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Epidemiology of fungal infections in HIV infected individuals in France : *P jirovecii* pneumonia and invasive aspergillosis in FHDH ANRS CO4

Blandine Denis

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**THESE DE DOCTORAT DE
L'UNIVERSITE PIERRE ET MARIE CURIE**

Spécialité

Epidémiologie Clinique

Ecole doctorale Pierre-Louis de santé publique à Paris : Epidémiologie et Sciences de
l'Information Biomédicale

Présentée par

Blandine DENIS

Pour obtenir le grade de

DOCTEUR de l'UNIVERSITÉ PIERRE ET MARIE CURIE

Sujet de la thèse :

Epidemiology of fungal infections in HIV infected individuals in France :
P. jirovecii pneumonia and invasive aspergillosis in FHDH ANRS CO4

Directeur de thèse : Mme Dominique COSTAGLIOLA

Soutenue le 15 mars 2016 devant le jury composé de :

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A ma famille,

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Résumé

Depuis la disponibilité des combinaisons antirétrovirales (cART) en 1996, l'incidence des infections opportunistes classantes SIDA (IO), dont la pneumocystose (PCP), a très fortement diminué. Malgré tout, chez les patients infectés par le VIH, la PCP était la 2^{ème} IO la plus fréquente en France en 2001-2003 et les infections fongiques, avec 1 million de nouveaux cas/an de cryptococcose, restent un problème de santé publique majeur au niveau mondial. Cependant, depuis l'ère des cART, peu de recherches épidémiologiques sur les infections fongiques dans les pays industrialisés ont été entreprises. C'est dans ce contexte que nous avons mené une étude épidémiologique de 2 infections fongiques chez les patients infectés par le VIH en France sur la French Hospital Database on HIV ANRS CO4 (FHDH) : la pneumocystose et l'aspergillose invasive. Concernant la pneumocystose, sur la période 2004-2011, dans la base FHDH, la moitié des 1259 cas de PCP étaient survenus chez des patients qui avaient interrompu leur suivi, et, pour ceux qui avaient déjà eu une IO avant la PCP, leur mortalité était de 25% à 3 ans. Pour l'aspergillose invasive (AI), après un retour national aux dossiers des cas déclarés sur 20 ans sur la base FHDH, un comité d'experts a validé 242 cas d'AI. Les données montrent que, chez les patients infectés par le VIH, seulement la moitié des AI validées répondaient aux critères EORTC. La mortalité à 3 mois après une AI s'est améliorée après l'ère des cART et un rôle protecteur du voriconazole sur la survie à 3 mois a également été démontré pour la 1^{ère} fois chez les patients infectés par le VIH.

Abstract

The advent of combined antiretroviral therapy (cART) in 1996 resulted in a dramatic fall in the incidence of AIDS-defining illness (ADI), including *Pneumocystis jirovecii* pneumonia (PCP). Nevertheless, PCP was the second most frequent ADI in France in 2001-2003 and fungal infections remain a major threat for HIV-infected individuals worldwide. Epidemiological data on fungal infections in the late cART period in resource-rich settings are scarce. The purpose of our work was to study changes in the epidemiology of fungal infections among HIV-infected individuals in France in the late cART period, focusing on PCP and invasive aspergillosis (IA) in the French Hospital Database on HIV ANRS CO4 (FHDH). In the FHDH, during the 2004-2011 period, half of the 1259 PCP cases occurred among HIV-infected individuals who had waning adherence to care, and for those who had a prior ADI before PCP the 3-year mortality rate was 25%. For the second study on IA, a review committee validated IA cases among all the cases that included a diagnostic code for aspergillosis (ICD-9 or ICD-10) in the FHDH over a 20-year period. Our study demonstrated that only half of validated IA cases among HIV-infected individuals met EORTC criteria. The 3-months survival rate after IA diagnosis improved after the advent of cART and a protective role of voriconazole was observed in the period after 2001.

Key words : HIV, AIDS, fungal infections, *Pneumocystis*, *Aspergillosis*, incidence, outcome

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Résumé long

INFECTIONS FONGIQUES CHEZ LES PATIENTS INFECTES PAR LE VIH A L'ERE DES COMBINAISONS ANTIRETROVIRALES (cART) : ETUDE DES PNEUMOCYSTOSES ET ASPERGILLOSES INVASIVES SUR LA BASE FHDH

Les infections fongiques sont les plus fréquentes des infections opportunistes (IO) au cours de l'infection par le VIH même si leur incidence a beaucoup diminué depuis l'instauration des traitements antirétroviraux hautement actifs (cART) (Palella FJ et al, 1998). Elles s'observent le plus souvent chez des patients naïfs de traitement, en échec thérapeutique ou non observants et sont un bon marqueur de la sévérité du déficit immunitaire. Au début de l'épidémie du VIH, la pneumocystose pulmonaire (pneumocystose) représentait 2/3 des infections classantes SIDA (ADI) et il était estimé que 75% des patients infectés par le VIH développeraient une pneumocystose au cours de leur suivi (Hay JW JAIDS 1988, Morris A Emerg Inf Dis 2004). Même si son incidence a beaucoup diminué depuis la mise en route des prophylaxies préventives et l'accès aux cART, la pneumocystose reste l'une des infections opportunistes les plus fréquentes dans les pays industrialisés et sur la base FHDH elle était la 2^{ème} IO classante SIDA en 2001-2003 (Grabar S et al, 2008). Par ailleurs, la cryptococcose est un problème majeur à l'échelon mondial avec 1 million de nouveaux cas /an (Park et al, 2009), c'est la 1^{ère} cause de méningite en Afrique du Sud et la 4^{ème} cause de mortalité infectieuse dans le monde. Plus de 500000 décès par an en Afrique Subsaharienne en 2008 étaient liés aux cryptococcoses neuro-méningées. Malgré la fréquence et mortalité importantes des infections fongiques invasives, seulement 2-2.5% des budgets alloués par les fonds anglo-saxons aux maladies infectieuses sont destinés à la recherche sur les infections fongiques (Brown GD, Sci Transl Med 2012). Les recherches sur les infections fongiques sont insuffisantes dans les pays à ressources limitées mais également dans les pays industrialisés où peu d'études ont été publiées depuis 10 ans sur

les infections fongiques chez les patients infectés par le VIH. C'est dans ce contexte que nous avons entrepris une étude épidémiologique de deux infections fongiques chez les patients infectés par le VIH en France sur la base FHDH : la pneumocystose et l'aspergillose invasive. Deux infections fongiques qui ont en commun de survenir chez des patients ayant un profond déficit immunitaire. La 1^{ère} est très fréquente mais il n'y avait pas de grande étude récente sur l'évolution des pneumocystoses chez les patients infectés par le VIH, la seconde portait sur l'aspergillose invasive, infection très rare mais de pronostic effroyable avant l'ère des cART avec une médiane de survie de 2-4 mois. Il n'y avait pas d'étude sur les aspergilloses invasives depuis l'accessibilité des cART. Nous voulions savoir quelle était l'évolution des aspergilloses invasives depuis 20 ans et si les critères EORTC, utilisés en onco-hématologie pour définir les aspergilloses invasives, étaient applicables à la population des patients infectés par le VIH présentant une aspergillose invasive.

La base FHDH collecte les données de patients infectés par le VIH depuis 1992, et regroupe actuellement des données de 70 hôpitaux français répartis sur 26 des 28 COREVIH (Coordination régionale de la lutte contre le VIH). Des données sur plus de 128000 patients entre 1992 et 2012 ont été recueillies et la cohorte FHDH-ANRS CO4 représente la plus grande cohorte prospective mondiale de suivi de patients infectés par le VIH. Par sa taille, la base FHDH offre la possibilité d'étudier un événement rare comme l'aspergillose invasive mais également d'avoir un bon reflet de l'épidémiologie récente des pneumocystoses en France.

Nous avons d'abord regardé sur la période 2004-2011 l'évolution de l'incidence des premières pneumocystoses déclarées sur la base FHDH, les caractéristiques des patients au diagnostic, l'évolution en terme de reconstitution immunitaire, contrôle virologique et mortalité à 3 ans après une pneumocystose. Comme sur la base FHDH 82% des patients

suivis sont sous traitement antirétroviral depuis au moins 6 mois, notre première hypothèse était que la majorité des patients présentant une pneumocystose sur cette période seraient des patients diagnostiqués tardivement avec une pneumocystose inaugurale du VIH et non des patients déjà suivis sur la base FHDH.

