec mon inscription en capacité de Médecine Tropicale puis en doctorat d'Université.

Ainsi, depuis 2009 et mes premiers pas dans l'équipe du CIC AG, j'ai participé activement et humblement au développement de la thématique histoplasmose et VIH. Avec le soutien du Pr P. Couppié, en partenariat avec l'ensemble de mes collègues et de mes responsables localement, nous avons pu poursuivre certains projets déjà engagés et donner un nouvel élan à cette thématique en Guyane française et dans la région.

Avec l'ignorance ou l'inconscience du débutant à propos des difficultés à venir, j'étais plein de motivation et d'énergie pour relever les défis. Développer cette thématique de mycologie médicale était une gageure dans la compétition générale pour l'obtention des financements. Gageure stricto sensu, de par un contexte multilingue sur le plateau des Guyanes et l'absence totale d'intérêt de ces pays pour les infections fongiques invasives. La plupart des acteurs en santé de ces pays ne suspectait absolument pas la présence de l'histoplasmose et encore moins l'ampleur de son impact sur la mortalité des patients au stade SIDA de l'infection par le VIH. De plus, évoluant dans les systèmes publics de santé défaillants des pays voisins (manque de personnel, turnover d'équipes peu expérimentées et salaires bas), nos partenaires professionnels de santé avaient très peu de temps à donner au développement de projets. Déjà débordés par la prise en charge complexe et chaotique de leurs patients, il a fallu convaincre ces personnes et les responsables locaux de la santé publique du bien fondé de notre démarche. Il est vrai qu'il était peu évident au premier abord de juger de la pertinence de nos hypothèses, en l'absence de données chiffrées ou de recommandations internationales !

Si il était nécessaire et pertinent d'inscrire d'emblée notre développement dans une perspective régionale, c'était méconnaître les difficultés en tout genre, inhérentes à la mise en œuvre de tout projet de recherche et pour certaines probablement un peu spécifiques à notre région, le temps interminable passé sur les routes et dans les aéroports, le bricolage permanent pour déplacer et former les équipes, les procédures d'achat interminables et des livraisons parfois chaotiques avec blocage en douanes, les difficultés d'échanges d'échantillons à l'international etc. la liste n'est pas exhaustive !

Fin 2013, le Pr B. Carme, alors responsable du CIC AG, m'avait dit au moment de partir à la retraite : « Je ne sais pas comment vous avez réussi à développer vos projets avec les pays voisins, cela fait quinze ans que j'essaie ». Si la gageure était sérieuse, les travaux et projets réunis dans ce manuscrit étaient le reflet d'une véritable aventure humaine, à la fois personnelle et collective. Personnelle, car elle m'a nourri de l'humanité des relations tissées avec nos partenaires et de moments de joie dans la réalisation d'objectifs majeurs comme les premières identifications d'*H. capsulatum* au Suriname et au Guyana. Collective, car tous les travaux et projets que nous avons menés sont dans la droite ligne des travaux antérieurs de mes aînés en Guyane française et, collective également car une initiative est en marche à l'échelle du continent dans l'objectif de faire vivre cette thématique et améliorer la survie des patients.

BIBLIOGRAPHIE

1. Tudela JLR, Denning DW. Recovery from serious fungal infections should be realisable for everyone. The Lancet Infectious Diseases. 2017.
2. Ajello L. The medical mycological iceberg. HSMHA Health Rep. 1971;86(5):437-48.
3. Darling ST. A protozoon general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymphnodes. Journal of the American Medical Association. 1906;46:1283-5
4. Nikiel N. [Histoplasmosis.]. Pol Tyg Lek (Wars). 1956;11(3):126-30.
5. Ribi E, Salvin SB. Antigens from the yeast phase of Histoplasma capsulatum. I. Morphology of the cell as revealed by the electron microscope. Experimental cell research. 1956;10(2):394-404.
6. Darling ST. The Morphology of the Parasite (Histoplasma Capsulatum) and the Lesions of Histoplasmosis, a Fatal Disease of Tropical America. The Journal of experimental medicine. 1909;11(4):515-31.
7. Daniel TM, Baum GL. Drama and discovery : The story of histoplasmosis. Greenwood press ed. London: Greenwood press; 2002. 174 p.
8. De Monbreun WA. Cultivation and cultural characteristics of Darling's Histoplasma capsulatum. Am J Trop Med Hyg. 1934(14):93–125.
9. Dodd K, Tompkins EH. Case of histoplasmosis of Darling in an infant. Am J Trop Med Hyg. 1934(14):127–37.
10. Parsons RJ, Zarafonetis CJD. Histoplasmosis in man: report of seven cases and a review of seventy-one cases. Arch Intern Med. 1945(75):1–23.
11. Emmons CW. Isolation of Histoplasma capsulatum from soil. Public Health Rep. 1949;64(28):892-6.
12. Cain JC, Devins EJ, Downing JE. An unusual pulmonary disease. Arch Intern Med. 1947(79):626–41.
13. Furcolow ML, Bunnell IL, Tenenberg DJ. A complement fixation test for histoplasmosis preliminary results with human sera. Public Health Rep. 1948;63(6):169-73.
14. Goodwin RA, Jr., Shapiro JL, Thurman GH, Thurman SS, Des Prez RM. Disseminated histoplasmosis: clinical and pathologic correlations. Medicine (Baltimore). 1980;59(1):1-33.
15. Palmer CE. Non tuberculosis pulmonary calcification and sensitivity to histoplasmin. Pub Health Rep. 1945;60:513–20.
16. Palmer CE. Geographic differences in sensitivity to histoplasmin among student nurses. Pub Health Rep. 1946(61):475–87.
17. Furcolow ML, Brasher CA. Chronic progressive (cavitary) histoplasmosis as a problem in tuberculosis sanatoriums. Am Rev Tuberc. 1956;73(5):609-19.
18. Post GW, Jackson A, Garber PE. Histoplasmosis on a small tuberculosis service in a general hospital. Dis Chest. 1957;31(6):688-95.
19. Walls K, Furcolow ML, Lehan PH. Histoplasmosis as a problem in tuberculosis sanatoriums throughout the United States. J Lab Clin Med. 1958;51(2):266-70.
20. Zeidberg LD, Ajello L, Dillon A, Runyon LC. Isolation of histoplasma capsulatum from soil. Am J Public Health Nations Health. 1952;42(8):930-5.
21. Emmons CW. Association of bats with histoplasmosis. Public Health Rep. 1958;73(7):590-5.
22. Mochi A, Edwards PQ. Geographical distribution of histoplasmosis and histoplasmin sensitivity. Bull World Health Organ. 1952;5(3):259-91.
23. Edwards PQ, Klaer JH. Worldwide geographic distribution of histoplasmosis and histoplasmin sensitivity. Am J Trop Med Hyg. 1956;5(2):235-57.
24. Edwards PQ, Billings EL. Worldwide pattern of skin sensitivity to histoplasmin. Am J Trop Med Hyg. 1971;20(2):288-319.
25. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. Am Rev Respir Dis. 1969;99(4):Suppl:1-132.

26. Homei A, Worboys M. Fungal Disease in Britain and the United States 1850-2000 Mycoses and Modernity. 2013/11/15 ed. Centre for the History of Science TaM, University of Manchester, England (http://chstm.manchester.ac.uk), editor. Manchester, England: Palgrave Macmillan; 2013. 225 p.
27. Jones PG, Cohen RL, Batts DH, Silva J, Jr. Disseminated histoplasmosis, invasive pulmonary aspergillosis, and other opportunistic infections in a homosexual patient with acquired immune deficiency syndrome. Sex Transm Dis. 1983;10(4):202-4.
28. Centers for Disease Control and prevention C. From the Centers for Disease Control and prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. JAMA. 1993;269(4):460.
29. Fandiño-Devia E, Rodríguez-Echeverri C, Cardona-Arias J, Gonzalez A. Antigen Detection in the Diagnosis of Histoplasmosis: A Meta-analysis of Diagnostic Performance. Mycopathologia. 2016;181(3):197-205.
30. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. Infect Dis Clin North Am. 2016;30(1):207-27.
31. Johnson PC, Wheat LJ, Cloud GA, Goldman M, Lancaster D, Bamberger DM, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. Ann Intern Med. 2002;137(2):105-9.
32. Wheat J, Hafner R, Korzun AH, Limjoco MT, Spencer P, Larsen RA, et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trial Group. Am J Med. 1995;98(4):336-42.
33. McKinsey DS, Wheat LJ, Cloud GA, Pierce M, Black JR, Bamberger DM, et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. National Institute of Allergy and Infectious Diseases Mycoses Study Group. Clin Infect Dis. 1999;28(5):1049-56.
34. Benedict K, Derado G, Mody RK. Histoplasmosis-Associated Hospitalizations in the United States, 2001-2012. Open forum infectious diseases. 2016;3(1):ofv219.
35. Adenis A, Nacher M, Hanf M, Vantilcke V, Boukhari R, Blachet D, et al. HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority. PLoS Negl Trop Dis. 2014;8(8):e3100.
36. Peigne V, Dromer F, Elie C, Lidove O, Lortholary O. Imported acquired immunodeficiency syndrome-related histoplasmosis in metropolitan France: a comparison of pre-highly active anti-retroviral therapy and highly active anti-retroviral therapy eras. Am J Trop Med Hyg. 2011;85(5):934-41.
37. Murphy RA, Gounder L, Manzini TC, Ramdial PK, Castilla C, Moosa MY. Challenges in the management of disseminated progressive histoplasmosis in human immunodeficiency virus-infected patients in resource-limited settings. Open forum infectious diseases. 2015;2(1):ofv025.
38. Nacher M, Adenis A, Basurko C, Vantilcke V, Blanchet D, Aznar C, et al. Primary prophylaxis of disseminated histoplasmosis in HIV patients in French Guiana: arguments for cost effectiveness. Am J Trop Med Hyg. 2013;89(6):1195-8.
39. Bulmer AC, Bulmer GS. Incidence of histoplasmin hypersensitivity in the Philippines. Mycopathologia. 2001;149(2):69-71.
40. Antinori S. Histoplasma capsulatum: more widespread than previously thought. Am J Trop Med Hyg. 2014;90(6):982-3.
41. Bahr NC, Antinori S, Wheat LJ, Sarosi GA. Histoplasmosis infections worldwide: thinking outside of the Ohio River valley. Current tropical medicine reports. 2015;2(2):70-80.
42. Pan B, Chen M, Pan W, Liao W. Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases. Mycoses. 2013;56(3):212-21.
43. Armstrong-James D, Meintjes G, Brown GD. A neglected epidemic: fungal infections in HIV/AIDS. Trends Microbiol. 2014;22(3):120-7.

44. Ten year roadmap, 95/95 by 2025: Global Action Fund for Fungal Infection (GAFFI); 2015 [cited 2016 May 2]. Available from: http://www.gaffi.org/roadmap/.
45. Mathieu N, Antoine A. Proceedings of First Histoplasmosis in the Americas and the Caribbean Meeting, Paramaribo, Suriname, December 4–6, 2015. Emerging Infectious Disease journal. 2016;22(9).
46. Tropical Forests of the Guiana Shield: Ancient Forests in a Modern World. Hammond DS, editor2005. 1-528 p.
47. Malhi Y, Roberts JT, Betts RA, Killeen TJ, Li W, Nobre CA. Climate Change, Deforestation, and the Fate of the Amazon. Science. 2008;319(5860):169-72.
48. Shanley P, Medina G, eds. Fruit trees and useful plants in Amazonian life. Rome, Italy: FAO; 2011.
49. Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, et al. Terrestrial Ecoregions of the World: A New Map of Life on Earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. BioScience. 2001;51(11):933-8.
50. ter Steege H, Pitman NCA, Sabatier D, Baraloto C, Salomão RP, Guevara JE, et al. Hyperdominance in the Amazonian Tree Flora. Science. 2013;342(6156).
51. Guernier V, Hochberg ME, Guegan JF. Ecology drives the worldwide distribution of human diseases. PLoS Biol. 2004;2(6):e141.
52. Nacher M, Aznar C, Blanchet D, Demar M, El Guedj M, Vaz T, et al. AIDS-related disseminated histoplasmosis in the greater Caribbean: how frequent is it? AIDS. 2006;20(6):951-2.
53. Melin M. Surveillance des infections à VIH et Sida dans les territoires français d'Amérique. Bulletin de Veille Sanitaire Antilles Guyane. 2015(11):29.
54. Nacher M, Vantilcke V, Parriault MC, Van Melle A, Hanf M, Labadie G, et al. What is driving the HIV epidemic in French Guiana? Int J STD AIDS. 2010;21(5):359-61.
55. Guyane RP. Exploitation annuelle 2015 du Registre d'Issue de Grossesse Informatisé (RIGI) de la région Guyane. Cayenne: Réseau Périnat Guyane, 2016.
56. Nacher M. Rapport d'activités 2015 du COREVIH Guyane. Cayenne: Comité de Coordination Régionale de la Lutte contre l'Infection par le VIH de Guyane (COREVIH), 2016.
57. Melin M. Infection par le VIH dans les DFA. Bulletin de Veille Sanitaire Antilles Guyane. 2014(6):13.
58. Morlat P, Roussillon C, Henard S, Salmon D, Bonnet F, Cacoub P, et al. Causes of death among HIV-infected patients in France in 2010 (national survey): trends since 2000. Aids. 2014;28(8):1181-91.
59. Lewden C, Sobesky M, Cabie A, Couppie P, Boulard F, Bissuel F, et al. [Causes of death among HIV infected adults in French Guyana and the French West Indies in the era of highly active antiretroviral therapy (HAART)]. Med Mal Infect. 2004;34(7):286-92.
60. InVS IdVS-. Actualités de la tuberculose. In: InVS, editor. Bulletin épidémiologique hebdomadaire - BEH. Bulletin épidémiologique hebdomadaire - BEH ed. Saint Maurice: Institut de Veille Sanitaire - InVS; 2009. p. 106-24.
61. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D, et al. Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). J Acquir Immune Defic Syndr. 2008;48(5):590-8.
62. Floch H. Revue critique des investigations et de la littérature mycologiques pour les annés 1946–1956 en Guyane Française. Mycopathologia et mycologia applicata. 1957;8(3):194-205.
63. Silverie C, Duchassin M. [Study of the incidence in French Guiana of histoplasmosis in a military environment, using histoplasmin skin tests]. Bull Soc Pathol Exot Filiales. 1966;59(2):199-207.
64. Crenn Y, Rieux D, Hulin A, Zeller H. Histoplasma capsulatum septicemia and acquired immunodeficiency syndrome. Report of one case Médecine et Maladies Infectieuses. 1986;16(12):770-1.