De façon surprenante, parmi les 1259 cas de 1^{ères} pneumocystoses durant la période 2004-2011, seulement 50% des cas avaient une pneumocystose inaugurale du VIH. L'autre moitié concernait des patients déjà suivis dans la base FHDH, avec une médiane de suivi de 8 ans par rapport à la date d'inclusion dans la base FHDH, 68% avaient déjà reçus au moins une année de cART avant le diagnostic de pneumocystose, avec dans 2/3 des cas, des CD4 > 350/mm³ notés dans leur historique immunovirologique. Au moment du diagnostic de pneumocystose, la médiane des CD4 était de 38/mm³, la charge virale VIH à 5.2 log copies/ml tout comme les patients présentant une pneumocystose inaugurale du VIH (médiane des CD4 à 53/mm³, charge virale VIH à 5.1 log copies/ml).

Le fait que 50% des patients étaient déjà suivis dans la base FHDH et qu'ils avaient pour la majorité bénéficié de cART avec une immunité correcte avant le diagnostic de pneumocystose nous a amené à étudier l'observance chez les patients suivis dans la base FHDH. Nous avons décidé de prendre comme critère de suivi optimal d'avoir une mesure de CD4 au moins tous les 6 mois. La proportion de suivi optimal avant la pneumocystose a ainsi été estimée et comparée avec la proportion de suivi optimal chez tous les patients suivis dans la base FHDH. La médiane de proportion de suivi optimal était de 85% (IQR, 66-96) pour tous les patients suivis dans la base FHDH, alors qu'elle n'était que de 45% (IQR,1-81) dans les 2 ans précédant la pneumocystose pour les cas étudiés. Ainsi, pour certains patients, nous avons pu démontrer que l'observance diminuait au cours du temps avec le risque de développer une ADI lors de la reprise du suivi. Il nous semble donc important de travailler sur l'évolution comportementale des patients suivis pendant de

nombreuses années pour une maladie chronique et de comprendre pourquoi certaines personnes sortent du suivi après de nombreuses années. La base FHDH ne recueille pas de données psychosociales permettant d'approfondir les facteurs de risques sociaux pouvant éventuellement participer à la rupture du suivi, mais nous pensons, tout comme d'autres auteurs (Masur H, 2015), que des indicateurs de suivis devraient être intégrés dans les services prenant en charge des personnes infectées par le VIH et que ces indicateurs devraient faire parti des bonnes pratiques de suivi des patients. Nous sommes actuellement en train de travailler sur un indicateur de suivi et aimerions créer un système d'alerte dès que le patient commence à espacer son suivi. Nous devons également travailler avec les structures non hospitalières, et en 1^{er} lieu avec les médecins généralistes afin de fluidifier les passerelles entre la ville et l'hôpital. Le délai optimal de suivi entre 2 consultations par un spécialiste reste débattu et les médecins de proximité sont d'indispensables relais quand le patient commence à espacer son suivi.

Par ailleurs, notre étude a montré que la reconstitution immunitaire étaient moins bonne chez les patients déjà suivis dans la base FHDH avec un diagnostic de pneumocystose que chez les patients présentant une pneumocystose inaugurale du VIH (HR d'avoir des CD4 >200/mm³ après le diagnostic : 0.7 (0.6-0.9) pour les patients déjà suivis dans la base FHDH mais sans ADI avant la pneumocystose et 0.6 (0.4-0.8) chez les patients déjà suivis dans la base FHDH mais sans ADI avant la pneumocystose, référence : patients ayant une pneumocystose inaugurale du VIH). De plus, pour les patients suivis dans la base FHDH et ayant déjà eu une ADI avant la pneumocystose, la mortalité à 3 ans était élevée (25%), alors qu'elle était de 8% chez les patients suivis dans la base FHDH et sans ADI avant la pneumocystose et de 9% chez les patients ayant une pneumocystose inaugurale du VIH. En comparant les patients suivis dans la base FHDH et ayant déjà eu une ADI avant la pneumocystose avec les patients ayant une pneumocystose inaugurale du VIH, le pronostic

restait péjoratif, même après ajustement sur le contrôle virologique et la reconstitution immunitaire (HR, 2.4 (IC 95%, 1.5-3.7)). Chez les patients ayant déjà présentés une ADI avant la pneumocystose, le déficit immunitaire persiste au-delà d'une remontée des CD4 sérique et il serait intéressant d'étudier plus finement les caractéristiques immunologiques de ces patients. Des alternatives thérapeutiques, notamment sur le choix de traitement antirétroviral (en privilégiant notamment les anti-intégrases) pourraient être proposées dans cette population de patients ayant déjà présentés une ADI afin d'optimiser au maximum la reconstitution des cellules CD4 naives et mémoires de l'organisme et la baisse de l'inflammation résiduelle observée chez les patients en échappement virologique.

En conclusion de ce premier travail, nous avons pu démontrer que si nous voulons améliorer l'incidence et le pronostic des patients présentant une pneumocystose, nous devons réussir à optimiser le suivi à long terme, et notamment pour les patients suivis depuis de longues années qui avaient déjà fait une ADI dans le passé. Nous devons mettre en place des indicateurs de qualité de suivi et les intégrer dans les bonnes pratiques médicales de suivi de patients infectés par le VIH. Par ailleurs, comme 50 % des patients présentaient une pneumocystose inaugurale du VIH, des efforts particuliers doivent être mis en œuvre afin de détecter les patients qui ignorent leur séropositivité et nous devons réfléchir à de nouvelles politiques de dépistage du VIH en France.

Notre second travail portait sur les aspergillose invasives (IA) chez les patients infectés par le VIH. Les publications avant l'ère des cART avaient montré que cette infection fongique était très rare chez les patients infectés par le VIH et de pronostic catastrophique, avec une médiane de survie à 3 mois. L'aspergillose invasive survenait chez des patients avec des taux de CD4 bas, et dans la moitié des cas les facteurs classiques prédisposant à l'aspergillose invasive (neutropénie, corticothérapie) étaient absents. Depuis ces publications, le nombre de diagnostic de cancers a augmenté chez les patients infectés par le VIH, avec des neutropénies post chimiothérapie et des corticothérapies prescrites, ceci pouvant potentiellement favoriser la survenue d'aspergillose invasive.

Dans ce contexte, nous avons souhaité étudier l'évolution de l'incidence de l'aspergillose invasive chez les patients infectés par le VIH. Nous avons également étudié les caractéristiques des patients au diagnostic d'aspergillose invasive, les méthodes diagnostic et regardé comment la classification EORTC habituellement utilisée en hémato-oncologie pour classer les aspergillose invasives pouvait être utilisée dans ce contexte. Un retour national sur dossiers a été entrepris afin d'avoir les données nécessaires à la validation de tous les cas déclarés en regardant plus spécifiquement les critères EORTC. Quand les cas d'aspergillose invasives correspondaient à la définition EORTC, ils étaient classés en « EORTC IA », sinon, les cas validés après analyse des données cliniques, microbiologiques et radiologiques et ne rentrant pas dans la définition EORTC étaient classés en « HIV related IA ». Sur une période de 20 ans, 242 cas d'aspergillose invasives ont ainsi été validés sur la base FHDH.

L'incidence de l'aspergillose invasive dans la FHDH a diminuée sur la période d'étude, passant de 19/10000 PatientAnnée (PA) ((95% CI, 16.0-23.0) sur la période 1992-1995 à

2.2 /10 000 PA (95% CI, 1.8-2.6) en 2002-2011. Le ratio d'EORTC IA sur le nombre de cas d'IA validés est resté stable au cours du temps (50.4% en 1992-1995, 48.1% en 1996-2001, et 54.4% en 2002-2011 (p=0.7)). La moitié des patients n'avait pas les critères d'hôte correspondant à la définition EORTC. La médiane de CD4 au diagnostic d'aspergillose invasive a augmenté au cours du temps mais restait inférieure à 100/mm³ (médiane à 9/mm³ (IQR, 4-26) en 1992-1995, 18/mm³ (IQR, 9-80) en 1996-2001 et 82/mm³ (IQR, 12-327) en 2002-2011 (p< 0.0001)), alors que la médiane de taux de polynucléaires neutrophiles était supérieure à 1500/mm³ dans les 2 dernières périodes étudiées (1263/mm³ (760–2535) en 1992-1995, 1500/mm³ (700–3004) en 1996-2001, 1810/mm³ (500–3920) en 2002-2011 (p=0.5)). Afin de faciliter le diagnostic d'aspergillose invasive chez les patients infectés par le VIH, nous proposons d'inclure le paramètre "avoir des CD4 inférieurs à 100/mm³" comme facteur d'hôte prédisposant, mais cette proposition devra être validée par une étude prospective.