65. Gerard Y, Couppie P, del Giudice P, Mille H, Cuchet T, Sainte-Marie D, et al. [Disseminated histoplasmosis: 2 cases in patients with human immunodeficiency virus infection in French Guiana]. Rev Med Interne. 1995;16(10):767-70.
66. Clyti E, Aznar C, Couppie P, Sainte-Marie D, Lemoine C, Huerre M, et al. [Disseminated histoplasmosis detected by lingual and tonsillar erosions in an immunocompetent patient]. Ann Dermatol Venereol. 1999;126(10):709-11.
67. Couppie P, Clyti E, Nacher M, Aznar C, Sainte-Marie D, Carme B, et al. Acquired immunodeficiency syndrome-related oral and/or cutaneous histoplasmosis: a descriptive and comparative study of 21 cases in French Guiana. Int J Dermatol. 2002;41(9):571-6.
68. Sarazin E, Nacher M, Toure Y, Clyti E, El Guedj M, Aznar C, et al. [Dermatologic manifestations associated with immune reconstitution syndrome in HIV+ patients starting HAART: a retrospective study in French Guiana]. Bull Soc Pathol Exot. 2005;98(3):187-92.
69. Huber F, Nacher M, Aznar C, Pierre-Demar M, El Guedj M, Vaz T, et al. AIDS-related Histoplasma capsulatum var. capsulatum infection: 25 years experience of French Guiana. AIDS. 2008;22(9):1047-53.
70. Putot A, Perrin S, Jolivet A, Vantilcke V. HIV-associated disseminated histoplasmosis in western French Guiana, 2002-2012. Mycoses. 2015;58(3):160-6.
71. Couppie P, Sobesky M, Aznar C, Bichat S, Clyti E, Bissuel F, et al. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. Clin Infect Dis. 2004;38(1):134-8.
72. Nacher M, Sarazin F, El Guedj M, Vaz T, Alvarez F, Nasser V, et al. Increased incidence of disseminated histoplasmosis following highly active antiretroviral therapy initiation. J Acquir Immune Defic Syndr. 2006;41(4):468-70.
73. Bonnet D, Balandraud P, Lonjon T, Rey P, Van de Walle JP, Cador L, et al. [Round pulmonary lesions after returning from French Guyana. Six cases of american pulmonary histoplasmosis]. Med Trop (Mars). 1995;55(1):55-60.
74. Cuguillière A, N'Guyen G, Maslin J, Lonjon T, Miltgen J, Bonnet D. Tumor form of histoplasmosis in subjects returning from French Guiana. Treatment of an immunocompetent patient. Presse medicale (Paris, France: 1983). 1997;26(36):1724-.
75. Bonnet D, Nguyen G, De Pina JJ, Martet G, Miltgen J, Cuguilliere A, et al. [American pulmonary histoplasmosis. Prospective study with 232 soldiers after a 2-year assignment in Guiana]. Med Trop (Mars). 2002;62(1):33-8.
76. Barthes N, Morin F, Pommier dSV, Briolant S. Lung nodule in French Guiana in a immunocompetent patient. Medecine et sante tropicales. 2017;27(1):26.
77. Cormier H, Perez N, Blanchet D, Couppié P, Carme B, Aznar C. Fatal histoplasmosis in a non-HIV patient in French Guiana. Journal de Mycologie Médicale/Journal of Medical Mycology. 2012;22(2):189-91.
78. Grabar S, Le Moing V, Goujard C, Leport C, Kazatchkine MD, Costagliola D, et al. Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. Ann Intern Med. 2000;133(6):401-10.
79. Mosnier E. Diagnostic et prise en charge des PVVIH en zones isolées et frontalières en Guyane. Bulletin de Veille Sanitaire Antilles Guyane. 2015(11):7.
80. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813-21.
81. Couppie P, Aznar C, Carme B, Nacher M. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. Curr Opin Infect Dis. 2006;19(5):443-9.
82. Kasuga T, White TJ, Koenig G, McEwen J, Restrepo A, Castaneda E, et al. Phylogeography of the fungal pathogen Histoplasma capsulatum. Mol Ecol. 2003;12(12):3383-401.

83. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, Brewer J, et al. Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. Clin Infect Dis. 1997;24(6):1195-203.
84. Moquet O, Blanchet D, Simon S, Veron V, Michel M, Aznar C. Histoplasma capsulatum in Cayenne, French Guiana. Mycopathologia. 2012;174(4):331-4.
85. Hajjeh RA, Pappas PG, Henderson H, Lancaster D, Bamberger DM, Skahan KJ, et al. Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. Clin Infect Dis. 2001;32(8):1215-20.
86. Maubon D, Simon S, Aznar C. Histoplasmosis diagnosis using a polymerase chain reaction method. Application on human samples in French Guiana, South America. Diagn Microbiol Infect Dis. 2007;58(4):441-4.
87. Simon S, Veron V, Boukhari R, Blanchet D, Aznar C. Detection of Histoplasma capsulatum DNA in human samples by real-time polymerase chain reaction. Diagn Microbiol Infect Dis. 2010;66(3):268-73.
88. Scheel CM, Samayoa B, Herrera A, Lindsley MD, Benjamin L, Reed Y, et al. Development and evaluation of an enzyme-linked immunosorbent assay to detect Histoplasma capsulatum antigenuria in immunocompromised patients. Clin Vaccine Immunol. 2009;16(6):852-8.
89. Arango-Bustamante K, Restrepo A, Cano LE, de Bedout C, Tobón AM, González A. Diagnostic value of culture and serological tests in the diagnosis of histoplasmosis in HIV and non-HIV Colombian patients. The American journal of tropical medicine and hygiene. 2013;89(5):937-42.
90. 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(12):941-3.
91. Wheat LJ, Hackett E, Durkin M, Connolly P, Petraitiene R, Walsh TJ, et al. Histoplasmosis-associated cross-reactivity in the BioRad Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2007;14(5):638-40.
92. Xavier MO, Pasqualotto AC, Cardoso IC, Severo LC. Cross-reactivity of Paracoccidioides brasiliensis, Histoplasma capsulatum, and Cryptococcus species in the commercial Platelia Aspergillus enzyme immunoassay. Clin Vaccine Immunol. 2009;16(1):132-3.
93. Riviere S, Denis B, Bougnoux ME, Lanternier F, Lecuit M, Lortholary O. Serum Aspergillus galactomannan for the management of disseminated histoplasmosis in AIDS. Am J Trop Med Hyg. 2012;87(2):303-5.
94. Girouard G, Lachance C, Pelletier R. Observations on (1-3)-β-D-glucan detection as a diagnostic tool in endemic mycosis caused by Histoplasma or Blastomyces. Journal of medical microbiology. 2007;56(7):1001-2.
95. Organization WH. Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children: December 2011. 2011.
96. Larue R, Datta K, Nosanchuk J, Marr KA, editors. 1433Diagnosis of Latent Histoplasmosis Using Interferon-γ Release Assays. Open forum infectious diseases; 2014: Oxford University Press.
97. Mazurek M, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. MMWR Recomm Rep. 2016;59(RR-5):1-25.
98. Sterling T, Jenkins C, Jayathilake K, Gotuzzo E, Veloso V, Cortes C, et al., editors. Culture-negative TB is associated with increased mortality in HIV-infected persons. Conference on Retroviruses and Opportunistic Infections; 2015.

ANNEXE 1

RICAI 2013
33è Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse
CNIT, Paris la Défense - France
21-22 novembre 2013

CERTIFICAT DE COMMUNICATION

L'A.C.A.I. (Association de Chimiothérapie Anti Infectieuse) représenté par JCD CONSEIL comme Organisateur de Congrès, certifie que :

➢ **Antoine Adenis** a officiellement présenté une communication orale :

ORAL N° 33

Histoplasmose et infection par le VIH.

A. Adenis
Hôpital général de Cayenne, France

à la **33è REUNION INTERDISCIPLINAIRE DE CHIMIOTHÉRAPIE ANTI-INFECTIEUSE.**

Cette réunion a eu lieu les 21 et 22 novembre 2013 au CNIT, Paris La Défense, France.

JCD Conseil S.A
26 Rue Salomon de Rothschild
92156 Suresnes Cedex
Tél. : 01 58 47 77 00 / Fax : 01 58 47 77 [illegible]
Capital : 1.000.000 € - Immatriculation IM[illegible]
N° TVA Intracommunautaire : FR 55 320 268 568

Alexia NOLLENT
Responsable secrétariat scientifique
JCD Conseil - Publicis Groupe
Département Congrès / PCO
26 rue Salomon de Rothschild - 92150 Suresnes – France
Standard : +33 (0)1 58 47 77 00
Fax : +33 (0)1 58 47 79 57
Email: alexia.nollent@jcdconseil.com

JCD CONSEIL

RICAI

ANNEXE 2

CERTIFICATE of PARTICIPATION

This certifies that

Dr A. Adenis presenting oral communication #64

attended the ASTMH 62nd Annual Meeting in
Washington, DC, USA, November 13-17, 2013 at the
Marriott Wardman Park Hotel

Advancing global health since 1903

Karen Goralski
Executive Director

David H. Walker, MD
ASTMH President

64

AMERICAN HISTOPLASMOSIS IN HIV-INFECTED PATIENTS: A STUDY OF PROGNOSTIC FACTORS ASSOCIATED WITH EARLY DEATH

Antoine A. Adenis[1], Mathieu Nacher[1], Matthieu Hanf[1], Christine Aznar[2], Olivier Lortholary[3], Bernard Carme[2], Pierre Couppié[2]

[1]Inserm CIE802, Centre Hospitalier de Cayenne, Cayenne, French Guiana, [2]EA 3593, Université des Antilles et de la Guyane, Cayenne, French Guiana, [3]Institut Pasteur de Paris, Paris, France

American histoplasmosis is a endemic fungal infection in French Guiana. In persons with AIDS, it is the most frequent opportunistic infection and the leading cause of death. In order to reduce deaths, it is important to identify prognostic factors associated with early mortality so that appropriate therapy can be given. We looked at a one of the largest series of patients available to determine risk factors for early mortality. A retrospective study was conducted to identify persons with HIV/AIDS infected with *Histoplasma capsulatum* var. *capsulatum* and admitted to one of the three main hospitals of French Guiana between 1992 and 2011. Early mortality was defined by death occurring within 30 days after antifungal treatment initiation. Data were collected on standardized case report forms and analysed using multivariable logistic regression models. A total of 274 patients with HIV/AIDS were identified with histoplasmosis during 1992-2011. Forty six patients met the criteria for early death. The final multivariate model found several factors associated with an increased risk of early death: dyspnea OR=11.36 [4.28-30.17], acute renal failure OR=7.23 [1.47-35.71], WHO performance status score > 2 OR=4.05 [1.86-8.82] and platelet count ≤ 100 000/mm3 OR=3.51 [1.34-9.16]. Cases found during 2005-2011, OR=0.02 [0.01-0.12], and those from Cayenne General Hospital, OR=0.13 [0.04-0.47], were associated with a reduced risk of early death. This is the largest case series looking at factors associated with early death for histoplasmosis, and for the first time after adjusting for CD4 counts. These results are consistent with other reports from the Americas. The factors identified can provide clinicians arguments about early and aggressive intervention with antifungal therapy in order to prevent early death due to histoplasmosis.

ANNEXE 3

RESUME DE LA RECHERCHE – PROJET ANRS 12260 EDIRAPHIS

PROMOTEUR	Institut national de la santé et de la recherche médicale - Agence Nationale de Recherches sur le Sida et les hépatites virales (Inserm-ANRS) 101 rue de Tolbiac, 75013 Paris
INVESTIGATEUR COORDONNATEUR	Pr Mathieu Nacher, CIC EC Antilles Guyane, CH de Cayenne, Av. des flamboyants, BP 6006, 97306 Cayenne CEDEX, France Tel : +594 594 39 50 24
TITRE	L'histoplasmose à *Histoplasma capsulatum* var. *capsulatum* sur le plateau des Guyanes et aux Antilles : Evaluation de la prévalence chez les patients infectés par le VIH à l'aide d'une méthode diagnostique rapide ELISA de détection d'antigènes sériques et urinaires d'*Histoplasma..*
TITRE ABREGE	ANRS 12260 EDIRAPHIS
NUMERO D'IDENTIFICATION	Numéro d'enregistrement sur Clinicaltrials.gov : NCT01884779
VERSION DU PROTOCOLE	Version 1.6 du 23/01/2014
JUSTIFICATION / CONTEXTE	L'histoplasmose à *Histoplasma capsulatum* var. *capsulatum* est la première cause de syndrome d'immunodéficience acquise (Sida) et de décès en Guyane et probablement en Amazonie. Le diagnostic de cette pathologie nécessite des gestes invasifs, des laboratoires performants, et des délais jusqu'à plusieurs semaines. La Mycotic Diseases Branch des Centers for Disease Control and prevention (CDC) a mis en place un test ELISA rapide, sensible et spécifique sur des prélèvements de sang et d'urines qui semble intéressant dans les pays en zone d'endémie, et plus particulièrement dans les pays en développement..
OBJECTIFS	**Objectif principal :** Mesurer la proportion de patients infectés par le VIH hospitalisés ou vus en consultation dans l'attente d'une hospitalisation pour une suspicion de syndrome infectieux ayant un test positif pour la détection d'antigènes sérique et/ou urinaire d'*Histoplasma capsulatum* var. *capsulatum*. **Objectifs secondaires** - Evaluer, chez les patients infectés par le VIH ayant bénéficié d'une culture fongique, la sensibilité et la spécificité de la détection d'antigène d'Histoplasma capsulatum var. capsulatum sur des prélèvements urinaires et sanguins. - Evaluer, chez les patients infectés par le VIH ayant bénéficié d'une culture fongique, les valeurs prédictives positive et négative de la détection d'antigène d'Histoplasma capsulatum var. capsulatum sur des prélèvements urinaires et sanguins. - Comparer la concentration en antigène d'Histoplasma des prélèvements urinaire et sérique suivant la gravité des cas d'histoplasmose à Histoplasma capsulatum var. capsulatum. - Identifier les facteurs pronostiques d'une évolution péjorative à court terme des cas d'histoplasmose à Histoplasma capsulatum var. capsulatum confirmés par le test ELISA et/ou par la culture fongique. - Identifier des facteurs de risque d'exposition environnementale associés à la survenue d'un épisode d'histoplasmose à Histoplasma capsulatum var.

	capsulatum confirmé par le test ELISA et/ou par la culture fongique. - Mesurer la fréquence de l'histoplasmose à Histoplasma capsulatum var. capsulatum par rapport aux autres diagnostics principaux des patients infectés par le VIH hospitalisés pour un syndrome infectieux. - Description comparative des caractéristiques des cas d'histoplasmose à Histoplasma capsulatum var. capsulatum confirmés par le test ELISA et/ou la culture fongique entre les différents centres d'inclusion. - Constituer une collection d'échantillons biologiques à partir des cultures fongiques positives pour Histoplasma capsulatum var. capsulatum, et des prélèvements sanguin et urinaire de l'étude pour la réalisation d'études ancillaires sur les marqueurs de l'infection à Histoplasma capsulatum var. capsulatum au cours de l'infection par le VIH.
SCHEMA DE LA RECHERCHE	L'étude suit un protocole de recherche non interventionnelle avec constitution d'une collection d'échantillon biologique et données personnelles associées.
CRITERES D'INCLUSION	- Adulte (âge supérieur ou égal à 18 ans) - Patient vu en hospitalisation ou en consultation dans l'attente d'une hospitalisation. - Infection par le VIH1 ou le VIH2 confirmée par les techniques validées en France et au Suriname, soit antérieure à l'épisode concerné, soit de découverte concomitante. - Patient présentant au minimum un des trois éléments suivants : une altération de l'état général (cotation selon l'échelle OMS de Performance Status ≥ 1) et/ou une fièvre et/ou des symptômes suspects de syndrome infectieux. - Prescription médicale dans les 15 jours après l'admission en hospitalisation de prélèvements sanguins et urinaires à la recherche d'un agent infectieux. - Recueil du consentement écrit d'accord de participation à l'étude
CRITERES DE NON INCLUSION	- Refus de participer à l'étude. - Patient dans un état clinique critique ne permettant pas aux médecins de recueillir son consentement éclairé et, n'ayant pas de tiers pour le représenter et signer un consentement de participation à l'étude pour le patient. - Traitement antifongique en cours ou arrêté dans le mois précédant la date d'inclusion (en dehors des traitements antifongiques topiques, non considérés). - Patient incarcéré dans un centre pénitentiaire lors de son admission à l'hôpital.
DEROULEMENT DE LA RECHERCHE	Tout patient infecté par le VIH,connu ou non, nécessitant une hospitalisation dans un des centres investigateurs pour une fièvre et/ou une altération de l'état général (PS OMS ≥ 1), associées ou non à des symptômes non spécifiques potentiellement liés à un syndrome infectieux, se verra proposer de participer à l'étude. Le médecin investigateur de chaque site informera le patient et recueillera son consentement. Le médecin prescrira les explorations qu'ils jugent nécessaires au vu de l'état du patient ainsi que les traitements adaptés suivant les pratiques habituelles propres à chacun des centres d'inclusion. Les données personnelles associées seront recueillies dans un cahier d'observation anonymisé. La seule différence pour le patient sera qu'une fraction des prélèvements sanguins et urinaires, réalisés habituellement dans un contexte de suspicion de pathologie infectieuse, sera utilisée pour l'évaluation en double insu du test ELISA de détection

	antigénique d'*Histoplasma capsulatum* var. *capsulatum*(ni le médecin ni le patient n'auront les résultats du test ELISA à l'étude).La durée de participation pour un patient sera de 3 mois. Chaque participant fera l'objet d'une visite d'inclusion à J0 et, d'un suivi à J 30 et J 90 après l'inclusion.
VARIABLES ETUDIEES	Les résultats du test ELISA pratiqué sur les urines et le sérum seront étudiés pour répondre à l'objectif principal. Pour répondre aux objectifs secondaires, les éléments de l'interrogatoire, les données cliniques, paracliniques et thérapeutiques disponibles dans le dossier médical du patient ainsi que l'exposition environnementale seront recueillies. Par ailleurs le statut vital à 30 jours et 90 jours après l'inclusion sera recueilli par contact téléphonique pour étudier la sévérité de l'épisode infectieux.
NOMBRE DE SUJETS NECESSAIRES	727 patients.
NOMBRE PREVU DE CENTRES	7 centres d'inclusion : 4 en France et 3 au Suriname.
DUREE DE LA RECHERCHE	La durée de la recherche est de 36 mois avec 24 mois prévus pour la phase de recrutement des participants.
ANALYSE STATISTIQUE DES DONNEES	L'estimation de la prévalence de la positivité du test ELISA de détection d'antigènes sérique et urinaire d'Histoplasma capsulatum var. capsulatum sera calculée avec un intervalle de confiance bilatéral à 95%. Deux groupes seront individualisés en fonction de la positivité ou non du test ELISA sur l'ensemble des patients puis au sein de chaque pays. Ainsi une description comparative des caractéristiques socio-démographiques, cliniques, paracliniques, thérapeutiques et de devenir sera réalisée à l'aide des tests statistiques standards. Au préalable, une analyse de la concordance des résultats du test ELISA sera réalisée pour les 20% de prélèvements ayant fait l'objet d'un contrôle qualité par l'expertise externe de l'équipe de la Mycotic Diseases Branch du CDC. Parmi les patients ayant bénéficié d'une culture fongique de tissus ou tout autre fluide corporel positive à la recherche d'Histoplasma capsulatum var capsulatum, technique Gold standard pour définir la maladie histoplasmose, la sensibilité, la spécificité, les valeurs prédictives positive et négative du test ELISA sur les prélèvements urinaires et sériques seront déterminées. Le groupe de référence sera donc constitué par les patients ayant bénéficié d'une culture fongique. Un seuil optimal de densité optique maximisant la sensibilité et la spécificité du test ELISA pour la détection d'antigènes sérique et urinaire sera recherché à l'aide de courbes ROC. L'identification d'un lien entre la concentration en antigène et la sévérité du tableau clinique sera réalisée en comparant la distribution des concentrations d'antigène d'Histoplasma des prélèvements urinaire et sérique entre deux groupes définis par la gravité ou non des cas d'histoplasmose à Histoplasma capsulatum var. capsulatum. La gravité sera définie par le statut vital (décès oui ou non) trente jours après l'inclusion. Selon la distribution des concentrations d'antigène la comparaison utilisera le test de Mann Whitney ou le test t de Student. Ces groupes de comparaisons permettront également d'identifier les facteurs pronostiques de décès précoces (dans les trente jours suivant l'inclusion) à l'aide de modèles multivariés de régression logistique ou autre modèle de Cox. Une analyse similaire sera réalisée en utilisant le statut vital à 90 jours après l'inclusion comme critère définissant la gravité, ceci afin de

	comparer avec les données de la littérature. La fréquence de l'histoplasmose par rapport aux autres diagnostics principaux sera définie suivant trois groupes de comparaisons : test ELISA et culture fongique positifs, test ELISA positif et culture fongique négative, test ELISA négatif et culture fongique positive. Dans chacun des groupes une estimation de la fréquence sera calculée avec un intervalle de confiance bilatérale à 95%. L'identification des facteurs de risque d'exposition environnementale se fera à l'aide d'une analyse descriptive première au sein de deux groupes : un groupe histoplasmose confirmée par le test ELISA ou la culture fongique versus un groupe histoplasmose négative au test ELISA ou à la culture fongique. Puis un modèle de régression logistique pas à pas sera déterminé à l'aide du test du maximum de vraisemblance. L'adéquation globale du modèle sera vérifiée à l'aide du test de Goodness of fit de Hosmer et Lemeshow.
RETOMBEES ATTENDUES	- Déterminer l'importance respective de l'Histoplasmose parmi les autres infections opportunistes chez les patients infectés par le VIH hospitalisés sur le plateau des Guyanes et aux Antilles. - Mesurer la fréquence et la morbi-mortalité associées à l'histoplasmose au Suriname, en améliorer le diagnostic et la prise en charge. - Diminuer la morbidité et la mortalité imputable à l'histoplasmose par un diagnostic plus précoce, moins invasif et simple à réaliser quelque soit les ressources du pays. - Identifier des facteurs pronostiques associés au décès précoce des cas d'histoplasmose afin guider le choix thérapeutique initial. - Transfert de technologie au plan des pratiques médicales courantes (cliniques, diagnostiques, thérapeutiques) et au plan de la recherche épidémiologique. - Etablir des mesures de prévention adressées aux patients immunodéprimés pour réduire leur exposition aux agents fongiques de l'environnement. - Discuter l'intérêt d'adapter les recommandations nationales françaises en matière de prophylaxie primaire antifongique en fonction du taux d'incidence de l'histoplasmose. **Mais également :** - la création d'un réseau international de services cliniques et de laboratoires. - une collaboration scientifique internationale France-Suriname-USA dans le domaine de la Médecine Tropicale. - une collaboration universitaire internationale (1 PhD au Suriname et en Guyane). - une extension de l'étude dans un deuxième temps à d'autres pays de la région Amazonienne, comme le Guyana ou le Brésil qui ont manifesté leur intérêt.

ANNEXE 4

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** Section 1.04 **No.** **Section 1.03 INFORME FINAL DE PROYECTO CTP**	**Section 1.05 Date: Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

	Report Date / Fecha del Reporte: 12/04/2015

1. GENERAL INFORMATION / INFORMACIÓN GENERAL

Project title/ Título del proyecto:	**Country Office Leading / Oficina de País Líder:**
Control of Histoplasmosis on HIV infected patients in the Guiana Shield	French Guiana, Office in Barbados
Participating countries/ Paises participantes	**Start date / Fecha de inicio:**
French Guiana (France), Suriname and Guyana	11/2013
	End date / Fecha de finalización
	12/2015

2. PROJECT BACKGROUND AND ORIGIN / ANTECEDENTES Y ORIGEN DEL PROYECTO

a) Context/ Contexto

Histoplasmosis is a disease caused by the fungus Histoplasma capsulatum that can be found throughout the world, but is common in the Americas. Many people who are infected with the fungus do not show any symptoms. In otherwise healthy people, Histoplasmosis sometimes causes a mild pneumonia, that will in general resolve without treatment. However, persons at risk can develop severe disease that can lead to death. This disseminated form is most frequent in people who have weakened immune systems, such as people with immunosuppressive therapy and, in the Guiana Shield, people living with HIV. For these HIV infected patients, disseminated Histoplamosis is included in the CDC list of AIDS defining illnesses since 1987.

In the Guiana Shield, where HIV prevalence exceeds 1%, disseminated Histoplasmosis is a real public health problem. A recent publication showed that disseminated Histoplasmosis was the first AIDS-defining event in French Guiana. Despite well-equipped laboratories, with a mycology department for research and development of new tests, Histoplasmosis is reported to be the first cause of AIDS-related deaths in French Guiana. A recent prospective study in all HIV patients hospitalised in Saint Laurent du Maroni on the border between Suriname and French Guiana (from 2008 – 2010) showed that 41% of HIV patients admitted a CD4 count < 200/µl were admitted because of Histoplasmosis and that for patients with a CD4 count <50/µl the proportion increased to 85% . Other authors in Colombia, Guatemala, Argentina, Peru, Panama, and Brazil have also underlined the importance of this pathology in HIV infected patients. There are several publications from Suriname most of which concerned the sixties. The most recent one described HIV patients from Suriname hospitalized in Holland for a febrile illness that proved to be disseminated Histoplasmosis . No publications on disseminated Histoplasmosis have been identified for Guyana or for the Amapa State, Brazil, which share a similar environment with French Guiana and Suriname. However, histoplasmin sensitivity tests have showed widespread exposure in the region: in Guyana 29% of the population had positive histoplasmin tests, 30% in Brazil and 42 % in Trinidad. It is thus suspected that Histoplasmosis is also affecting HIV infected patients in these countries with identical severe consequences. Although improvements in HIV testing and HAART availability have a favourable impact on histoplasmosis through immune restauration, there are still patients suffering and dying from this treatable disease. The present project will thus lead to additional gains in the fight against HIV/AIDS.

Despite the information available, Histoplasmosis in HIV infected patients is not considered to be a major public health problem in the region. It is frequently undiagnosed and untreated, or with late diagnosis and late treatment, often leading to death. The similarity of the clinical presentation often eludes clinicians to treat histoplasmosis as smear negative tuberculosis. Moreover, several studies have shown that Histoplasmosis is frequently associated with tuberculosis, up to 15% of the cases, and this may explain the absence of improvement in suspected TB patients on effective tuberculostatic therapy.

Disseminated Histoplasmosis requires aggressive diagnostic explorations, and prompt treatment with antifungals (itraconazole or liposomal amphotericin B for the more severe cases) in order to prevent death. Diagnosis however, is not easy. The best tool is expensive and only available in the USA. In French Guiana, it relies on direct examination of fluid or tissue samples (with a low sensitivity)and culture (which may take weeks to obtain a diagnosis) while death may occur quickly. Clinicians must therefore be aggressive in collecting samples (bone marrow, biopsies, urine, etc) and prompt to deliver presumptive treatment. This attitude is grounded on the knowledge of the local epidemiology of opportunistic infections. In some countries, there is scepticism and even denial of Histoplasmosis infection among physicians because there are no diagnostic facilities and therefore they have never “seen it” even if a significant number of their HIV patients die without diagnosis. If it’s not perceived to be a problem, clinicians do not prescribe examinations and the laboratories do not develop their diagnostic capacity.

This lethal vicious circle makes Histoplasmosis a neglected cause of AIDS and AIDS related deaths in the region. Suriname has recently started cooperating with French Guiana on this subject, and clinicians begin to be more prone to start presumptive treatment or prescribe fungal cultures or Gomori-Grocott staining of pathological samples. As a consequence, more cases are diagnosed and treated. But there is still room for improvement.

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** Section 1.04 **No.** **Section 1.03 INFORME FINAL DE PROYECTO CTP**	**Section 1.05 Date: Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

In this perspective, French Guiana and Suriname have been working close together during 2011 and early 2012 in order to implement two different strategies.
First, in the context of a research project, French Guiana obtained funds from the French National Research Agency on AIDS (ANRS-Inserm) mid 2011 and the European Union by 10/2011, to evaluate prevalence of histoplasmosis in HIV-infected patients hospitalized in Suriname and French Guiana. This research project used a new ELISA diagnostic test developed by the Centers for Disease Control and Prevention (CDC). This test only requires a urine sample and provides a result in 4 hours whereas fungal culture (Gold Standard method for the diagnosis of histoplasmosis) often requires 4 weeks and invasive procedure. The test is designed for countries with limited resources and is transferred readily by the CDC. The test has been validated in Guatemala in the context of a prospective trial versus fungal culture (sensitivity 81% and specificity 95%), and was used for the fisrt time in the context of the Guiana Shield. After a 24 months recruitment period in Suriname and French Guiana, 490 patients were recruited and finalization of data entry will occur in december 2015. The results of this study are intended to shape the future of histoplasmosis diagnostic procedures in the region.
Second, apart from the fact that this research gave impetus for the trans border cooperation between French Guiana and Suriname, funds were obtained from the French Agency for Development (AFD) and the Regional Public Health Agency of French Guiana (ARS) in order to implement the development of cooperation on the standard practices for the diagnosis of fungal diseases in HIV-infected patients in the Guiana Shield (direct examination and fungal culture). This trans border cooperation project was divided into two parts. On one side, it focused on harmonizing the fungal culture laboratory practices by using the same laboratory protocols and equipments. This implies protocols translation, laboratory technicians' trainings and the purchase of equipments for Suriname. On the other side, this cooperation project focused on the medical practices for the diagnosis of fungal diseases in HIV-infected patients, and particularly on the diagnosis of histoplasmosis. This included trans border medical meetings with exchanges on the medical practices in order to implement common strategies and to increase awareness of physicians about histoplasmosis in Suriname. Whereas the first axis (not part of the TCC) focused on diagnostic methods for the future, this second axis was not research and focused on diagnostic techniques that needed to be implemented as soon as possible.

For both projects, it was expected to associate Guyana since Histoplasmosis is found all over the Guiana Shield environment. Negotiations with the National AIDS program of Guyana to include Guyana in these projects (research and cooperation) were fruitful through this TCC for the trans border cooperation project but the "research project" was not implemented since we didn't manage to obtain funds from ANRS-Inserm or elsewhere despite submitting proposals for funding.

Hence, the TCC fund was requested in order to include Guyana in the trans border cooperation project (laboratory and medical practices) and to strengthen capacity building in French Guiana, Suriname and Guyana in the context of the trans border cooperation project on the standard practices for the diagnosis of fungal diseases. It has also strengthened the objective of raising awareness of histoplasmosis on the Guiana Shield by funding the implementation of a common report on the strategy to control this disease, a common network of health practitioners and an international meeting in Paramaribo with presentation of data obtained in the different countries taht are part of the TCC project. By using staff already defined for the management and the coordination of the two projects up cited, the TCC project was a highly effective tool, complementary to ongoing projects at the time manuscript was submitted (01/2013 with an approval in 11/2013).

b)Purpose/ Propósito
Mortality and morbidity due to American Histoplasmosis in the Guiana shield reduced.
As a summary, in the context of ongoing projects that were already funded for French Guiana and Suriname at the time the TCC manuscript was submitted, the TCC funds were used to achieve the following objectives:

- ✓ Financing the inclusion of Guyana in the laboratory and clinical trainings of the transborder cooperation project on fungal diseases.
- ✓ Supporting the implementation of the new CDC ELISA test in Guyana in terms of laboratory training and small equipment.
- ✓ Measuring the true burden of Histoplasmosis in Suriname, Guyana and French Guiana, with the preparation of a detailed situation analysis using data from the gold standard method for the diagnosis of histoplasmosis.
- ✓ Developing adapted strategies to control Histoplasmosis on HIV infected patients on the Guiana Shield, based on the information collected.
- ✓ Increasing the awareness on histoplasmosis in the whole Region, by sharing the situation analysis through an international meeting with other interested countries in the Americas, the Caribbean and beyond.

c) Expected results/ Resultados esperados

- Awareness of the problem of American Histoplasmosis in HIV-positive patients increased on the Guiana Shield
- Diagnostic capacity building for histoplasmosis improved on the Guiana Shield
- Clinical practice in HIV patients on the Guiana Shield improved
- Strategy for Histoplasmosis control developed

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** **Section 1.03 INFORME FINAL DE PROYECTO CTP** Section 1.04 **No.**	**Section 1.05 Date: Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

- Network of trained health professionals reinforced in the Guiana Shield.

The project has a direct correlation with Biannual Work Plan (BWP) 2012-13 for the FDAs which include Suriname and French Guiana, and contributed to the OSER FDA S02.05 "Coordination between the FDAs and the Sub-Region for HIV prevention and control strengthened".
Suriname's BWP is linked to Region Expected Result (RER) 2.4. And the Office Specific Expected Result (OSER) is 02.04: *"Surveillance, monitoring & evaluation systems strengthened on TB, HIV/AIDS and Malaria"*.
Guyana's BWP is linked to Region Expected Result (RER) 2.4. And the Office Specific Expected Result (OSER) is 02.04: *"Surveillance data for HIV, malaria and TB disaggregated by sex and age reported to PAHO/WHO"*.