Avec une médiane de survie à 3 mois après le diagnostic d'aspergillose invasive à l'ère des pré-cART, nous avons postulé que la survie serait meilleure après la disponibilité des cART à partir de 1996. Le pronostic des patients infectés par le VIH s'est transformé après 1996, et le pronostic des patients atteints d'aspergillose invasive en oncologie s'est amélioré après la disponibilité de l'amphotéricine B liposomale en 1997 puis du voriconazole en 2002. Ainsi, nous avons défini 3 périodes d'étude pour le suivi évolutif des aspergilloses invasives : pré-cART et pré-voriconazole (1992-1995), post-cART et pré-voriconazole (1996-2001), et post-cART et post-voriconazole (2002-2011). Nous avons confirmé que la médiane de survie après une aspergillose invasive aspergillosis s'est améliorée depuis l'accès des cART, passant de 1.9 mois (IQR, 0.9-3.1 months) pendant la 1ère période, à 10.3 mois (IQR, 2->100 mois) et 29 mois (IQR, 2.2-98.2 mois) durant les 2 dernières périodes. Après 2002, les patients sous voriconazole ont

vu leur survie s'améliorer avec une diminution importante du risque de mortalité à 3 mois (HR= 0.1 (95% CI, 0.01-0.80). Ces résultats plaident pour l'utilisation du voriconazole en 1ère intention dans l'aspergillose invasive chez les patients infectés par le VIH.

L'aspergillose invasive reste de diagnostic difficile chez les patients infectés par le VIH, l'addition de la donnée "avoir des CD4 bas" comme facteur d'hôte pourrait en faciliter le diagnostic. Un taux de CD4 inférieur à 100/mm³ semble être approprié pour une nouvelle définition des facteurs d'hôte, mais d'autres études seraient nécessaires pour valider cette proposition. Nous planifions également de comparer les aspects radiologiques des patients classés en EORTC IA et en HIV related IA afin d'évaluer les différences éventuelles et de les comparer aux critères radiologiques de la définition EORTC 2008.

En conclusion, nous avons démontré que les infections fongiques restaient un sujet majeur en 2016, même dans les pays industrialisés. Nous planifions maintenant de reprendre les cryptococcoses sur la base FHDH, ces infections fongiques ayant également la particularité de survenir chez des patients avec des CD4 très bas. La cryptococcose méningée est un problème de santé publique dans les pays à ressources limitées, avec une mortalité d'environ 50% dans les 2 1ères semaines dans ces pays. C'est une infection rare chez les patients suivis dans les pays industrialisés. Dans la base FHDH, l'incidence de la cryptococcose a été divisée par 10 depuis 1996, passant de (47.5 (42.5-52.5) /10 000 PA) en 1992-1995 à (4.5 (3.9-5.2) /10 000 PA) en 2002-2011. Nous allons également étudier qui est à risque de nos jours, l'évolution de la mortalité au cours du temps, ainsi que le délai d'initiation entre le début du traitement de la cryptococcose.

Au-delà de nos résultats, ces études montrent que le screening VIH doit être améliorée, que nous devons améliorer le suivi à "très long terme" de certains patients et nous proposons qu'un suivi adéquat tous les 6 mois fasse partie des indicateurs de qualité du suivi chez les patients infectés par le VIH.

Glossary

cART/ HAART : combined antiretroviral therapy

ADI : AIDS defining illness

ANRS : Association Nationale de Recherche sur le SIDA et les hépatite virales

ARV : Antiretroviral treatment

BAL : Broncho alveolar lavage

CDC : Centern for Diseases Control

CMV : Cytomegalovirus

CNS : Central Nervous System

Cr Ag : Cryptococcal Antigen

FHDH-ANRS CO4 : French Hospital database on HIV

GM : Galactomannan

IA : Invasive aspergillosis

ICU : Intensive Care Unit

IFI : invasive Fungal Infection

IQR : Interquartile Range

INSERM : Institut National de santé de la recherché médicale

IRIS : Immune Resconstitution Inflammatory Response

IVDU : Intravenous Drug User

KS : Kaposi sarcoma

LN : Lymph Node

MAC : Mycobacterium avium mycobacteria

MSM : Men who have Sex with Men

NHL : Non Hodgkin Lymphoma

OI : Opportunistic Infection

PCP : Pneumocystis jirovecii Pneumonia

PY : Person Year

VL : Viral load

WHO : World Health Organization

Introduction

Fungi are usually harmless in a context of normal host responses, but immune deficiencies, particularly in HIV-infected individuals, result in significantly increased susceptibility to many fungal infections. During the early years of the AIDS pandemic, *Pneumocystis jirovecii* pneumonia (PCP) accounted for two-third of AIDS-defining illnesses among patients in the USA, and an estimated 75% of HIV-infected individuals developed PCP during their lifetime (Hay et al., 1988; Morris et al., 2004). After the advent of cART, many studies showed a dramatic fall in the incidence of AIDS-defining clinical illness (ADI) and also an improvement in survival (Palella et al., 1998). Nevertheless, PCP remains one of the most common AIDS-defining illnesses in resource-rich settings and was the second most frequent ADI in France in 2001-2003 (Grabar et al., 2008; Kaplan et al., 2000; Morris et al., 2004).

Fungal infections remain a major threat for HIV-infected individuals worldwide. Approximately one million cases of cryptococcal meningitis occur globally every year, with over 500 000 related deaths in sub-Saharan Africa in 2008 (Park et al., 2009). It has been estimated that HIV/AIDS results in nearly 10 million cases of oral thrush and 2 million cases of esophageal fungal infections annually (Brown et al., 2012a, 2012b). Probably about one-and-a-half million people die from fungal infections every year, as many as from tuberculosis or malaria. Despite the high global burden of invasive mycoses, only 2–2.5% of the infectious-disease research budgets of major funders in the UK and USA are targeted at human fungal infections (Brown et al., 2012a). A meeting on AIDS-related mycoses in 2013 in Cape Town, South Africa (Brown et al., 2014), fixed several urgent priorities in order to reduce morbidity and mortality due to fungal infections.

Research on fungal infections in resource-limited settings are lacking, and epidemiological data on fungal infections in the late cART period in resource-rich settings are also scarce. In

this context, we examined changes in the epidemiology of fungal infections among HIV-infected individuals in France in the late cART period, focusing on PCP and invasive aspergillosis. In this era of powerful cART regimens, PCP should no longer be observed if HIV-infected individuals are diagnosed early and remain in care. We thus analyzed trends in the incidence of PCP in the French Hospital Database on HIV (ANRS CO4) from 2004 to 2011. Studies published before the cART era showed that invasive aspergillosis (IA) was rare, difficult to diagnose, occurred in patients with very low CD4 cell counts, and carried a very poor prognosis. No research has been undertaken in the late cART period, yet the situation has changed with the availability of cART and its impact on immune restoration, but also with population aging and the increase in cancer, as chemotherapy-induced neutropenia is a classical predisposing factor for IA. We retrieved all medical charts in the French Hospital Database on HIV (ANRS CO4) that included a diagnostic code for aspergillosis (ICD-9 or ICD-10). After a review committee had validated IA cases, we analyzed the incidence, diagnostic circumstances, risk factors, and trends in IA among HIV-infected individuals.