3. PROJECT EXECUTION / EJECUCIÓN DEL PROYECTO

a) Achievement of the expected results / Logros de los resultados esperados

1. Awareness of the problem of American Histoplasmosis in HIV-positive patients increased in the Guiana Shield

Since 2011, French Guiana and Suriname have been working close together in raising awareness among clinicians, laboratory practitioners, public health institutions and ministries, foundations and biotechnology companies. In 2013, Guyana joined the network and related activities through the present TCC project.
In the indicators table at Appendix 4, the number of trained professionnals in each country is important (240 professionals trained in different contexts), since efforts in organizing workshops, symposiums or CME meetings in each country were very important (16 events). Focal points in each country have worked close together to make this TCC programme a reality by coordinating all activities using mainly emails and teleconference. PAHO officers in each country were of great help in coordination along with the CDC MDB team in Atlanta. Protocols and project brochure were created and diffused each time we had a training or a project presentation respectively.
Since histoplasmosis awareness and diagnostic facilities have been improved in Suriname and Guyana (15 cases and 2 cases to date, respectively), the initiative launched will now grow by its own if public health authorities ensure support to the mycological examinations of HIV-patients in country.
The TCC workshop to be held in Suriname, 4-6 december 2015, is of great importance since a real initiative will be launched at an unexpected scale with 14 countries accross the Americas, the Caribbean and beyond now willing to participate in the network initiated through this TCC project.

2. Diagnostic capacity building for histoplasmosis on the Guiana Shield

A. Exploratory missions:

- Suriname: 1 exploratory mission of French Guiana coordinators in Paramaribo, Suriname, from 17 to 18 november 2011 was completed by a final drafting of the TCC trainings with a mission of Surinamese coordinators in Cayenne, French Guiana, from 21 to 22 May 2012 (Dr Hermelijn and Mr Mangroe).
- Guyana: 1 exploratory mission of French Guiana coordinators in Georgetown, Guyana, from 5 to 7 december 2012.
- French Guiana: 1 exploratory mission of French Guiana coordinators at CDC Mycotic Diseases Branch in Atlanta, USA, from 27 to 28 december 2011 (Dr Adenis and Pr Nacher).

B1. Laboratory trainings for Fungal Culture:

- Suriname:
1 training of 2 laboratory technicians of the AZP Hospital on the standard practices for fungal culture in Cayenne Mycology laboratory by Dr C. Aznar, from 22 to 25 october 2012.
1 training of 2 other laboratory technicians of the AZP Hospital on the standard practices for fungal culture in Cayenne Mycology laboratory by Dr C. Aznar, from 5 to 8 november 2012.
- Guyana:
1 training of 2 medical technologist of the National Public Health Reference Laboratory and the Georgetown Public hospital Corporation on the standard practices for fungal culture in Cayenne Mycology laboratory by Dr D. Blanchet, from 8 to 12 december 2014.

B2. Laboratory trainings for the CDC Histoplasma EIA test:

- Suriname:
1 training of 2 laboratory technician on the EIA test on urine by Dr B. Gomez, CIB Medellin (ex CDC MDB), from 14 to 18 october 2013 at the National Public Health Central Laboratory of Suriname.
1 training of 2 laboratory technician on the EIA test on serum by Dr C. Scheel, CDC MDB, from 2 to 6 february 2014 at at the National Public Health Central Laboratory of Suriname.
- French Guiana:
1 training of 2 laboratory technician on the EIA test on urine by Dr B. Gomez, CIB Medellin (ex CDC MDB), from 20 to

24 october 2013 at the Cayenne Hospital Parasitology and Mycology laboratory.
1 training of 2 laboratory technician on the EIA test on serum by Dr C. Scheel, CDC MDB, from 8 to 12 february 2014 at the Cayenne Hospital Parasitology and Mycology laboratory.
- Guyana: Not performed since no funds were available to implement the study protocol.

C1. Quality control for Fungal Culture:

- Suriname:
1 quality control of laboratory procedures and data entry by a French Guiana representative (C. Hermine) at the AZP Hospital from 26 to 30 august 2013.
1 quality control of laboratory procedures and data entry by a French Guiana representative (C. Hermine) at the AZP Hospital from 5 to 11 april 2014.

- Guyana:
1 study monitoring and evaluation mission by a French Guiana representative (A. Adenis) at the National Public Health Reference Laboratory from 28 to 29 august 2014.
1 quality control of laboratory procedures and data entry by a French Guiana representative (D. Blanchet) at the National Public Health Reference Laboratory from 26 september to 6 october 2015.

C2. Quality control for the CDC Histoplasma EIA test:

- Suriname:
1 quality control of laboratory procedures and data entry by a French Guiana representative (A.Lalliaume) at the National Public Health Central Laboratory of Suriname from 19 to 25 july 2014.
1 quality control of laboratory procedures and data entry by a French Guiana representative (A.Lalliaume) at the National Public Health Central Laboratory of Suriname from 25 to 31 january 2015.

- French Guiana:
1 quality control of laboratory procedures and data entry by a CDC MDB representative (C. Scheel) at the Cayenne Mycology laboratory from 2 to 6 february 2014.
1 quality control of laboratory procedures and data entry by a Colombian representative (D. Caceres) at the Cayenne Mycology laboratory from 13 to 29 november 2014.
- Guyana: Not performed since no funds were available to implement the study protocol.

D1. Translation of laboratory protocols for Fungal Culture:

- Suriname:
1 protocol was written and validated for the mycological examinations of bone marrow samples
1 protocol was written and validated for the mycological examinations of urine samples

- Guyana:
1 protocol was written and validated for the mycological examinations of bone marrow samples

D2. Translation of laboratory protocols for the CDC Histoplasma EIA test:

- Suriname:
1 protocol was written and validated for the EIA on urine
1 protocol was written and validated for the EIA on serum

- French Guiana:
1 protocol was written and validated for the EIA on urine
1 protocol was written and validated for the EIA on serum
- Guyana: Not performed since no funds were available to implement the study protocol.

E1. Equipment purchase for Fungal Culture:

- Suriname:
According to the validated protocol, equipments were purchased using the AFD funds

- Guyana:
According to the validated protocol, equipments were purchased using the TCC funds

E2. Equipment purchase for the CDC Histoplasma EIA test:

- Suriname:
According to the validated protocol, equipments were purchased using the ANRS-Inserm funds

- French Guiana:
According to the validated protocol, equipments were purchased using the European Union funds

- Guyana: Not performed since no funds were available to implement the study protocol.

3. Clinical practice in HIV patients in the Guiana Shield improved

A. Exploratory missions:

- Suriname: 1 exploratory mission of French Guiana coordinators in Paramaribo, Suriname, from 17 to 18 november 2011 was completed by a final drafting of the TCC trainings with a mission of French Guiana coordinators in Paramaribo, Suriname, from 23 to 26 January 2013.

- Guyana: 1 exploratory mission of French Guiana coordinators in Georgetown, Guyana, from 5 to 7 december 2012 was completed by a final drafting of the TCC trainings with a mission of French Guiana coordinators in Georgetown, Guyana, from 28 to 29 August 2014.

B. Clinical exchanges and trainings:

- Suriname:

1 clinical workshop with 2 representatives of French Guiana and 15 physicians of Suriname in BOG meeting room, from 22 to 24 november 2012.

1 clinical workshop with 2 representatives of French Guiana and 60 physicians from Suriname and Holland in AZP Hospital during the "18e Symposium Immunologie en infectieziekten" held from 24 to 26 january 2013 in Paramaribo, Suriname.

1 clinical workshop with 2 representatives of French Guiana and 60 physicians from Suriname and Holland in AZP Hospital during the "19e Symposium Immunologie en infectieziekten" held from 6 to 8 february 2014 in Paramaribo, Suriname.

- Guyana:

1 clinical workshop with 1 representative (Dr Adenis) of French Guiana and 15 physicians of Guyana in the National Public Health Insitute of Guyana, from 28 to 29 august 2014.

1 clinical workshop with trainings on bone marrow sampling and slide staining with 2 representatives (Dr Ramos and dr White) of Guyana and physicians of French Guiana in Cayenne Hospital, French Guiana, from 2 to 7 march 2015.

1 clinical workshop and 1 CME session with 1 representative (Dr Blanchet) of French Guiana and 30 physicians of Guyana in the National Public Health Insitute of Guyana, from 26 september to 6 october 2015.

- French Guiana:

1 clinical workshop with 2 representatives of the CDC MDB (Dr Chiller and Dr Scheel) and 15 physicians of French Guiana in Cayenne Hospital ER meeting room, from 8 to 12 february 2014.

1 clinical workshop with 1 representative of the National Reference Center for Mycoses and Antigungals at Paris Pasteur Institute and 50 physicians from French Guiana in Cayenne Hospital ER meeting room in the context of research seminar of Institut Pasteur de la Guyane entitled " Les infections fongiques profondes et plus particulièrement l'histoplasmose chez le patient infecté par le VIH" held from 14 to 16 january 2014.

C. Translation of clincial protocols:

- Suriname:

1 protocol was written and validated for the harmonization of bone marrow sampling (01/2012)

- Guyana:

1 protocol was written and validated for the harmonization of bone marrow sampling (03/2015)

4. Strategy for Histoplasmosis control developed

An international workshop entitled "Histoplasmosis in the Americas and the Caribbean, First meeting" (Appendix 3) will take place in Paramaribo, Suriname, from 4 to 6 december 2015, with the following objectives:

- To communicate about the Histoplasmosis situation in the Guiana Shield to clinicians and public health experts based on the data collected in the context of the present TCC and subsequent situation analysis conclusions.
- To share information on Histoplasmosis with health professionals from others interested countries from the Americas, the Caribbean and beyond.
- To prepare a strategy to control Histoplasmosis in HIV infected patients.

This international workshop is organized in 2 separate sessions:

- 4 december 2015, a one day workshop with 6 persons from the Guiana Shield countries involved in the TCC (2 persons per country), 3 PAHO representatives (CPC, PWR/SUR and PWR/GUY) and some international experts (8 persons, including CARPHA) to evaluate the present TCC and elaborate the strategic plan for Histoplasmosis control to be discussed with a broader group of experts during the following two days conference. Hence, by the end of this 3 days workshop, based on the meeting conclusions, a post meeting session is scheduled to consolidate the conclusions of the TCC evaluation and the drafting of the strategic plan for Histoplasmosis control.
- 5 to 6 december 2015, a two days conference with the full attendance for communication and discussion on Histoplasmosis.

The persons attending this workshop have been selected among professionals having published data on the subject and/or having participated or committed to participate to the international network being built since 2011 (i.e, HIV program managers of the Region, National and International organizations, including PAHO, CARPHA and the CDC etc.). Along with the Guiana Shield (including Venezuela and the Amapa State from Brazil), countries of the Amazon, the Americas and the Caribbean have been the priority target of this workshop. Between 75 and 100 persons will be attending the conference with at least 30 attendees coming from abroad.

Situation analysis required for the drafting up of the strategic plan is based on data collected in French Guiana, Guyana and Suriname in the context of the present TCC according to the monitoring and evaluation indicators listed in the approved project manuscript. The indicators to be analysed are summarized in the table in Appendix 4. However, caution should be taken when analyzing the purpose indicators: since not all histoplasmosis cases are currently being diagnosed in Suriname and Guyana, the first years are likely to be followed by an increase in the number of cases.

5. Network of trained health professionals reinforced in the Guiana Shield.

Since 2011, French Guiana coordinators have been traveling to the partner countries in the funded project and to the different reference centers for histoplasmosis, i.e. mainly those teams who have published HIV-associated case series in the literature, accross the Americas and the Caribbean, in order to identify areas of collaborations and build strong partnerships in the context of a network.

Here under are listed these centers:

- SURINAME*:

The Academisch Ziekenhuis Paramaribo (AZP), the Public Health Central Laboratory of Suriname, the Diakonessenhuis Hospital, the 'sLands hospital and the Foundation for Scientific Research Suriname (SWOS), Paramaribo, with monthly exchanges regarding several ongoing projects led close together (onsite missions in both countries, email, video and/or teleconference),

- GUYANA*:

The National Public Health Institute of Guyana (National Public Health Reference Laboratory and National Care and Treatment Center), the National HIV/AIDS Programme Secretariat of the Ministry of Health in Guyana, the Georgetown Public Hospital Corporation (GPHC), the Guyana Regional office of the Centers for Disease Control and Prevention, Georgetown, Guyana, with 3 onsite missions (2012, 2014, 2015), and since monthly exchanges regarding ongoing projects led close together (onsite missions in both countries, email, video and/or teleconference),

- USA*:

The Mycotic Diseases Branch of the Centers for Disease Control and Prevention, Atlanta, with 2 onsite missions (12/2011 and 10/2015) and 1 mission of CDC representatives in Paramaribo and Cayenne (02/2014), and monthly exchanges regarding several ongoing projects led close together (onsite missions in both countries, email, video and/or teleconference),

IMMY biotechnology company, Oklahoma City, with 1 onsite mission (23 to 25 october 2015) and priorly regular exchanges on technical support and submission of collaborative projects to NIH call for funds, unsuccessfully in 2014 (email, video and/or teleconference)

- COLOMBIA*:

The Corporación para Investigaciones Biológicas (CIB), Medellin, and the Del Rosario University, Bogota, with 1 onsite mission 1 to 7 april 2014 and since regular exchanges regarding several ancillary studies of the ANRS12260 research project and on a collaborative project together with the Colombian team (onsite mission, email,video and/or teleconference),

- GUATEMALA:

The Asociacion de Salud Integral and the Clínica Familiar "Luis Angel García", Asociación de Salud Integral, Guatemala City, with 1 onsite mission 25 to 30 May 2015 and since regular exchanges regarding a collaborative project together with the Colombian team (onsite mission, email, video and/or teleconference),

- BRAZIL:

Macapa, state of Amapa*,

The National Public Health Laboratory of Amapa (LACEN AP), with 1 onsite mission 19 to 26 august 2012, several visit of brazilian counterparts in Cayenne in the context of the trans border cooperation between France and Brazil, regular exchanges regarding technical support and updates on our activities in order to extend ANRS 12260 research project and a molecular epidemiology project (onsite mission, trans border cooperation meetings, email),

Belem, state of Para*,

The Instituto Evandro Chagas - IEC/SVS/MS Seção de Bacteriologia e Micologia- SABMI Laboratório de Micologia, Belem, with 1 onsite mission 19 to 26 august 2012 and since regular exchanges regarding technical support and updates on our activities in order to extend ANRS 12260 research project and a molecular epidemiology project (onsite mission, trans border cooperation

meetings, email),

Fortaleza, state of Ceara,

The Federal University of Ceará, Fortaleza, with 1 onsite mission 15 to 17 june 2015 and since regular exchanges regarding technical support and updates on our activities in order to extend ANRS 12260 research project and a molecular epidemiology project (onsite mission, email),

Manaus, state of Amazonas,

The Instituto Leônidas e Maria Deane (Fiocruz - Amazônia) and the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil, with 1 onsite mission 20 to 26 june 2015 and since regular exchanges regarding technical support and updates on our activities in order to extend ANRS 12260 research project and a molecular epidemiology project (onsite mission, email),

Porto Alegre, state of Rio Grande Do Sul,

The Hospital Dom Vicente Scherer (HDVS), Molecular Biology Laboratory, Santa Casa de Porto Alegre, with regular exchanges regarding technical support and updates on our activities (email),

Rio de Janeiro, state of Rio de Janeiro,

The Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, with 1 onsite mission 5 to 9 november 2013 and since regular exchanges regarding technical support and updates on our activities in order to extend ANRS 12260 research project and a molecular epidemiology project (onsite mission, email),

- TRINIDAD & TOBAGO*:

The HIV and AIDS Coordinating Unit of the Ministry of Health of Trinidad & Tobago and the Caribbean Public Health Agency (CARPHA), Port of Spain, with 1 onsite mission 30 april to 3 May 2013 and since regular exchanges regarding technical support and updates on our activities (onsite mission, email),

- ARGENTINA:

The INEI-ANLIS ‘‘Dr. Carlos G. Malbran’’ and the Hospital de Clinicas "Jose de San Martin", Infectious Diseases Division, Buenos aires, with regular exchanges regarding technical support and updates on our activities (email),

- VENEZUELA:

The Instituto Nacional de Hygiene Rafael Rangel, Caracas, with regular exchanges regarding technical support and updates on our activities (email),

- PANAMA:

The Hospital Santo Tomas, Infectious Diseases Division, Panama City, with regular exchanges regarding technical support and updates on our activities (email),

- MEXICO:

The Universidad Nacional Autonoma de Mexico, Laboratorio de Immunologia de Hongros, Mexico City, with regular exchanges regarding technical support and updates on our activities (email),

- FRANCE mainland:

The National Reference Center for Mycoses and Antigungals, Pasteur Institute in Paris, and the Mycology Laboratory of Necker Hospital, Paris, 1 onsite mission (9 to 16 november 2015) and the visit of Pr Lortholary in Cayenne (14 to 16 february 2014), and regulard exchanges on tehcnical support and collaborative project led close together on B-D-Glucan and Platelia Aspergillus BIORAD screening (onsite missions in both countries, email, video and/or teleconference),

- FRENCH GUIANA (French overseas department)*:

Part of the network are representatives of the Centre Hospitalier de Cayenne Andrée Rosemon, the Centre Hospitalier de l'Ouest Guyanais Franck Joly, the Université de Guyane, the Centre d'Etudes de la Biodiversité Amazonienne (CEBA)

- SWITZERLAND:

The Global Action Fund for Fungal Infection (GAFFI), Geneva, with exchanges regarding technical support and updates on our activities (email),

Hence, uplisted countries represented with a (*) were first gathered in the context of an international network meeting in Suriname "The First Histoplasmosis International Network (HiNET) meeting", held in Paramaribo, Suriname, 17-18 October 2013 (Appendix 1), and all of uplisted countries and instutions will be gathered in the context of the TCC workshop held in Paramaribo, Suriname, from 4 to 6 december 2015, entitled: "Histoplasmosis in the Americas and the Caribbean, First meeting" (Appendix 3).

b) Specific products / Productos específicos

- Implementation of an international network meeting in Suriname "The First Histoplasmosis International Network (HiNET)" meeting, held in Paramaribo, Suriname, 17-18 October 2013, leading to a strategic plan document written by attendees (Appendix 1),

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** Section 1.04 **No.** **Section 1.03 INFORME FINAL DE PROYECTO CTP**	**Section 1.05 Date:** **Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

- Constitution of a biological sample collection of urines and serum (in Suriname and French Guiana) for ancillary studies with a potential of international collaborations:
  1. Urine samples were already used to test the first version of a Histoplasmosis Monoclonal EIA test in collaboration with the biotechnology company IMMY, Oklahoma City, USA.
  2. Serum samples were alreday tested with a serology immunodiffusion test developped by the Corporación para Investigaciones Biológicas (CIB), Medellin, Colombia, with a B-D-glucan test and a Platelia Aspergillus BIORAD test in partnership with Paris Pasteur Insitute, France, and with the CrAg LFA test developped by IMMY, USA.
  3. A submission to the 2016 first NIH call for funds of a proposal on an international validation study (Suriname, French Guiana together with USA, Guatemala and Colombia) for a Histoplasmosis monoclonal EIA test developped by the US biotechnology company IMMY is being written.
- Constitution of a biological sample collection of Histoplasma strains identified in culture tubes (13 in Suriname, 2 in Guyana and 80 in French Guiana) for ancillary studies with a potential of international collaborations:
  1. One PhD programme is currently funded in French Guiana with a doctoral grant from the Université de Guyane and a programme grant from the Ministère français de l'Outre-Mer (23 000€) . The objective is to describe the molecular epidemiology of Histoplasma strains identified on the Guiana Shield (French Guiana, Suriname and Guyana),
  2. International collaboration are ongoing (Colombia, Guatemala, the Brazilian states of Amapa, Para, Ceara, amazonas and Rio de Janeiro) with research teams who sent strains to Cayenne in order to extend the project to a South American scale,
- International publications with representatives of the network built in the context of the TCC (Appendix 2),
- Implementation of a "Epidemiology and Biostatistics in Tropical Medicine" summer course, held in Paramaribo, Suriname, 26-30 January 2015, with 20 Surinamese attendees and one trainer (Pr M. Nacher, Inserm CIC1424, Cayenne, French Guiana).

c) Additional achievements / Logros adicionales

- 1 PhD student leading a programme on the epidemiology of histoplasmosis in Suriname,
- 1 PhD student leading a programme on the epidemiology of histoplasmosis in French Guiana,
- 1 PhD, Microbiologist, from the Trinidad & Tobago National Public Health Laboratory was trained on the CDC Histoplasma EIA in the National Public Health Central Laboratory of Suriname, 14-18 October 2013,
- 1 Symposium entitled "Histoplasmosis and HIV in the Americas: The fight against a neglected fungal disease" was held at the 64th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Philadelphia, 28 october 2015.
- 1"Colloque" Grant from ANRS-Inserm (3000€), 1 "Meeting support" grant from GAFFI (1500 USD) and 1 "Meeting support" grant from IMMY (3500 USD) were obtained as additional funds for the organization of the 2015 TCC workshop (Appendix 3).
- The advocacy work, the scientific publications on the topic of Histoplasmosis in Latin America have raised the interest of diagnostic companies, who have an interest in developping diagnostic tools (ELISA, dipstick) that are adapted to this potential new market. This will help achieve the goal of controlling histoplasmosis.

d) Limitations / Limitaciones

Despite answering ANRS-Inserm call for funds two times in 2014 and trying to get CDC headquarters funding the activity through CDC regional office in Guyana, we didn't manage to find any money to achieve the objective of "supporting the implementation of the new CDC ELISA test in Guyana in terms of laboratory training and small equipment purchase" in order to extend the research activity led by Suriname and French Guiana on the CDC Histoplasma EIA on urine and serum of HIV-infected individuals in Georgetown, Guyana.

Laboratory equipment purchase in Suriname and Guyana took a long time (at least one year), explaining the delays in the implementation of trainings.
International cooperation in the region is very labor intensive since few people already crowded with work are available to develop new strategies and since traveling is time consuming due to the distance between centers.
As long as countries do not include histoplasmosis as a strategic objective, with indicators to monitor and evaluate their actions, it will be difficult for clinicians on the ground to overcome the lack of diagnostic tools, lack of effective treatments, lack of data. This would be facilitated if UNAIDS PAHO and other international organizations made recommendations to amend strategic plans and add the reduction of histoplasmosis mortality as a strategic objective.

e) Total budget and amount spent / Presupuesto total y ejecutado

60 000 USD asked and 50 000 USD obtained in 11/2013.
At the time of monitoring and evaluation of the TCC, 100% of the TCC budget per country was spent.

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** Section 1.04 **No.** **Section 1.03 INFORME FINAL DE PROYECTO CTP**	**Section 1.05 Date: Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

Year	Activities	CPC	PWR/SUR	PWR/GUY	Total
2013	Laboratory and clinical training			20,500 US$	20,500 US$
2014	Laboratory and clinical training			8,000 US$	8,000 US$
2015	Situation analysis and sub-regional workshop	2,000 US$	15,000 US$	1,500 US$	18,500US$
	Evaluation	3,000 US$			3,000 US$
Total given by PAHO (11/2013)		5,000 US$	15,000 US$	30,000 US$	**50,000 US$**
Total spent by the evaluation of the TCC (12/2015)		5,000 US	15,000 US$	30,000 US$	**50,000 US$**

4. STAKEHOLDERS INVOLVED, E.G. MINISTRIES, INSTITUTES, FOUNDATIONS, NGOS / ACTORES INVOLUCRADOS, EJ: MINISTERIOS, INSTITUTOS, FUNDACIONES, ONGS.

Academisch Ziekenhuis Paramaribo, Suriname,
Agence Régionale de Santé Guyane, Cayenne, France,
Agence Française de Développement, AFD, Paramaribo, Suriname,
Asociacion de Salud Integral, Guatemala City, Guatemala,
Caribbean Public Health Agency (CARPHA), Port of Spain, Trinidad & Tobago,
Centre d'Etudes de la Biodiversité Amazonienne, Labex CEBA, Cayenne, France,
Centre Hospitalier de Cayenne Andrée Rosemon (CHAR), Cayenne, France,
Central America Regional Office of the Centers for Diseases Control and prevention, Guatemala City, Guatemala,
Clinical Investigation Center Clinical Epidemiology Inserm CIC1424, Cayenne, French Guiana,
Clínica Familiar "Luis Angel García", Asociación de Salud Integral, Guatemala city, Guatemala
Corporación para Investigaciones Biológicas (CIB), Medellin, Colombia,
Del Rosario University, Bogota, Colombia,
Diakonessenhuis, Paramaribo, Suriname,
Federal University of Ceará, Fortaleza, Brazil
Foundation for Scientific Research Suriname (SWOS), Paramaribo, Suriname,
Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil,
Georgetown Public Hospital Corporation (GPHC), Georgetown, Guyana,
Global Action Fund for Fungal Infection (GAFFI), Geneva, Switzerland,
HIV and AIDS Coordinating Unit, Ministry of Health of Trinidad & Tobago, Port of Spain, Trinidad & Tobago,
Hospital Dom Vicente Scherer (HDVS), Molecular Biology Laboratory, Santa Casa de Porto Alegre, Brazil,
Hospital de Clinicas "Jose de San Martin", Infectious Diseases Division, Buenos aires, Argentina,
Hospital Santo Tomas, Infectious Diseases Division, Panama City, Panama,
IMMY biotechnology company, Oklahoma City, USA,
Instituto Nacional de Hygiene Rafael Rangel, Caracas, Venezuela,
Instituto Leônidas e Maria Deane (Fiocruz - Amazônia), Manaus, Brazil,
Instituto Evandro Chagas - IEC/SVS/MS Seção de Bacteriologia e Micologia- SABMI Laboratório de Micologia, Belem, Brazil,
INEI-ANLIS ''Dr. Carlos G. Malbran'', Buenos Aires, Argentina,
Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, Brazil,

Section 1.01 **PWR:**	Section 1.02 **TCC PROJECT FINAL REPORT** **Section 1.03 INFORME FINAL DE PROYECTO CTP** Section 1.04 **No.**	**Section 1.05 Date: Fecha:**

Section 1.06 Pan American Health Organization/Organización Panamericana de la Salud
Article II. TCC FINAL REPORT / INFORME FINAL DE PROYECTO CTP
Article III. SUMMARY SHEET / RESUMEN

Minsitry of Health in Suriname, Paramaribo, Suriname,
Ministry of Health in Guyana, Georgetown, Guyana,
Mycotic Diseases Branch of the Centers for Disease Control and Prevention, Atlanta, USA,
National HIV/AIDS Programme Secretariat, Ministry of Health, Georgetown, Guyana,
National Public Health Institute of Guyana, Georgetown, Guyana
National Public Health Reference Laboratory, Georgetown, Guyana
National Care and Treatment Center, Georgetown, Guyana,
National Public Health Laboratory of Amapa, Macapa, Brazil,
National Reference Center for Mycoses and Antigungals, Pasteur Institute, Paris, France
Public Health Central Laboratory of Suriname, Paramaribo, Suriname,
Regional office of the Centers for Disease Control and Prevention, Georgetown, Guyana,
'sLands Hospital, Paramaribo, Suriname,
Universidad Nacional Autonoma de Mexico, Laboratorio de Immunologia de Hongros, Mexico City, Mexico,
Université de la Guyane, Cayenne, France,
Western French Guiana Hospital, Saint Laurent du Maroni, French Guiana.

5. LESSONS LEARNED / LECCIONES APRENDIDAS

1. Histoplasmosis is prevalent in HIV individuals in Suriname, Guyana and French Guiana.
2. Histoplasmosis might be the 1st AIDS-defining event in Suriname and Guyana, as it is in French Guiana,
3. Mortality due to histoplasmosis is high and mostly underestimated in Suriname and Guyana,
4. Strategy to control histoplasmosis is feasible since this TCC was implemented with great achievements due to the motivation and collaborative strong will of the three teams,
5. International collaboration accross the Guiana Shield is expensive and labor intensive due to the time required to travel from one place to another,
6. International regulations for airplane transportation of Class III infectious agents, like Histoplasma, make it difficult and expensive sharing strains between countries for international collaborations,
7. Although this TCC concerns the Guiana Shield, the networking activities and the published literatura show that the problem is one that concerns most of Latin America. Each year the burden of HIV-associated histoplasmosis is expected to represent 5 000-10 000 deaths in Latin America, an order of magnitude that is on par with HIV-associated tuberculosis deaths. Yet histoplasmosis is not a strategic objective in most endemic countries's HIV/AIDS plan.

6. CONCLUSIONS AND RECOMMENDATIONS / CONCLUSIONES Y RECOMENDACIONES

Conclusions

1. Histoplasmosis is highly prevalent in HIV individuals accross the Guiana Shield (as long as you look for it!).
2. HIV-infected individuals experiencing histoplasmosis are dying from an undiagnosed and untreated disease since confusion with tuberculosis is the rule,
3. Histoplasmosis is a neglected disease accross the Guiana Shield and the Americas due to the lack of awareness of physicans, the lack of Medical Mycology facilities and a difficult access to effective antifungal therapeutics, out of a few number of medical centers with both the knowledge and the factilities for the care and treatment of patients.
4. The networking activities around the TCC have accelerated the realization that a major cause of death of HIV patients has been overlooked. This has raised the attention of the industry. The national and international public health authorities should also take it from here to scale up this low cost pilot initiative.

Recommendations

Non invasive affordable tests are coming on the market, more are being devised. This should facilitate data collection for the mapping of the burden of histoplasmosis throughout Latin America. This data will accelerate awareness. The marked increase in the number of cases implies treatment. The best treatment for severe histoplasmosis is liposomal amphotericin B (Ambisome) which is not available for histoplasmosis in Latin America except in French Guiana. Lobbying efforts should be performed with Gilead to obtain price reductions. Data will help such lobbying efforts.
In our networking experience, public health authorities, and AIDS programmes are still largely oblivious or ignorant of the burden of histoplasmosis, which does not appear in HIV/AIDS plans' strategic objectives throughout the continent. Ultimately all plans should be amended to include this neglected disease, the strategic objective could be: reduce the mortality of HIV patients due to disseminated histoplasmosis.
PAHO is ideally placed to spearhead this recommendation throughout Latin America.
Histoplasmosis confounds the interpretation of tuberculosis statistics among HIV patients. Because it looks like tuberculosis, it inflates tuberculosis statistics of incidence, resistance, and mortality. Having a clear view of histoplasmosis would thus help getting a clear view of tuberculosis in HIV patients. Ideally TB clinics throughout Latin America should systematically screen for histoplasmosis.

APPENDIX 1:

First HiNET meeting, October 17th-18th, 2013, Paramaribo

Meeting Report

In the Guianas and the Amazon Basin, the prevalence of human immunodeficiency virus (HIV) is approximately 1%. Given an estimated incidence of histoplasmosis in HIV patients of 1.5 per 100 person years and a historical mortality rate of 30% (can be higher!) then, in the Amazon region alone 1500 HIV patients would be expected to develop histoplasmosis and 600 die from it, every year. The development of diagnostic capacity and the adaptation of clinical guidelines to entail this hidden killer are necessary to reduce deaths from histoplasmosis. This is the ultimate goal of the HiNET meeting that took place in Paramaribo between partners of Brazil, Colombia, French Guiana, Guyana, Suriname, and Trinidad and Tobago. The meeting was funded by the French Guiana Health Regional Agency (ARS) and PAHO graciously facilitated the meeting. It was simultaneous to the training of Suriname's Central Laboratory technicians for the CDC ELISA test in the context of the ANRS/EU EDIRAPHIS 12260 research project.
The workshop aimed to find concrete steps and goals for each country, and to identify funding needs. Short presentations allowed participants to get a glimpse of other countries situation regarding HIV and histoplasmosis.