Chapter 1. State of the art: fungal infections and HIV

a. PCP and the discovery of the HIV/AIDS pandemic

i. “An outbreak of community-acquired *Pneumocystis carinii* pneumonia: Initial manifestation of cellular immune dysfunction”

The *Morbidity and Mortality Report* (MMWR) reported the first cases of what would become the HIV pandemic in June 1981: 5 previously healthy homosexual men (MSM) in Los Angeles were found to have biopsy-confirmed *Pneumocystis jirovecii* pneumonia as well as confirmed CMV disease or virus shedding within 5 months of the diagnosis of *Pneumocystis* pneumonia and candida mucosal infections (Centers for Disease Control (CDC), 1981a). This report in the MMWR alerted the medical and public health communities 4 months before the first peer-reviewed article was published (Hymes et al., 1981). Scientists in the Parasitic Diseases division of CDC's Center for Infectious Diseases were already concerned with an increase in requests for pentamidine isethionate to treat unusual cases of PCP in New York. One month later, a new MMWR report included 26 cases of Kaposi's sarcoma in young male homosexuals, 20 in New York and 6 in San Francisco, 7 of whom also had PCP, CMV or other opportunistic infections (OI) (Centers for Disease Control (CDC), 1981b). In July 1981, another 10 cases of PCP (without Kaposi's sarcoma) were identified. The CDC then formed a task force on Kaposi's sarcoma and opportunistic infections. Results from active surveillance in the United States rapidly established that the syndrome was new, and showed that the number of cases was increasing rapidly (“Epidemiologic aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections,” 1982). In England, the first case of PCP and acquired cellular immunodeficiency was reported in 1981 in a MSM with PCP who had been to the USA (du Bois et al., 1981). In France, the first reported case was that of a MSM who was admitted to hospital in 1981 with PCP (Rozenbaum et al., 1982). The name “acquired immunodeficiency syndrome” (AIDS) was first used in 1982. By the end of 1982, the case

distribution strongly suggested that AIDS was caused by an agent transmitted through sexual contact between men (Centers for Disease Control (CDC), 1982a; Jaffe et al., 1983) and between men and women (Centers for Disease Control (CDC), 1983a; Harris et al., 1983), and also through blood exposure among injecting-drug users and recipients of blood or blood products (Centers for Disease Control (CDC), 1982b, 1982c, 1982d). Cases were also identified among infants born to women with AIDS or at risk of AIDS (Centers for Disease Control (CDC), 1982e), and persistent unexplained lymphadenopathy emerged as a frequent feature (Centers for Disease Control (CDC), 1982f). To prevent transmission of AIDS, in 1983 the USA Public Health Service used epidemiological information about the condition to recommend that sexual contact be avoided with persons known or suspected to have AIDS, and that persons at increased risk for AIDS refrain from donating plasma or blood (Centers for Disease Control (CDC), 1983b, 1982d). Work was intensified toward developing safer blood products for persons with hemophilia. These recommendations were developed and published only 21 months after the first cases had been reported and before the first published report by F Barre-Sinoussi and Luc Montagnier's team identifying the causative retrovirus (Barré-Sinoussi et al., 1983). By 1984, the National Heart, Lung, and Blood Institute (NHLBI) had analyzed the types and frequencies of pulmonary diseases in 441 patients with AIDS and found that 373 (85%) had PCP (27). In 1986, the French and Americans agreed to call this retrovirus HIV. By the end of 1986, 38 401 cases of AIDS had been identified by WHO: 31 741 in the USA, 3858 in Europe, 2323 in Africa, 395 in Oceania, and 395 in Asia.

ii) PCP: characteristics

(*Pneumocystis* workshop 10th anniversary eukaryotic cell 2009)

Pneumocystis spp. are extracellular, obligate, host-specific, yeast-like parasitic fungi virtually restricted to lung tissues. The morphological forms range in size from 1 to 10 µm. Members

of the fungal genus now known as *Pneumocystis* were first identified in 1909 by Carlos Chagas in the lungs of guinea pigs that had been experimentally infected with *Trypanosoma cruzi*. In 1910, Carini was studying Norway rats infected with another trypanosome, *Trypanosoma lewisi*, and noted the same cystic aspect. In 1912, Delanoë and Delanoë noted the presence of cysts in the lungs of Paris sewer rats free of trypanosome infection and suggested that they were due to a new parasitic species that they named *Pneumocystis carinii*.

In humans, epidemic forms of interstitial plasma cell pneumonitis were described in the 1940s, and a relationship with *Pneumocystis* was suggested by Van der Meer and Brug in 1942. In 1951 and 1952, Vanek and Jirovec reported the association between pneumocystis and interstitial pneumonitis among premature and malnourished children. In the 1950s and 1960s, *Pneumocystis spp* was described as an opportunistic pathogen among children with acute leukemia or congenital immunodeficiencies that impaired T-cell function.

P. carinii was first thought to be a protozoan, but in 1988 Edman and Stringer showed that ribosomal RNA sequences of *P. carinii* were related to fungal RNAs and this was confirmed by phylogenetic analyses. The 18S rRNA sequences from human-derived *Pneumocystis* and rat-derived *Pneumocystis* differed by 5%. At the 2001 International Workshop on Opportunistic Protists in Cincinnati, the name *Pneumocystis jirovecii* was chosen for the form that infects humans and *Pneumocystis carinii* for 1 of the 2 forms infecting rats.

Remark: For this study, we have decided to keep the acronym “PCP” for *Pneumocystis jirovecii* pneumonia, as it is still used in most publications. However, PjP should be used in future.

Pneumocystis species are classified within the phylum Ascomycota. They are atypical fungal microorganisms unable to grow in vitro in fungal culture media. They respond to antiparasitic agents (cotrimoxazole, pentamidine), and their cell wall contains cholesterol rather than

ergosterol. There are 2 predominant life cycle forms, the trophic form and the cyst form. In an infected host, *Pneumocystis* exists almost exclusively within the alveoli of the lung. Alveolar macrophages are the primary resident phagocytes that mediate the clearance of *Pneumocystis* in the lung. Immune control of *Pneumocystis* also involves production of chemokines and inflammatory cytokines by alveolar macrophages and epithelial cells. CD4 T cells are essential for the control of *Pneumocystis* infection.

Clinical manifestations of PCP are nonspecific. Patients classically present with a fever of several days or weeks duration, nonproductive cough, and progressive dyspnea, often with normal pulmonary auscultation. Symptoms are usually of longer duration before diagnosis in HIV-infected individuals than in other immunosuppressed patients.

Radiological presentation: on chest radiography, PCP usually manifests as bilateral diffuse reticular opacities. Chest radiography may be normal at diagnosis in more than 1/3 of cases. Computed tomography (CT) shows ground-glass opacities with a patchy distribution, predominating in perihilar regions. Multiple and bilateral cysts are observed in 10 to 34% of cases.

Microbial diagnosis: As *Pneumocystis* cannot be cultured, the diagnosis continues to rely on microscopic demonstration of *Pneumocystis* in BAL fluid or induced sputum. Trophic forms can be stained with Papanicolaou, Giemsa or Gram-Weigert. Cysts can be stained with Gomori-methenamine silver, toluidine blue O or Calcofluor white. Fluorescein-labeled monoclonal anti-*Pneumocystis* antibodies are commercially available and can be used to detect both trophic forms and cysts; they now represent the gold standard method for detection. Quantitative PCR is broadly used but the cut-off between *Pneumocystis* carriage and PCP still needs to be determined. Serum (1-3) β D glucan is an adjunctive noninvasive marker of PCP with good negative predictive value. If clinical signs are compatible with a

PCP, having a serum (1-3) β D glucan above 500 pg/ml makes PCP diagnosis highly probable. However, the number of false positives is high and this reagent cannot be used to monitor the response to therapy. The detection of serum (1-3) β D glucan and *P. jirovecii* DNA could be usefully combined as serum markers for the diagnosis of PCP in patients with severe diseases or other conditions precluding the BAL procedure (Costa et al., 2012).

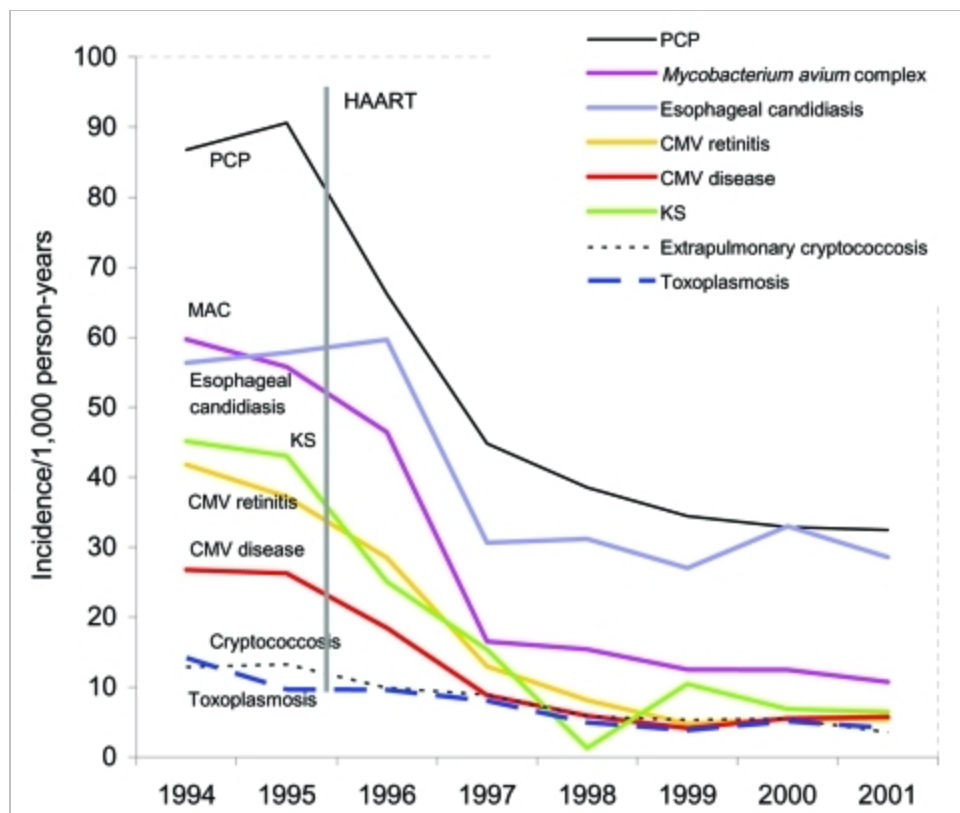
Treatment: Trimethoprim-sulfamethoxazole (TMP-SMX) is the first-line agent of choice. Parenteral pentamidine is the most extensively studied alternative but is very poorly tolerated. No clinical trials have compared atovaquone, clindamycin-primaquine or dapsone-TMP with TMP-SMX for the treatment of moderate to severe PCP. For patients with mild to moderate PCP, clindamycin-primaquine and dapsone-TMP exhibit comparable efficacy and toxicity to TMP-SMX, while atovaquone is less effective but better tolerated than TMP-SMX, and as effective as pentamidine.

Corticoid administration starting within the first 72 h after diagnosis improves survival among HIV-infected patients whose arterial oxygen pressure is ≤ 70 mm Hg.

iii) PCP: epidemiological changes

During the early years of the AIDS pandemic, PCP accounted for 2/3 of AIDS-defining illnesses in patients in the USA, and an estimated 75% of HIV-infected individuals developed PCP during their lifetime (Hay et al., 1988; Morris et al., 2004). Rates of PCP were as high as 20/100 PY among those with CD4 <200/mm³. The first decline in the incidence of PCP occurred after the introduction of anti-*Pneumocystis* prophylaxis in 1989 (Jones et al., 1999). The advent of cART resulted in further declines in PCP and other opportunistic infections (figure 1). Many studies showed a dramatic fall in the incidence of AIDS-defining clinical illness (ADI) and improved survival (Palella et al., 1998). In Europe, the Euro SIDA study (Mocroft et al., 2000; Weverling et al., 1999) showed a reduction in the incidence of PCP from 4.9 cases/100 PY before March 1995 to 0.3 cases/100 PY after March 1998. Yet PCP remains one of the most common AIDS-defining illnesses in resource-rich settings and was the second most frequent ADI in France in 2001-2003 (Grabar et al., 2008; Kaplan et al., 2000; Morris et al., 2004). One large prospective study showed that PCP survival at 3 years rose from 51% in the pre-cART era to 87% in 2001-2003 (Grabar et al., 2008). Another study in France showed that PCP was the second invasive fungal disease, and while PCP's incidence decreased in HIV infected individuals, PCP's incidence increased in HIV-seronegative patients between 2001 and 2010 (Bitar et al., 2014).

Figure 1



Yearly opportunistic infection rates per 1000 person-years, CDC Adult and Adolescent Spectrum of Disease Project, 1994–2001.

CMV, cytomegalovirus; HAART, highly active antiretroviral therapy; KS, Kaposi's sarcoma; MAC, *Mycobacterium avium* complex; PCP, *Pneumocystis pneumonia*. Data are standardized to the population of AIDS cases reported nationally in the same year by age, sex, race, HIV exposure mode, country of origin, and CD4+ lymphocyte count.

b) Other fungal infections in HIV-infected individuals

This section is inspired by the chapter *Fungal infections in patients with AIDS* of the Oxford Textbook of Infectious Disease and Microbiology: Medical Mycology (B. Denis, F. Lanternier, O.Lortholary), to be published soon.

Invasive fungal infections (IFIs) are a major cause of HIV-related mortality globally. Despite widespread rollout of combined antiretroviral therapy, there are still up to 1 million deaths annually from IFIs, accounting for 50% of all AIDS-related deaths. Fungal infections are the most frequent opportunistic diseases occurring during the course of HIV infection. Untreated individuals living with HIV have an immune deficit that worsens with time, allowing mycotic diseases to develop (Cunliffe and Denning, 1995; Perfect et al., 1993; Spellberg and Edwards, 2001). *Pneumocystis jirovecii*, *Candida albicans* and *Cryptococcus neoformans* are the three major fungal pathogens in patients with AIDS (Catherinot et al., 2010; Mitchell and Perfect, 1995). In endemic areas, infections due to dimorphic fungi also represent an important group. *Histoplasma capsulatum*, *Coccidioides* species and *Penicillium marneffeii* are the most important endemic pathogens. In some AIDS patients, mycotic disease is the consequence of reactivation several years after a primary infection (Wheat, 1995). Some fungal infections, such as oropharyngeal candidiasis, are relatively benign, but others such as cryptococcal meningitis and invasive aspergillosis are usually severe and carry a poor prognosis.

i) Cryptococcosis

C. neoformans var. *grubii* (serotype A) is responsible for most cases of cryptococcosis in AIDS patients. It will be referred to as *C. neoformans* in this section.

Epidemiology

While the incidence of cryptococcosis has declined dramatically among HIV-infected patients in resource-rich settings since the availability of cART (Dromer et al., 2004), cryptococcosis is

an increasing concern in Africa and Southeast Asia. It is encountered in up to 15-20% of hospitalized AIDS patients in sub-Saharan Africa (Jarvis et al., 2008; Micol et al., 2007). Many countries in Africa and Asia have limited access to antifungal agents, and mortality rates $\geq 50\%$ within the first 15 days have been observed in low-resource countries (Loyse et al., 2013a, 2013b). In France, mortality remains at about 17% (Dromer et al., 2007).

Pathogenesis

Quantitative and/or qualitative CD4 T cell deficiency is a major risk factor for cryptococcosis, and HIV infection is the most prevalent underlying disease. The median CD4 cell count at diagnosis is $20/\mu\text{L}$ (Dromer et al., 2007). The portal of entry is mainly pulmonary and the interaction of cryptococci with alveolar macrophages determines pulmonary infection and subsequent systemic dissemination.

Clinical manifestations

Meningoencephalitis is the most frequent clinical form of cryptococcosis in HIV-infected patients. Neurological symptoms are most often subacute. Fever and headache are common, while meningeal symptoms are not inconsistent. Cerebral imaging is abnormal in 50% of cases and MRI is more sensitive than computed tomography (CT) (Charlier et al., 2008). Measurement of CSF opening pressure is mandatory, as the prognosis is tightly associated with the degree of intracranial hypertension.

Pneumonia can be associated with cough and dyspnea and, less commonly, with chest pain and hemoptysis. The acute respiratory distress syndrome is associated with poor outcome. Standard chest radiographs and CT show interstitial infiltrates, nodules, pleural effusion, mediastinal lymphadenopathy, and/or cavitory lesions. Disseminated cryptococcosis should be suspected in febrile HIV-infected patients who present with skin lesions. Urinary tract

involvement is common in men but is usually asymptomatic. The prostate can serve as a sanctuary for *C. neoformans*.

Diagnosis

Analysis of CSF with India ink shows encapsulated yeasts in more than 80% of HIV-infected patients with cryptococcosis. Samples should be incubated for at least 4 weeks at 35-37°C, and sensitivity increases with the volume of CSF. Blood and urine culture should be done systematically.