Vision: to improve diagnosis and reduce mortality from disseminated histoplasmosis through regional collaboration

Mission:
- To develop training for clinicians on the diagnosis and treatment of disseminated histoplasmosis in HIV patients
- To develop training for laboratory staff regarding the implementation of standard mycology (direct examination and culture) and the CDC ELISA test for Histoplasma antigen detection.
- To meet at regular intervals to exchange data and update regional objectives regarding histoplasmosis

Workshop .Participants:

Group 1: Ayanna Sebro, Sigrid Mac Donald, Chantal Hermine, Merril Wongsokarijo, Vincent Vantilcke, Mathieu Nacher, Rachida Boukhari, Rachel Eersel, Laure Garancher, Shanti Singh, Mathieu Nacher

Group2 : Margarete Gomes, Rosiléne Malcher, Maurimélia Costa, Silvia Marques, Christine Aznar, Pierre Couppié, Anaïs Lalliaume, Gustavo Bretas, Antoine Adenis

Common diagnostic methods
Standard mycology (direct examination and culture) & CDC ELISA Histoplasma antigen detection
Expand the ongoing project between French Guiana and Suriname to the region, piggyback or expand planned trainings.

Training
Brazil, Guyana and Trinidad

Laboratory practices:
2 trainings a year (initial training and quality control visit) for 2 participants/country max

Standard mycology (direct examination and culture)
Trainers: Christine Aznar, Denis Blanchet, Rachida Boukhari, Maurimélia Costa, Silvia Marques
Trainees : 2 maximum per country laboratory with english skills
Prior to the Standard mycology training, French Guiana and Para should first harmonize their practices using the protocol for the treatment of a bone marrow sample built within French Guiana and Suriname.
Then 2 persons from LACEN Amapa will be trained in Evandro Chagas Institute with a trainers team from Evandro Chagas Institute and the Cayenne Mycology Laboratory. The initial training will last 4 daysI in Evandro Chagas Institute.
A few months after starting treating samples in standard mycology in LACEN Amapa, an onsite visit in LACEN Amapa will allow to follow the implementation and to help resolving field problems in Macapa.
However, prior to training, the LACEN must acquire equipment in order to be able to implement culture (a laminar flow cabinet, 2 incubators, one variable temperature "bain marie" and cytocentrifugation equipment). French Guiana will work in close collaboration with LACEN Amapa and Evandro Chagas Institute in order to finalize the equipement list to be purchased.

CDC ELISA test for the polyclonal detection of Histoplasma antigen in urine
Trainers: Beatriz Gomez, Christina Scheel.
Trainees : 2 maximum per country laboratory with english skills and serology skills
2 from Guyana (fisrt training) and 2 from Trinidad (first training) in Port of Spain or Georgetown
2 from Suriname (second training) in Paramaribo
One from French Guiana (second training) in Cayenne
2 from Belem and 2 from Amapa in Belem, which will facilitate the translations (first training)

Proficiency tests, protocols, standard operating practices, branded manuals are important for administrators notably if funds are to be obtained for transportation of reagents and for trainers to come in the country.
Panel of samples of proven histoplasmosis cases (identified by fungal culture) should be blinded and sent to collaborating laboratories at regular intervals to control quality.
Once trainees have practiced the ELISA test several times and that quality control (trainers onsite visit occurring 6 months after starting running the ELISA test) shows that they are proficient, they may start giving results to clinicians.
For Brazil, first Evandro Chagas will implement and validate the ELISA test for the region (including Macapa). LACEN Amapa trainees will follow the training in Belem first in order to implement the ELISA test in Macapa in a short time after validation of the test by the Evandro Chagas Institute.

Also, the Evandro Chagas Institute Mycology unit expressed interest in starting the production of polyclonal antibodies to make the test locally.

It is important that staff from a given country be trained together (personnel turnover) on the site were they will be using the technique. By the same way, it would also be important to train trainers so that, in a region where staff turnover is high, new lab technicians could take over.

Clinical or medical practices

Trainers: Vincent Vantilcke and Pierre Couppié
Trainees: medical doctors, surgeons, residents and medical students involved in the care and treatment of HIV-infected patients in each country
For Brazil, one infectious disease physician from Macapa and from Belem could come first to Cayenne in order to look at the local practices/investigations and to define the detailed medical practices training programme to be implemented in their respective context: framework for the visit of physicians from French Guiana in Belem and Macapa for on site training.
For Amapa, there is a need to train the clinicians on the procedure of bone marrow aspiration.
Physicians must be trained to develop local care and treatment algorithms for histoplasmosis.
Thus, it **would be important to develop guidelines** for them and harmonize practices between Guiana Shield stakeholders.

It would be good, if possible, if laboratory and clinical trainings occurred at the same time.

Favour staff meetings with both laboratory and clinical representatives, apply for a special session in Guyana that would deliver CME credits to attending physicians.
E-learning, website could be implement so that protocols up to date and experiences could be shared by stakeholders.
For Belem, Brazil, the problem of clinicians not prescribing fungal explorations upon patient admission is an important problem. Reaching clinicians is key to implement the medical practices strategies in order to improve the patients prognosis. French Guiana team, will meet Belem clinicians interested in Mycology on November 4th 2013.

Immediate Actions

Acquire equipment for standard mycology (send detailed list and invoices to Dr Singh, Dr Sebro and LACEN Amapa) in Amapa state, in Guyana, and in Trinidad. Harmonize culture methods between Cayenne and Belem (Cayenne can send protocol).

Acquire equipment for CDC ELISA test (send detailed list and invoices to Dr Singh, Dr Sebro and LACEN Amapa) in Amapa state, in Guyana, and in Trinidad. For Evandro Chagas institute all seems ready for training but verify if all elements in the detailed list are available. Dr Gomez proposed that prior to the trainings an accurate evaluation of the equipment available and those to be purchased could be made by her and/or Dr Scheel using teleconference system.

Identify medical focal point for Belem.
Identify trainees in Guyana and Trinidad.
Once mandatory equipment and reagents available on site, start trainings

Start testing patients as soon as possible, control quality before giving test results to physicians.

Monitoring and evaluation

Number of persons trained
Number of patients tested
-standard mycology
-ELISA test
Number of AIDS patients per year
Number of patients with proven (culture) and probable (ELISA) histoplasmosis per year
Treatment of patients (itraconazole, amphotericin B)
Proportion of patients with histoplasmosis that are alive at one month
Sensitivity specificity versus gold standard
What antifungals available in the region and what is their cost
For Guyana and Trinidad data collection should not be a problem. For French Guiana and Suriname it is included in the research project. For Amapa it should not be a problem. For Para no problem for the laboratory part but for the clinical part a focal point should be identified. A physician with a master's degree in mycology is a potential candidate and should be approached to determine if she could collect the data on the clinical side (decision on November 4th 2013 in Belem).

Research

Difficult at this stage to do more than the M&E data but fungal culture strains and urine/serum biological collections can be pooled and exchanged to look at molecular epidemiology or other studies on histoplasmosis.
For the ELISA test in Amapa, research is an interest and there are potential PhD students. For Guyana, Trinidad and Para the ELISA is intended for immediate routine use in diagnosis to be able to give an earlier diagnosis so that clinicians have incentives to prescribe more diagnostic tests.

Expected risks

CDC supplying enough reagents for the region should not be a problem. Beatriz Gomez is trying to start producing reagents in Columbia which would be a backup safety net. If there was a need to obtain more reagents pooling resources to facilitate production for the region. However, once awareness is there even if there is no more CDC test, other tests will be there, it is possible that in a future a dipstick will exist but that should not stop from starting diagnosing and treating patients as soon as possible.

Hi-NET & legal structure

The legal structure is mostly intended to receive funds facilitating the goals of meeting, and networking between members of the working group.
In French Guiana an Association was created (HiNET Guyane) but there are no funds so far (none demanded).
Suriname has an existing Foundation (SWOS) that could be used.

What funds

The TCC project, funded by PAHO, will fund standard mycology equipments and trainings for Guyana.
Another TCC could help fund the initiation with Trinidad.
The French Guiana Health Regional Agency (ARS) funds if they are sustained could help
For Amapa, funds are needed for equipment (standard mycology and ELISA) and for transportation of trainees to Belem, trainers to Belem, ELISA reagents from CDC to Belem and Macapa, samples from Macapa to Belem.
Other possible funders have to be identified in each country by stakeholders

Coordinating structure

Simple Email could inform different focal points about progress, enquire about training dates, problems…
Website could help.

Next meeting

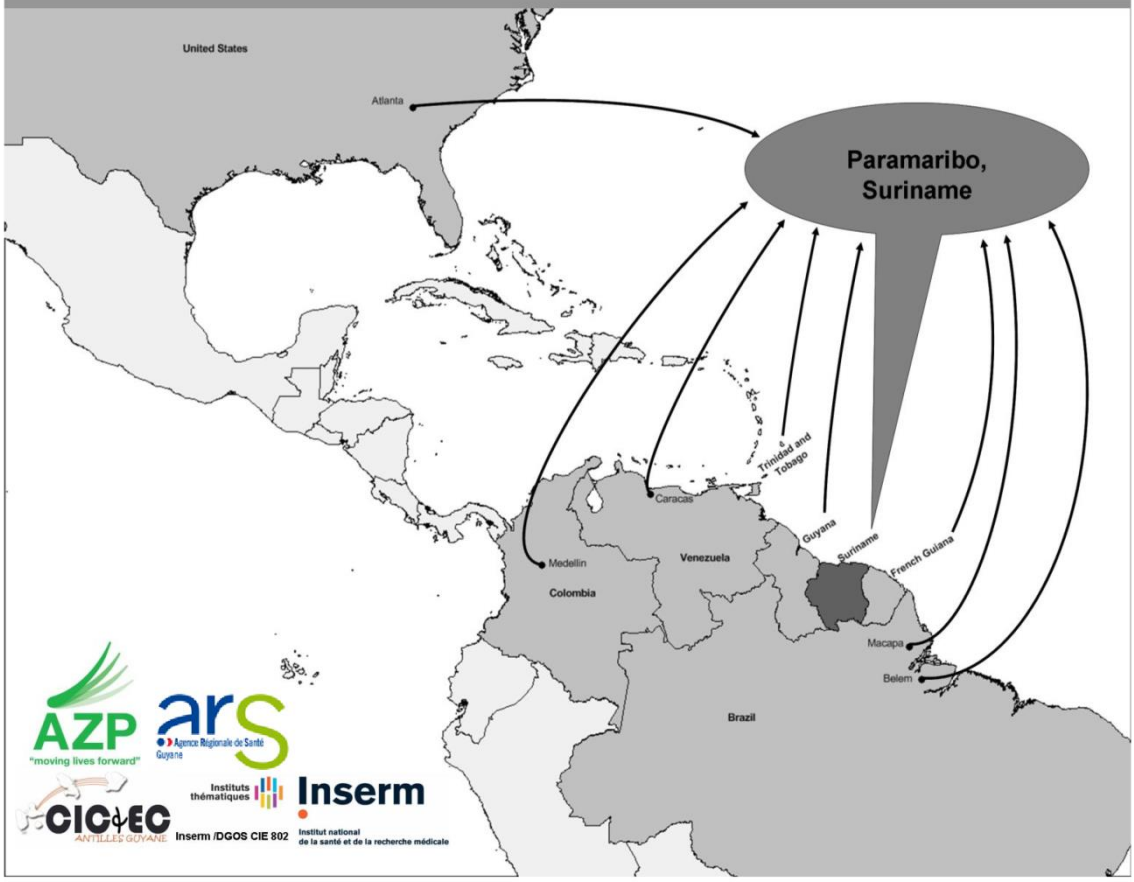
At the end of the TCC Suriname/Guyana/French Guiana on histoplasmosis, in 2015, an international meeting is planned to show data from the different countries this would be a suitable occasion for another HiNET meeting.

	Brazil	Guyana	Trinidad	Suriname	French Guiana
Training	Training in Evandro Chagas institute for standard method for LACEN AMAPA (standardization of culture with French Guiana) + ELISA Evandro Chagas+ LACEN AMAPA (dates to be determined once required equipment is acquired by LACEN Amapa)	Training in FG for standard method (TCC project) + ELISA training in Georgetown (dates to be determined once required equipment is received)	Training in FG for standard method + ELISA training in Port of Spain (dates to be determined once required equipment is received)	Standard method n°2 (1st semester 2014) ELISA Training n°2 in February (tentative date)	ELISA Training n°2 in February (tentative date)
Purchases	In AMAPA need for Equipment for standard mycology and for ELISA Transportation (trainees, trainers, reagents, samples)	Guyana to acquire equipment for standard mycology (TCC) and for ELISA	Equipment for standard mycology and for ELISA		
Funds	Amapa must secure funds to acquire equipment for standard mycology and for ELISA (estimate 100 000 euros)	Standard mycology (TCC funds) ELISA test, funds to be identified	Trinidad must secure funds to acquire equipment for standard mycology and for ELISA		
	Funds for transport & stay of trainers	Funds for transport & stay of trainers	Funds for transport & stay of trainers		

Histoplasmosis International NETwork (HiNET)

1st Meeting in Paramaribo, Suriname,

October 17th & 18th 2013



Coordination & contacts :

Dr S. Vreden, MD, PhD, Foundation for Scientific Research Suriname (SWOS), stephenvreden@yahoo.com,
Pr Nacher, MD, PhD, Cayenne General Hospital, mathieu.nacher@ch-cayenne.fr,
Dr Mac Donald, MD, Foundation for Scientific Research Suriname (SWOS), sigi_macdonald@live.com,
Dr Adenis, MD, MPH, Cayenne General Hospital, antoine.adenis@gmail.com,

The Foundation for Scientific Research in Suriname
and
The Clinical Investigation Center Clinical Epidemiology Inserm CIC1424
are glad to announce the

Histoplasmosis International Network (HiNET)
1st meeting

Date:

October 17th & 18th 2013

Location:

Academisch Ziekenhuis Paramaribo Hospital Conference Room, Paramaribo, Suriname.

Programme:

Thursday 17th October 2013 Public Meeting – Open access	Friday 18th October 2013 Technical Meeting – Restricted access
19:00-19:20 Registration	**16:00-16:20 Registration**
19:20-19:30 Opening speech	**16:20-16:30 Opening speech**
19:30-20:45 Situation analysis of histoplasmosis and fungal diseases on the Guiana Shield	**16:30-18:45 Countries SWOT analysis for the diagnosis of histoplasmosis (Part 1 and 2)**
20:45-21:00 Break	**18:45-18:55 Break**
20:50-22:00 Clinical management and current options for the diagnosis of histoplasmosis. Overview of ongoing projects in the greater Caribbean and the Guiana Shield.	**18:55-20:00 Discussions on the perspectives and the next steps for the diagnosis of histoplasmosis Current options for a network of practitioners in the field of fungal diseases.**

International Guests institutions:

Academisch Ziekenhuis Paramaribo, Suriname,
Biological Investigation Corporation (CIB), Medellin, Colombia,
Cayenne General Hospital, Cayenne, French Guiana,
Centers for Disease Control and Prevention, Atlanta, USA,
Clinical Investigation Center Clinical Epidemiology Inserm CIE 802, Cayenne, French Guiana,
Del Rosario University, Bogota, Colombia,
Diakonessenhuis, Paramaribo, Suriname,
Evandro Chagas Insitute, Belem, Brazil,
Foundation for Scientific Research Suriname (SWOS), Paramaribo, Suriname,
French Guiana Health Regional Agency, Cayenne, French Guiana,
HIV and AIDS Coordinating Unit, Ministry of Health of Trinidad & Tobago, Port of Spain, Trinidad & Tobago,
Instituto Nacional de Hygiene Rafael Rangel, Caracas, Venezuela,
National HIV/AIDS Programme Secretariat, Ministry of Health, Georgetown, Guyana,
National Public Health Laboratory of Amapa, Macapa, Brazil,
Public Health Central Laboratory of Suriname, Paramaribo, Suriname,
's Lands Hospital, Paramaribo, Suriname,
Western French Guiana Hospital, Saint Laurent du Maroni, French Guiana.

Histoplasmosis International Network (HiNET)
1st meeting

17th October 2013 – Day 1 Programme

Time	Topic	Facilitators Drs M. Van Eer & Prof M. Nacher
19:00–19:20	**Registration**	
19:20–19:30	**Welcome**	Director of AZP
19:30-19:50	Epidemiology and situation analysis of histoplasmosis in the Guyana Shield	Prof. M. Nacher (Inserm CIE802, French Guiana)
19:50-20:10	Epidemiology and situation analysis of histoplasmosis in the Americas	Dr T. Chiller & Dr C Scheel (CDC Mycotic Diseases Branch, USA)
20:10-20:30	Clinical Histoplasmosis and antifungal therapy management	Drs M. Van Eer (SWOS, Suriname)
20:30-20:45	Discussion	
20.45-21.00	**Break**	
21:00-21:30	Current options for the diagnosis of Histoplasmosis in immunosuppressed and non immunosuppressed patients	Prof B. Gomez (Universidad del Rosario, Colombia)
21:30-21:50	Ongoing Histoplasmosis projects in the Guiana Shield - ANRS 12260 EDIRAPHIS and, - Cooperation projects.	Drs M.S. Mac Donald (SWOS, Suriname) & Drs A. Adenis (Inserm CIE802, French Guiana)
21:50–22:20	Discussion	
22:20–22:30	**Closing remarks**	

Histoplasmosis International Network (HiNET)
1st meeting

18th October 2013 – Day 2 Programme

Time	Topic	Facilitators Drs M. Van Eer & Prof M. Nacher
16:00–16:20	**Registration**	
16:20–16:30	**Welcome** **and introduction on the objectives of the meeting**	Drs M. Van Eer (SWOS, Suriname) and Prof. M Nacher (Inserm CIE802, French Guiana)
16:30–16:40	**Brazil, Para**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Marques and Drs Da Costa (IEC, Belem, Brazil)
16:40–16:50	**Brazil, Amapa**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Gomes and Drs Malcher (LACEN AP, Macapa, Brazil)
16:50–17:00	**Suriname**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Van Eer (Center of Excellence, Suriname) and Drs Hermelijn (AZP Hospital, Suriname)
17:00–17:10	**Guyana** situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Singh (NAPS, Guyana)
17:10–17:20	**Trinidad & Tobago** situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Sebro (HACU, Trinidad & Tobago)
17:20–17:30	**Venezuela** situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Mrs Garcia (INHRR, Venezuela)
17:30–17:45	**Break**	
17:45–17:55	**French Guiana, Cayenne**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Prof Couppié and Drs Aznar (Cayenne Hospital, French Guiana)
17:55–18:05	**French Guiana, Saint Laurent du Maroni**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Drs Vantilcke and Drs Boukhari (Cayenne Hospital, French Guiana)
18:05–18:15	**Colombia**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Prof Gomez (Universidad del Rosario, Colombia)
18:15–18:25	**USA**, situation analysis for the diagnosis of histoplasmosis: SWOT analysis and perspectives	Dr T Chiller & Dr C Scheel (CDC Mycotic Diseases Branch, USA)
18:25–18:40	**Summary of countries SWOT analysis**	Prof. M Nacher (Inserm CIE802, French Guiana)
18:40–18:55	**Break**	
18:55–19:45	**Discussions on the next steps:** - perspectives for the diagnosis of Histoplasmosis in the Guiana Shield - current options for a network of practitioners in the field of fungal diseases	
19:45–20:00	**1st HiNET Meeting conclusions**	**Drs M. Van Eer** (SWOS, Suriname)

APPENDIX 2:

Publication #1

OPEN ACCESS Freely available online

PLOS | NEGLECTED TROPICAL DISEASES

Editorial

Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage

Mathieu Nacher[1,2]*, Antoine Adenis[1,2], Sigrid Mc Donald[3], Margarete Do Socorro Mendonca Gomes[4], Shanti Singh[5], Ivina Lopes Lima[4], Rosilene Malcher Leite[4], Sandra Hermelijn[3], Merril Wongsokarijo[6], Marja Van Eer[7], Silvia Marques Da Silva[8], Maurimelia Mesquita Da Costa[8], Marizette Silva[9], Maria Calvacante[9], Terezinha do Menino Jesus Silva Leitao[10], Beatriz L. Gómez[11], Angela Restrepo[11], Angela Tobon[11], Cristina E. Canteros[12], Christine Aznar[2], Denis Blanchet[2], Vincent Vantilcke[13], Cyrille Vautrin[13], Rachida Boukhari[13], Tom Chiller[14], Christina Scheel[14], Angela Ahlquist[14], Monika Roy[14], Olivier Lortholary[15,16], Bernard Carme[1,2], Pierre Couppié[2], Stephen Vreden[3]

1 Centre d'Investigation Clinique Epidémiologie Clinique Antilles Guyane (Inserm/DGOS CIE 802), Centre Hospitalier de Cayenne, Cayenne, French Guiana, France, 2 Epidemiologie Parasitoses et Mycoses Tropicales, EA 3593, Université Antilles Guyane, Cayenne, French Guiana, 3 Academisch Ziekenhuis Paramaribo Hospital, Paramaribo, Suriname, 4 Laboratório Central de Saúde Pública do Amapá, Macapa, Brazil, 5 National AIDS Program, Georgetown, Guyana, 6 Public Health Central Laboratory of Suriname, Paramaribo, Suriname, 7 Diakonessenhuis Hospital, Paramaribo, Suriname, 8 Instituto Evandro Chagas, Belém, Brazil, 9 Hospital de Clinicas Dr. Alberto Lima, Macapa, Brazil, 10 Universidade Federal do Ceara, Faculdade de Medicina, Departamento de Saude Comunitaria, Fortaleza, Ceara, Brazil, 11 Corporación para Investigaciones Biológicas, Medellín, Colombia, 12 INEI-ANLIS "Dr. Carlos G. Malbrán," Buenos Aires, Argentina, 13 Centre Hospitalier de l'Ouest Guyanais, Saint Laurent du Maroni, French Guiana, France, 14 Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, 15 Institut Pasteur, National Reference Center for Mycoses and Antifungals, Molecular Mycology Unit, Paris, France, 16 CNRS URA3012, Paris, France

Publication #2 (accepted in AIDS journal, 11/2015)

Disseminated histoplasmosis in Central and South America, the invisible elephant: The lethal blind spot of International Health organizations.

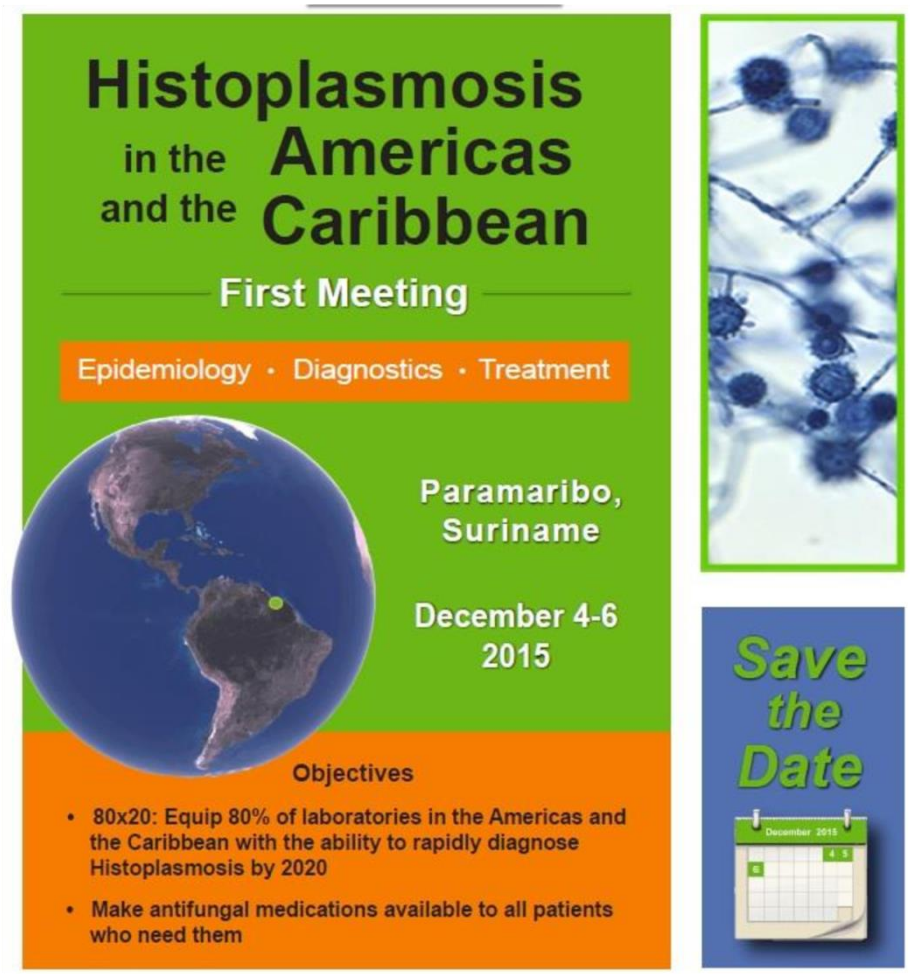
on behalf of "The neglected histoplasmosis in Latin America group"

Writing committee

Mathieu Nacher[a, b], Antoine Adenis[a,b], Eduardo Arathoon[c], Blanca Samayoa[c] ,Dalia Lau-Bonilla[c] , Beatriz Gomez[d], Angela Tobon[d], Diego Caceres[d], Silvia Marques da Silva[e], Maurimelia Mesquita da Costa[e], Rosely Zancope[f], Terezinha Silva Leitão[g], , Margarete Do Socorro Mendonca Gomes[h], Ivina Lopes Lima[h], Rosilene Malcher Leite[h], Stephen Vreden[i], Marja Van Eer[j], Sigrid Mac Donald[i], Sandra Hermelin[i], Magalie Demar[b,k], Denis Blanchet[b,k], Felix Djossou[b, l], Vincent Vantilcke[m], Maria Mercedez Panizo[n], Maribel Dolande[n], Christina Canteros[o], Marcus Lacerda[p,q], Pierre Couppié[br], Angela Restrepo[d]

a: Centre d'Investigation Clinique Antilles Guyane, CIC INSERM 1424, Centre Hospitalier de Cayenne, Cayenne, French Guiana
b: EA3593 Ecosystèmes Amazoniens et Pathologie Tropicale, Université de Guyane, Cayenne, French Guiana
c: Clinica Familiar "Luis Angel Garcia", Asociación de Salud Integral, Guatemala city, Guatemala
d:Corporacion para investigationnes biologicas, Medellin, Colombia
e: Instituto Evandro Chagas - IEC/SVS/MS Seção de Bacteriologia e Micologia- SABMI Laboratório de Micologia, Belem, Brazil
f: Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, Brazil.
g: Federal University of Ceará, Fortaleza, Brazil
h : Laborato´ rio Central de Saude Publica do Amapa´, Macapa, Brazil
i: Academic Hospital Paramaribo, Suriname
j : Diakonessenhuis, Paramaribo, Suriname
k: Laboratoire Hospitalo-Universitaire de parasitologie mycologie, Centre Hospitalier de Cayenne, Cayenne, French Guiana
l: Service des Maladies Infectieuses et Tropicales, Centre Hospitalier de Cayenne, Cayenne, French Guiana
m: Service de Médecine, Centre Hospitalier de l'Ouest Guyanais,Saint Laurent du Maroni, French Guiana
n: Departamento de Micología, Instituto Nacional de Higiene Rafael Rangel, Caracas, Venezuela
o: INEI-ANLIS ''Dr. Carlos G. Malbran,'' Buenos Aires, Argentina
p: Instituto Leônidas e Maria Deane (Fiocruz - Amazônia)
q: Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil
r: Service de Dermatologie-Vénéréologie, Centre Hospitalier de Cayenne, French Guiana

APPENDIX 3:


Histoplasmosis in the Americas and the Caribbean
First Meeting
Epidemiology · Diagnostics · Treatment
Paramaribo, Suriname
December 4-6 2015
Objectives
• 80x20: Equip 80% of laboratories in the Americas and the Caribbean with the ability to rapidly diagnose Histoplasmosis by 2020
• Make antifungal medications available to all patients who need them
Save the Date
Université de la Guyane
Ministerie van Volksgezondheid
gaffi
CDC
Centers for Disease Control and Prevention
CIC
Inserm CIC1424
Inserm
SWOS

« Histoplasmosis Initiative in the Americas »

December 4 to 6, 2015

Paramaribo, Suriname

Friday 4th December,

Pre-meeting TCC Histoplasmosis-HIV evaluation
(not for all attendees; Target audience: TCC coordinators)

2:00 – 5:00 PM Monitoring and evaluation
(CARPHA attendance is expected)

Pre-meeting clinical Workshop

7:00 – 7:30 PM : Registration

7:30 – 9 :30 PM : AIDS-related Histoplasmosis clinical and diagnostic implications

Clinical aspects of HIV-associated Histoplasmosis
Global epidemiology and impact of disease
(Chiller, 20 min)
Clinical picture and treatment options
(Nacher, 15 min)
Diagnostic toolbox
(Gomez, 15 min)

Clinical experiences from the field: exposing a killer and changing the outcome
10 years experience in Guatemala
(Arathoon, 20 min)
Becoming "Histo aware" in Suriname
(Vreden, 20 min)
Advocacy for histoplasmosis in the Americas and the Caribbean
(Nacher 20 min)

Open questions and discussion
(10 min)

Saturday 5th December,

8:00 – 8:30 AM: Registration

8:30 – 9:00 AM: Official opening
Welcome of Suriname Ministry of Health
Welcome of the meeting coordinators
Objectives: Dr Vreden (AZP, Suriname)
Introductions: Dr Chiller (CDC, USA) and Dr Nacher (Inserm, French Guiana)

9:00 – 9:30 AM: Keynote address
Histoplasmosis: a neglected disease state of the art and meeting context
(Dr Nacher, 30 min)

9:30 – 12:30 AM: Sharing local experiences on histoplasmosis
(Chairs: Dr Chiller and Dr Nacher)
(10-15 min/presentation, discussion and wrap up)
List according to attendees registered

Suriname (Mac Donald)
Guyana
French Guiana
Brazil (Macapa-Belem)
Brazil (Rio)
Brazil (Porto Alegre)
Brazil (Fortaleza, North East)
Brazil (Manaus, Amazon)
Argentina
Colombia
Guatemala
Panama
Peru
Mexico
USA
Venezuela
Dominican Republic (by CDC)
Trinidad & Tobago

12:30 – 1:30 PM: Lunch break

1:30 – 3:00 PM: Identifying the killer: current and future tools
(Chair: Samayoa, Tiraboschi, 1h10)

Diagnostic toolbox overview
(by Gomez, 30 min)
Diagnostic perspectives from a public institution
(by Zancope, 20 min)
Diagnostic perspectives from the private sector
(by Bauman, 20 min)
Discussion (20 min)

3:00 – 3:10: Break

3:10– 4:10: Identifying and treating the patients
(Chair: Singh, Vreden, 1h00, 20 min/presentation)

Challenges and outcomes in implementing a urine antigen test in Guatemala
(by Arathoon, 20 min)
Management of HIV and fever: identifying and treating histoplasmosis.
Practices in Suriname (by Van Eer, 10 min)
Practices in Brazil (by Silva Leitao, 10 min)
Are Tuberculosis and Histoplasmosis so similar in HIV patients?
(Adenis, 20 min)

4:10 – 4:20: Break

4:30 – 5:30: A review of global initiatives in fungal diseases
(Chair: Gomez, Silva Leitao, 1h00, 20 min/presentation)

Establishing fungal opportunistic infections surveillance in Central America
(by Forno, 20 min)
An update from the Mycosis study Group (MSG)
(by Chiller, 20 min)
An update from the Global Action Fund for Fungal Infection (GAFFI)
(by Denning, 20min)

Sunday 6th December

8:30 – 9:00 AM: Registration

9:00 – 9:40: Introduction on the meeting goals
(Chair: Mac Donald and Singh, 40 min)
9:00 – 9:20: Mapping the burden of histoplasmosis in South and Central America
(by Adenis, 20 min)
9:20 – 9:40: Cryptococcosis, a success story
(by Chiller, 20 min)

9:40 – 12:00 Achieving 80 by 20: The road map
(Chair: Nacher, Chiller, Vreden)

9:40 – 10:20 AM: Access to diagnosis
-Harmonizing the tool box
-Focal points
-Monitoring & Evaluation
-Funding
-Timeline & milestones