Cryptococcal antigen (CrAg) should be sought in serum and CSF. Commercially available tests are sensitive ($\geq 95\%$) and specific ($\geq 95\%$). A high serum CrAg titer is associated with a higher mycological failure rate, but a lack of decrease is not predictive of worse outcome. New CrAg detection methods such as CRAG Lateral Flow assays (LFA), particularly the kit developed by Immy, are easy to use and have improved the diagnosis of cryptococcal meningitis in Africa (Binnicker et al., 2012; Boulware et al., 2014b; Kabanda et al., 2014). A recent study showed that baseline CSF CrAg LFA titers predicted vital outcome at 2 and 10 weeks (Kabanda et al., 2014). More recently, a new LFA has been developed by Biosynex and Institut Pasteur, with the advantage of offering simultaneous diagnosis and semi-quantitation. Clinical studies comparing both tests are currently ongoing both as screening and diagnostic strategies in South Africa, Cameroon and Malawi/Kenya.

Treatment

Antifungal agents used to treat cryptococcosis include amphotericin B (including a liposomal formulation), flucytosine and fluconazole. For initial therapy, a combination of amphotericin B (1 mg/kg daily) and flucytosine (100 mg/kg daily), given for at least 14 days, has been shown to be superior to monotherapy, and flucytosine use is an independent predictor of better early mycological outcome (Day et al., 2013; Dromer et al., 2007; Perfect et al., 2010;

van der Horst et al., 1997). The largest study ever performed ACTA (MRC and ANRS) with 691 patients to be included should provide major informations regarding potential alternatives to amphotericin B and flucytosine as a first-line strategy.

If clinical outcome is favorable and CSF culture becomes negative, therapy can be switched to oral fluconazole 400 mg daily for 8-10 weeks. Maintenance therapy with fluconazole, 200 mg daily, should be given until persistent immune restoration is obtained. If the patient has renal failure, liposomal amphotericin B, 3-5 mg/kg daily, can be used.

Appropriate management of elevated CSF opening pressure (≥ 25 cmH₂O) strongly reduces early mortality, and repeated lumbar puncture with evacuation of up to 20 cc should be performed every 1-3 days until pressure normalizes (Boulware et al., 2014a; Perfect et al., 2010). In case of persistently increased intracranial pressure, a ventriculo-peritoneal shunt or lumbar drain should be used. Mannitol, acetazolamide and steroids have not been shown to be helpful (Graybill et al., 2000).

In case of an isolated positive serum CrAg titer, an exhaustive workup should be performed to search for a site of infection; fluconazole should be initiated if none is found and if the patient has a CD4 cell count $<200/\mu\text{L}$.

Maintenance therapy can be safely withdrawn in patients who have been treated for cryptococcosis for at least a year, who are on cART, and who have a CD4 cell count $>100/\mu\text{L}$, undetectable HIV viral load for at least 3 months, and a CrAg titer $< 1/512$ (Lortholary et al., 2006; Vibhagool et al., 2003).

Primary prophylaxis is not recommended in developed countries. WHO recommends cryptococcal antigen screening and pre-emptive therapy for patients who are CrAg-positive and have 100 CD4/mm³ in regions where there is a high prevalence of cryptococcal

antigenemia. Screening and pre-emptive treatment for cryptococcal infection has been shown to reduce mortality among HIV-infected patients in Africa when combined with counseling strategy (Mfinanga et al., 2015).

ii) Dimorphic fungi

Among fungal infections due to dimorphic fungi, only three -- histoplasmosis, coccidioidomycosis, and penicilliosis -- are frequently seen in AIDS patients.

1. Histoplasmosis

Histoplasmosis due to *H. capsulatum var. capsulatum* is endemic in the United States, the Caribbean, and Central and South America. It is much less frequent in Africa and South East Asia. Among HIV-positive patients living in endemic areas in midwestern United States, the incidence of histoplasmosis has ranged from 1% to 25% historically. It is a major threat in areas such as French Guyana and Surinam (Nacher et al., 2013) and more generally in South America. Outside endemic areas, it usually results from reactivation several years after the primary infection (Warnock et al., 1998). Histoplasmosis generally occurs in patients with CD4 counts <100/ μ L and is an AIDS-defining illness in its disseminated form.

Clinical manifestations

In AIDS patients, histoplasmosis is usually disseminated and manifests with fever (84%), weight loss, fatigue, and night sweats. Pneumonia is present in over half of cases, and hepatomegaly (40%), splenomegaly (40%), and lymphadenopathy (63%) are common findings (Peigne et al., 2011). Septic shock and respiratory failure are seen in about 10% of cases and are associated with a poor prognosis (Wheat et al., 2000). Central nervous system involvement (meningitis or a space-occupying lesion) occurs in approximately 15% of patients (Wheat et al., 1990). Various cutaneous lesions are seen, including maculopapular rash, pustules, papules and ulcers, and mucosal ulcers are typical. Gastrointestinal involvement is fairly frequent. Mediastinal granuloma and chronic cavitary lung disease are

unusual in this population. Death is associated with low CD4 counts and the absence of ARV treatment (Nacher et al., 2013).

Histoplasmosis due to *H. duboisii* is rarely observed in AIDS patients, and usually occurs among patients living in or having traveled to Central or West Africa or Madagascar (Loulergue et al., 2007).

Diagnosis

Pancytopenia is common at presentation. Chest radiography demonstrates patchy infiltrates, with diffuse interstitial pneumonitis in more severe cases. Microscopic examination of biopsy specimens or body fluid aspirates is the fastest diagnostic approach but its sensitivity varies. The highest diagnostic yield is from bone marrow and skin or mucosal lesions. The classic 2-4 µm budding yeasts are best visualized after periodic acid Schiff or methenamine silver staining. Samples suitable for culture of *H. capsulatum* include blood, bone marrow, bronchoalveolar lavage fluid and solid tissues. Cultures have to be handled in a P3 facility.

Histoplasma antigen detection in blood, urine or CSF is specific and sensitive in patients with disseminated disease. Antigenuria correlates well with the response to therapy and is useful for detecting relapsing histoplasmosis (Wheat et al., 2007). Serum galactomannan antigen can also be used for diagnosis and follow-up when *Histoplasma* antigen testing is negative (Rivière et al., 2012). More recently, specific PCR, targeting fungal RNA, methods have been developed.

Treatment

Amphotericin B and itraconazole are effective against both *H. capsulatum* and *H. duboisii* (Wheat et al., 2007). Among patients with severe disease, initial treatment should consist of liposomal amphotericin B, 3 mg/kg daily. When patients have improved, generally after

several weeks, they can be switched to oral itraconazole, 400 mg daily for 12 months. Milder illness can be treated initially with itraconazole, beginning with a loading dose of 200 mg thrice daily for 3 days, then 400 mg daily for 12 months (Wheat et al., 2007). Monitoring of serum itraconazole levels is mandatory in AIDS patients. Fluconazole is less effective than itraconazole but can be given, at a dosage of 400–800 mg daily, if itraconazole is not tolerated. The role of newer azoles has not been established.

Itraconazole can be withdrawn in patients who have been treated for at least 12 months, have at least 2 CD4 cell counts $> 150/\mu\text{L}$ in the preceding 6 months, and have received cART for at least 6 months (Goldman et al., 2004). Maintenance therapy should be restarted when CD4 cells number less than $100/\mu\text{L}$. Primary prophylaxis can be considered in areas in which the incidence of histoplasmosis is highest. An immune reconstitution inflammatory syndrome (IRI) can occur after ARV initiation (Breton et al., 2006)

2. Coccidioidomycosis

Coccidioides immitis and *Coccidioides posadasii* are dimorphic fungi found in semi-arid areas of the southwestern United States, Mexico, and Central and South America. Infection can occur after inhalation of arthroconidia present in soil, and may reactivate. Extrathoracic coccidioidomycosis involving sites other than the respiratory tract is an AIDS-defining illness.

Clinical manifestations

The most common symptoms of coccidioidomycosis in AIDS patients are fever, cough, and weight loss. Chest radiography reveals a variety of abnormalities, including focal pulmonary alveolar infiltrates, discrete nodules, hilar lymphadenopathy, and pleural effusion in more than 60% of patients. A diffuse reticulonodular infiltrate is present in 40% of patients.

Approximately 12% of patients develop meningitis. Lymphadenopathy, cutaneous lesions, and subcutaneous abscesses are common. Fungemia, thyroiditis, peritonitis, adrenal involvement, osteoarticular disease, and prostatic involvement have also been reported (Fish et al., 1990).