10 :20 – 11 :00 AM : Access to drugs
-The current arsenal against histoplasmosis
-Obtaining cheap Ambisome
-Focal points
-Timeline & milestones

11 :00– 12 :00 AM: Fitting histoplasmosis in HIV strategic plans across Americas
-Reaching the international organization
-Reaching National HIV/AIDS programmes
-Focal points
-Advocating & Lobbying for histoplasmosis
-Timeline & milestones

12:00 – 12:15 PM: HAM meeting closing remarks by Dr Vreden, Suriname and PAHO WDC or regional office PWR

12:15 – 2:00 PM: Lunch

Post meeting consolidation of the conclusions of the TCC evaluation (30 min)

Appendix 4

TABLE part #1

		FRENCH GUIANA				SURINAME				GUYANA			
Goal	**Indicator**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
Burden of AIDS-related deaths reduced in the Guiana shield	Annual number of AIDS-related deaths by country	48	24	12	12	"Not yet available"	89	"Not yet available"	"Not yet available"				
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
Purpose	**Indicators**	**Total**	**2013**	**2014**	**2015**	**Total**	**2013**	**2014**	**2015**	**Total**	**2013**	**2014**	**2015**
Mortality and morbidity due to Histoplasmosis in the Guiana Shield reduced.	Annual number of HIV-infected patients deaths due to disseminated histoplasmosis	2 of 31	2 of 16	0 of 12	0 of 3 (incomplete)	"Not yet available"	"Not yet available"	"Not yet available"	"Not yet available"				
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
	Annual % of HIV patients diagnosed for histoplasmosis and dying at 1 month after initiation of antifungal therapy against histoplasmosis.	13%	0%	13%	NA	"Not yet available"	"Not yet available"	"Not yet available"	"Not yet available"	33%	-	-	33%
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			
	✓ Annual % of HIV patients diagnosed with disseminated histoplasmosis	28 cases	13 cases	15 cases	NA	"Not yet available"	29%	31%	NA	3 cases	-	-	3 cases
	Data source:	COREVIH Guyane				National AIDS Programme				National AIDS Programme			

TABLE part #2.1

		FRENCH GUIANA				SURINAME				GUYANA			
Expected results	**Indicators**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
#1. Awareness of the problem of American Histoplasmosis in HIV-positive patients increased in Guiana Shield,	✓ Number of trained professionals												
	- Laboratory	2		2		10	7	3		2		2	
	- Clinicians and others	60		15 + 45		120	60	60		60		15	45
	✓ Number of meetings	3	1	2	0	8	3	3	2	5	1	2	2
	✓ Number of focal points	4	4	4	4	4	4	4	4	3	3	3	3
	✓ Number of Information Education and Communication (IEC) materials developed and disseminated	3	3	3	3	3	3	3	3	2	2	2	2
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#2. Diagnostic capacity for American Histoplasmosis improved in the Guiana Shield,	✓ Number of mycologic examinations for histoplasmosis in HIV-infected patients hospitalized (direct examination, cultures, pathology)	121	Not yet available	Not yet available	Not yet available	352	51	301	Not yet available	12	0	0	12
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			

TABLE part #2.2

	✓ Number of ELISA tests performed for histoplasmosis in HIV-infected patients hospitalized (urines + serum)	341	Not yet available	Not yet available	Not yet available	633	Not yet available	Not yet available	Not yet available				
	Data source:	Research project				Research project				Project Not Performed			
#3. Clinical practice in HIV patients in the Guiana Shield improved,	✓ Proportion of hospitalized HIV-infected patients screened for histoplasmosis (Fungal culture and ELISA test)	Not yet available	Not yet available	Not yet available	Not yet available	Not yet available	50-60%	>90%	Not yet available				
	✓ Number of HIV-infected patients hospitalized and treated for histoplasmosis	Not yet available	Not yet available	Not yet available	Not yet available	Not yet available	80 (AZP)	116 (AZP)	Not yet available				
	Data source:	Trans border cooperation & research project				Trans border cooperation & research project				TCC project			
#4. Strategy for Histoplasmosis control developed	✓ Document elaborated	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#5. Operational network of trained health professionals reinforced in the Guiana Shield.	✓ Network operational (at least 10 health professionals)	YES	1	1	1	YES	1	1	1	YES	1	1	1
	✓ Number of communications and publications on the topic.	9	3	4	2	5	3	1	1	3	2	0	1
	✓ Data Base information system specifically related to histoplasmosis and hiv wil be developed	YES	1	1	1	YES	1	1	1	YES	0	0	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			

TABLE part #3

		FRENCH GUIANA				SURINAME				GUYANA			
Activities	**Indicators**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**	**Total TCC**	**2013**	**2014**	**2015**
#1. Laboratory training	✓ Number of participants trained	2	0	2	0	10	7	3	0	2	0	2	0
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#2. Clinical training	✓ Number of participants trained (at least 10/country)	60	0	15 + 45	0	120	60	60	0	60	0	15	45
	Data source:	Trans border cooperation project				Trans border cooperation project				TCC project			
#3. Situation analysis	✓ Document produced	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#4. Workshop in Paramaribo	✓ Workshop held					2	1	-	1				
	✓ Number of participants (30 expected)					130	30	-	100				
	✓ Number of countries attending (at least 10 countries)					21	7	-	14				
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			
#5. Network of Health professionals	✓ Network operational (at least 2 health professionals from Brazil, French Guiana, Guyana and Suriname)	YES	-	-	-	YES	-	-	-	YES	-	-	-
	✓ Evaluation of the TCC completed.	YES	-	-	1	YES	-	-	1	YES	-	-	1
	Data source:	PAHO/WHO				PAHO/WHO				PAHO/WHO			

ANNEXE 5

FRANCE-GUYANE
www.franceguyane.fr

Actualité - Éducation / Santé / Environnement

Les infections passées au crible

T. F. Jeudi 16 janvier 2014

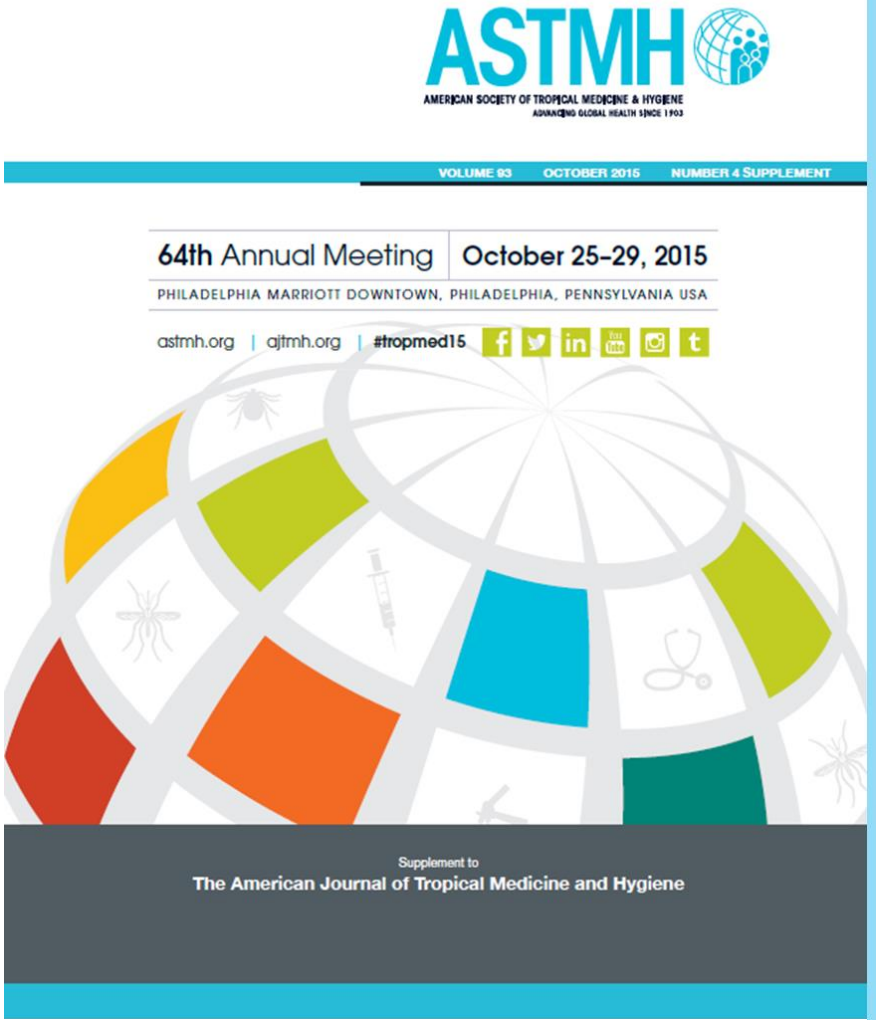
Les professeurs Olivier Lortholary et Mathieu Nacher, hier à l'Institut Pasteur. (TF)

Médecins et chercheurs se sont réunis hier matin dans les locaux cayennais de l'Institut Pasteur afin d'évoquer, lors d'un séminaire, les principales maladies infectieuses qui touchent la population. Et tout particulièrement l'histoplasmose.

Quand deux épidémiologistes se rencontrent en Guyane, aucun risque qu'ils viennent à manquer de sujets de conversation. Pour la simple et bonne raison que dans leur domaine de compétence, particulièrement dans cette région du monde, leur discussion peut porter sur un nombre incalculable de virus et autres maladies infectieuses. Par conséquent, réunis hier matin en séminaire dans les locaux cayennais de l'Institut Pasteur, médecins et chercheurs disposaient d'une multitude de choix. Si leur conversation a porté sur différentes questions liées aux infections fongiques invasives chez l'homme, c'est l'histoplasmose qui a retenu leur attention. L'histoplasmose est une maladie infectieuse sur laquelle les chercheurs présents dans le département planchent depuis de nombreuses années. « C'est une maladie que l'on connaît et que l'on diagnostique bien » , insiste le professeur Mathieu Nacher. Les études menées en Guyane portent sur les patients souffrant d'une immunodéficience, comme les personnes atteintes par le VIH. Dans neuf cas sur dix, l'infection se révèle très discrète. Il s'avère difficile de la détecter. En revanche, lorsqu'elle se manifeste chez un patient immunodéprimé, elle peut être mortelle.

COLLABORATION AMPLIFIÉE

En Guyane, 15 à 35 cas sont diagnostiqués chaque année. En 1997, le taux de mortalité chez les patients détectés s'élevait à 40%. Aujourd'hui, il est inférieur à 10%. Un progrès spectaculaire lié à l'amélioration des techniques de diagnostic, aux efforts de recherches mais aussi aux nombreux contacts et échanges initiés par les chercheurs guyanais. Ceux-ci sont parvenus à tisser une toile relationnelle qui s'étend de Rio à Georgetown en passant par le Suriname et les États-Unis. Une activité qui ne passe évidemment pas inaperçue. Hier, le professeur Olivier Lortholary, directeur du département des maladies infectieuses et tropicales de l'hôpital Necker (Paris), a tenu a souligner l'importance d'une collaboration « amplifiée » entre la Guyane et l'Hexagone. « Les échanges avec les pays voisins montés par les équipes de Guyane peuvent servir de modèle, précise-t-il. L'Institut Pasteur joue un rôle structurant pour ces réseaux. Il faut sensibiliser les médecins mais aussi la population, car il existe peut-être d'autres déficits immunitaires que l'on ne connaît pas encore. » Les avancées liées aux travaux des chercheurs guyanais sont donc primordiales.



Mid-Day Session 138

Tropical Medicine in the Arts

Marriott - Grand Ballroom Salon E
Wednesday, October 28, 12:15 p.m. - 1:30 p.m.

This session will highlight artistic representations of global health and infectious disease, the people and cultures affected, and the ways that important tropical medicine research and activities are being bolstered or otherwise helped by the myriad ways in which these artistic representations communicate their messages. This session will feature several artistic forms and mediums to represent this, and will focus upon poetry, the history of malaria in art, science journalism of tropical diseases designed researcher/artist interactions and getting the message across effectively through media. The goal of this symposium is to provide another "atypical" avenue to explore the infectious diseases and the global health issues that we as a Society have dedicated ourselves to. These presentations will invoke and explore the human impacts of these diseases, show the ways art can inform and illuminate public engagement, teach us how to effectively communicate to the people how tropical medicine can improve their lives, and help serve to remind us of the importance of our work and the social change that might come when we succeed. This symposium will hold broad appeal for those attending the conference, including those in the process of identifying what long-term role they will play in tropical medicine, those interested in global health communications, and for those researchers interested in seeing where their own work might intersect with the arts.

CHAIR
Scott E. Lindner
Pennsylvania State University, University Park, PA, United States

12:15 p.m.
MALARIA, POEMS
Cameron Conaway
Pennsylvania State University Brandywine, Media, PA, United States

12:30 p.m.
HISTORY OF MALARIA IN ART
Peter Billingsley
Sanaria, Inc., Rockville, MD, United States

12:45 p.m.
ARTISTS-IN-RESIDENCE IN A BUSY TROPICAL MEDICINE RESEARCH UNIT: EXPERIENCE AND LESSONS LEARNED
Phaik Yeong Cheah
Mahidol Oxford Research Unit, Bangkok, Thailand

1 p.m.
SCIENCE JOURNALISM OF TROPICAL DISEASES
Debora Miranda
London School of Tropical Medicine and Hygiene, London, United Kingdom

1:15 p.m.
GETTING THE MESSAGE ACROSS EFFECTIVELY THROUGH MEDIA
Richard Hatzfeld
Sabin Vaccine Institute, Washington, DC, United States

Burroughs-Wellcome Fund/ASTMH Fellowship Committee Meeting

Marriott - Room 304
Wednesday, October 28, Noon - 2 p.m.

CTropMed® Exam Committee Meeting

Marriott - Room 303
Wednesday, October 28, 12:15 p.m. - 1:30 p.m.

Membership Committee Meeting

Marriott - Room 301
Wednesday, October 28, 12:15 p.m. - 1:30 p.m.

Poster Session C Viewing

Convention Center - Ballroom AB
Wednesday, October 28, 1:45 p.m. - 7 p.m.

Symposium 139

Histoplasmosis and HIV in the Americas: The Fight Against a Neglected Fungal Disease

Marriott - Grand Ballroom Salon AB
Wednesday, October 28, 1:45 p.m. - 3:30 p.m.

This session will describe the burden of Histoplasmosis in HIV patients, where in many parts of the Americas it is the most common OI in hospitalized patients. The symposium will describe new diagnostic tests, risk factors, and strategies to reduce death in several different settings, as well as a new initiative for Histoplasmosis in the Americas.

CHAIR
Tom Chiller
Centers for Disease Control and Prevention, Atlanta, GA, United States
David Boulware
University of Minnesota, Minneapolis, MN, United States

1:45 p.m.
HISTOPLASMOSIS IN FRENCH GUIANA AND SURINAME: DESCRIBING A NEGLECTED KILLER
Mathieu Nacher
Hospital Cayenne, Cayenne, French Guiana

2:05 p.m.
RISK FACTORS AND DIAGNOSTIC CHALLENGES FOR HISTOPLASMOSIS IN A COLOMBIAN COHORT
Beatriz Gomez
Centro Investigaciones Biologicas, Medellin, Colombia

2:25 p.m.
DEVELOPING DIAGNOSTICS FOR NEGLECTED FUNGAL DISEASES
Sean Bauman
Immy Mycologics, Norman, OK, United States

2:45 p.m.
BUILDING CAPACITY FOR NEGLECTED FUNGAL DISEASES IN CENTRAL AMERICA: CHALLENGES AND SUCCESSES
Angela A. Cleveland
Centers for Disease Control and Prevention, Atlanta, GA, United States

ANNEXE 7

Certificate of Presentation

This certificate recognizes

Antoine Adenis

Presented

"Mapping the burden of Histoplasmosis in South and Central America"

In

Symposium 65. If You Neglect It, It Will Grow: Addressing Fungal Infections in Advanced HIV Care

At the

American Society of Tropical Medicine and Hygiene
65th Annual Meeting
November 13-17, 2016
Atlanta Marriott Marquis and Hilton Atlanta
Atlanta, Georgia USA

Karen A. Goraleski

Executive Director