Diagnosis

The CD4 cell count is usually $<200/\mu\text{L}$ and often $<50/\mu\text{L}$. Definitive diagnosis relies on visualization of the typical large spherules containing numerous endospores in clinical specimens, and isolation of *Coccidioides* in culture. *Coccidioides* cannot be handled in clinical microbiology laboratories that are not equipped with a P3 facility. In case of meningitis, cerebrospinal fluid analysis typically shows leukocytic pleocytosis, with the possible presence of eosinophils, decreased glucose and increased protein values. *Coccidioides* culture from CSF is often negative, but positive serology can establish the diagnosis of coccidioidal meningitis. More recently, specific PCR methods, targeting fungal RNA, have been developed.

Treatment

The drug of choice for severe or life-threatening coccidioidal infection is amphotericin B lipid formulation, 3-5 mg/kg daily (Galgiani et al., 2005). Once the disease is controlled, generally after 2 to 3 weeks, a switch to an oral triazole drug should be considered. Fluconazole 400 mg daily is a good alternative, particularly in patients with meningitis; itraconazole 400 mg daily has also given good results, especially in patients with skeletal disease (Galgiani et al., 2005). Lifelong maintenance therapy with a triazole (fluconazole 400 mg daily or itraconazole 400 mg daily) is necessary to avoid relapse in AIDS patients (Kaplan et al., 2009).

3. Penicilliosis

Penicillium marneffei is a dimorphic fungus present in soil in Southeast Asia. Several imported cases of infection have occurred in Europe. *Penicillium marneffei* is associated with several species of bamboo rats, but contact with soil seems to be the most important risk factor for exposure to this fungus. In northern Thailand, penicilliosis used to be the third most common AIDS-defining opportunistic disease, after tuberculosis and cryptococcosis (Supparatpinyo et al., 1994). Penicilliosis is frequent in South China, accounting for 4.8% of AIDS-related hospital admissions.

Clinical manifestations

Penicilliosis is frequently disseminated at presentation, and the CD4 cell count is usually below 50 cells/ μ L. In a study of 92 patients with disseminated penicilliosis seen at Chiang Mai University Hospital in Thailand, 86 (93%) of patients were HIV-infected (Supparatpinyo et al., 1994), and most were young men. Signs and symptoms included fever (93%), anemia (78%), pronounced weight loss (76%), skin lesions (68%), generalized lymphadenopathy (58%), hepatomegaly (51%), cough (49%), diarrhea (31%), splenomegaly (16%), and jaundice (8%). Skin lesions, especially a generalized papular rash, are very suggestive of penicilliosis in patients at risk. Some papules, with central umbilication, mimic lesions caused by molluscum contagiosum. Subcutaneous nodules, acne-like lesions and folliculitis may also be present. Genital ulcers and palatal papules have been reported. Chest radiography is abnormal in almost 30% of patients with disseminated penicilliosis. Penicilliosis accounted for 11% of mucocutaneous lesions in HIV-infected patients in Guangxi, China (Han et al., 2013), and 16.5% of all positive blood cultures in Ho Chi Minh City in 2005 (Nga et al., 2012). Cases of IRIS have been reported.

Diagnosis

Fever, with or without pancytopenia, skin and/or lung lesions may also suggest a diagnosis of leishmaniasis or disseminated histoplasmosis. For any HIV-infected individual, a history of residence in or travel to Southeast Asia or South China should evoke the possibility of penicilliosis (Warnock et al., 1998).

In the Chiang Mai series, diagnosis of penicilliosis was made by culture of the fungus from blood (76%), skin lesions (90%), bone marrow aspirate (100%), or sputum. The galactomannan test for *Aspergillus* antigen cross-reacts with *P. marneffei* (Zheng et al., 2015). A presumptive diagnosis of penicilliosis can be made from examination of sputum or biopsy material from bone marrow, skin or a lymph node.

Treatment

Itraconazole and amphotericin B are the mainstays of therapy for penicilliosis, regardless of immune status. Induction treatment with amphotericin B 0.6 mg/kg daily for approximately 2 weeks, followed by itraconazole 400 mg daily for 10 weeks, is the treatment of choice (Sirisanthana et al., 1998). Voriconazole has also been shown to be effective. Suppressing therapy with itraconazole, 200 mg daily, is recommended for all patients with persistently low CD4 cell counts (Supparatpinyo et al., 1998). In geographic areas in which *P. marneffei* is endemic, primary prophylaxis with itraconazole 200 mg daily was recommended before the ART era (Chariyalertsak et al., 2002).

4. Other dimorphic fungi

Paracoccidioidomycosis

Paracoccidioidomycosis is not commonly reported in AIDS patients, for unexplained reasons. All reported cases of AIDS-associated paracoccidioidomycosis have occurred in Brazil, apart from one case reported in Venezuela. Most patients had disseminated disease, with advanced AIDS and CD4 cell counts well below 200/ μ L.

Diagnosis of paracoccidioidomycosis is usually based on direct microscopic examination and culture of clinical specimens, most commonly skin and lymph nodes. Culture of blood, bone marrow, and sputum may also be positive. Tests for *P. brasiliensis* antigen and PCR for *P. brasiliensis* DNA are promising new diagnostic tools.

First-line therapeutic options include trimethoprim-sulfamethoxazole, amphotericin B, ketoconazole, and itraconazole. Lifelong maintenance therapy with trimethoprim-sulfamethoxazole or itraconazole is recommended (Paniago et al., 2005).

Blastomycosis

Blastomycosis is also uncommon among HIV-infected patients. Several cases of blastomycosis have been reported in patients with AIDS, most of whom had CD4 cell counts well below 200/ μ L. Amphotericin B is considered to be the initial treatment of choice for patients with severe disease and for all immunosuppressed patients (Chapman et al., 2008). Patients who are improved after 2–4 weeks on amphotericin B may reasonably be switched to oral itraconazole 200 mg twice daily. Suppressive therapy with itraconazole should be maintained indefinitely in patients who remain severely immunodeficient.

Sporotrichosis

In a review of 16 cases of disseminated sporotrichosis in HIV-infected patients, diffuse ulcerative skin lesions were always present. Other disease sites included the CNS, joints, eyes, spleen, and bone marrow. *Sporothrix schenckii* fungemia was noted in two patients. The mean CD4 cell count was 73/ μ L.

Examination of tissue biopsies or material aspirated from skin lesions usually leads to the diagnosis when the typical cigar-shaped yeast forms are seen. Culture of tissue or body fluids at 25°C reveals the mould form of *S. schenckii*.

The optimal therapy for disseminated sporotrichosis in HIV-positive patients has not been determined. The most reasonable treatment would be amphotericin B for the most severe cases, followed by itraconazole. Itraconazole should be considered for long-term maintenance therapy among patients who remain immunosuppressed (Kauffman et al., 2007).

Emmonsiosis

Emmonsia sp. is a novel dimorphic fungi recently reported to cause disseminated infections in South African HIV-infected patients with very low CD4 cell counts (average 16/mm³). All the patients had disseminated infection involving the skin and lungs in most cases. The yeasts could be seen on pathological examination. Most patients were treated with liposomal amphotericin B (71%) and the mortality rate was 48% (Kenyon et al., 2013; Schwartz et al., 2015; van Hougenhouck-Tulleken et al., 2014).

iii) Other opportunistic moulds: aspergillosis

Invasive aspergillosis has been reported among patients with advanced HIV disease, especially those with CD4 cell counts <50/ml (Denning et al., 1991; Khoo and Denning, 1994; Lortholary et al., 1993). The classical risk factors for invasive aspergillosis, namely neutropenia and corticosteroid therapy, were absent in many AIDS patients with *Aspergillus* infection. The lungs are the most common site of infection. In a study of 33 AIDS patients with invasive aspergillosis, most of whom had pulmonary disease, the predominant symptoms were fever, cough and dyspnea, while chest pain and hemoptysis were less frequent (Lortholary et al., 1993). Chest radiographs often show nodular lesions and cavitary infiltrates suggestive of aspergillosis. Bilateral interstitial infiltrates are also noted. Invasive necrotizing tracheobronchitis, manifested by acute dyspnea and wheezing, is a clinical form of aspergillosis also seen in AIDS patients. Bronchoscopy reveals necrotic ulceration of the trachea, with pseudomembranes (Kemper et al., 1993). Obstructing bronchial aspergillosis with chest pain, hemoptysis and dyspnea may precede the onset of necrotizing tracheobronchitis (Denning et al., 1991). The majority of patients have a prior history of pulmonary infection. Preexisting cystic pulmonary lesions and bullae may be risk factors for invasive pulmonary aspergillosis in patients with AIDS. Disseminated aspergillosis often involves the CNS and myocardium. *Aspergillus* endocarditis and sinusitis have also been reported among patients with AIDS.

There is a good correlation between *Aspergillus*-positive bronchoalveolar lavage fluid culture and histologically proven aspergillosis in immunocompromised patients, including AIDS patients. The *Aspergillus* species most often implicated are *fumigatus*, *flavus*, *niger* and *terreus*. Detection of galactomannan antigen in serum or bronchoalveolar lavage fluid, and/or *Aspergillus* PCR, may help to establish the diagnosis of invasive disease.

Although not specifically studied in AIDS patients, voriconazole has now become the recommended therapy for invasive aspergillosis in all patient groups (Walsh et al., 2008). Interactions with protease inhibitors and non-nucleoside reverse transcriptase inhibitors must be carefully monitored when azole agents are used. An alternative therapy is a lipid formulation of amphotericin B. The prognosis of invasive aspergillosis in AIDS patients is poor, with a mean survival time of only 2–4 months despite antifungal treatment. This opportunistic infection is very unusual among patients on effective cART.

c) Immunosuppression and fungal risk among individuals living with HIV

i) Immunosuppression among individuals living with HIV

The earliest reports of AIDS showed that profound depletion of circulating CD4 T cells was central to the immune deficiency that defined the syndrome (Gottlieb et al., 1981; Masur et al., 1981). The same investigators also recognized that, despite the profound immune deficiency, activation of T cells (Gottlieb et al., 1981) and B cells (Lane et al., 1983) was also characteristic of this syndrome. The pathology of HIV disease is complex and multifaceted. In addition to high levels of systemic viral replication, HIV infection results in chronic immune activation and overall immunological dysfunction, which are closely associated with progression to AIDS.

Alteration of mucosal tissue:

HIV directly infects and depletes CD4 cells. The depletion occurs first in mucosal tissues, where large numbers of activated memory T cells expressing CCR5 chemokine receptors are present. As the majority of T cells reside within mucosal tissues, early loss of memory T cells leads to the loss of most of the body's T cells (Brenchley and Douek, 2008). Around 90% of gut-associated lymphoid tissue (GALT) T cells are depleted within 2 weeks of simian immunodeficiency virus acquisition (Mattapallil et al., 2005). During acute HIV infection, the viral RNA is detectable in 0.01-1% of peripheral T cells, compared to 60% of mucosal memory CD4 T cells (Brenchley et al., 2004; Mattapallil et al., 2005). Slower depletion of CD4 T cells in peripheral tissues and blood ensues (Douek et al., 2003). While cART can in most cases restore immunity, reflected by CD4 cell recovery, T cells in the gastrointestinal tract usually fail to recover. HIV infection damages the tight epithelial barrier of the gastrointestinal tract, leading to microbial translocation, severe immunological dysfunction

and chronic immune activation (Brenchley et al., 2006; Brenchley and Douek, 2008; Mehandru et al., 2006; Tincati et al., 2009).

Altered functionality and turn-over of CD8 T cells, B cells and innate immune cells are also observed (Paiardini et al., 2004; Ribeiro et al., 2002). Altered B cell function affects antibody production (Moir and Fauci, 2009), while altered NK cell function reduces cytokine production and increases cytotoxicity (Alter et al., 2004; Reeves et al., 2010). Dendritic cells and macrophages are also dysfunctional (Estes et al., 2010; Wallet et al., 2010)

Alteration of naïve T cells:

Among CD4 and CD8 T cells, HIV infection leads to a loss of essential naïve and central memory cell populations, resulting in an increased frequency of short-lived effector cells (Klatt et al., 2013). T cell maturation is also affected, with low numbers of circulating naïve CD4 and CD8 T cells (Lederman et al., 2011). Zeng et al explained how, in secondary lymphoid tissue, immune activation leads to fibrosis and architectural distortion of the fibroblast reticular network, limiting access to IL-7 (Zeng et al., 2012). This fibrosis and decreased access to IL-7 would deplete naïve T cells and limit immune reconstitution, as shown in the figure below.

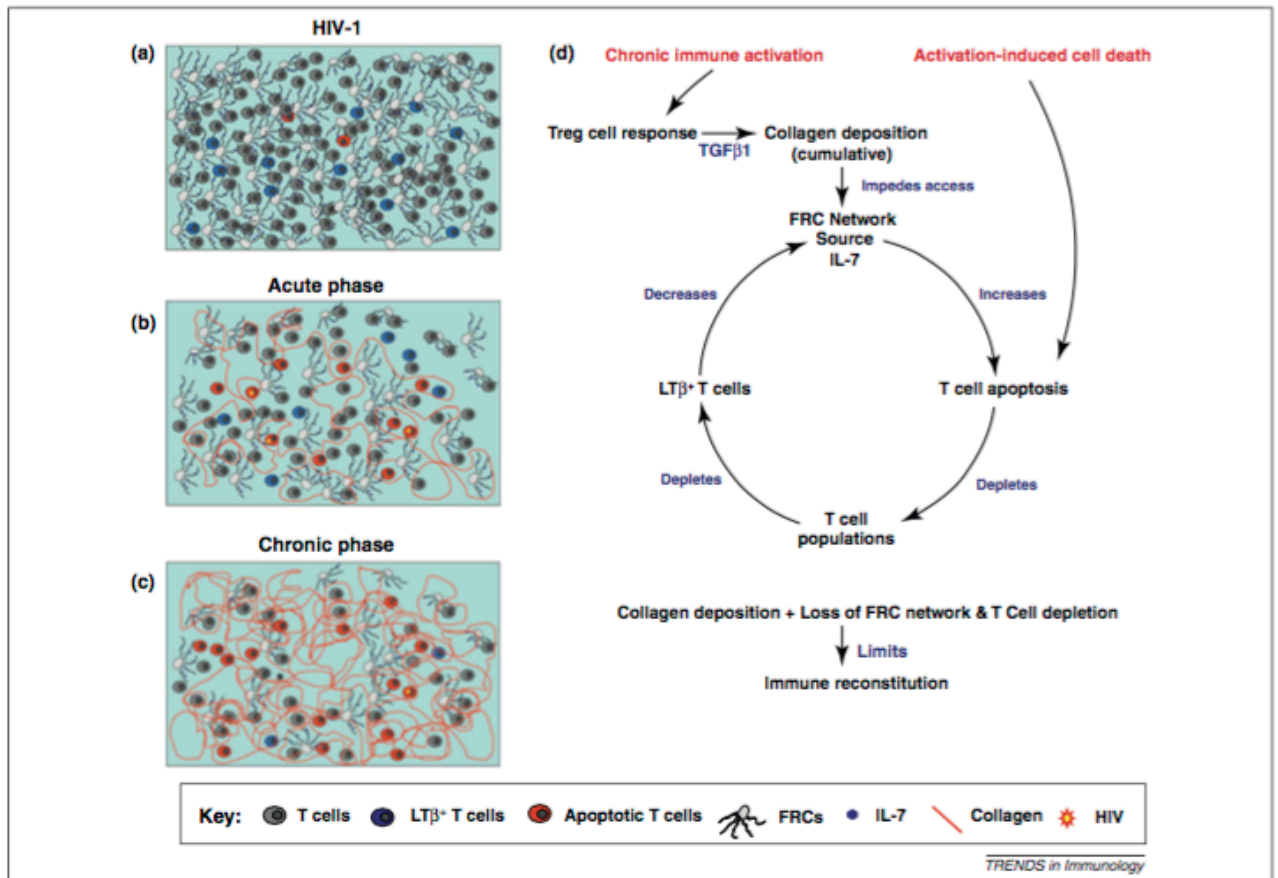


Figure 3. Collagen deposition and damage to the FRCn in HIV infection. (a) In the T cell zone of LNs from uninfected individuals, naïve T cell survival depends on interaction with IL-7 produced and ‘posted’ on the surface of FRCNs. (b) During the acute phase of HIV-1 infection, T cells are depleted by direct viral killing and other mechanisms such as AICD. However, immune activation also elicits a T regulatory response that activates TGFβ signaling in fibroblasts, resulting in cumulative collagen deposition. Collagen deposition and depletion of the FRCn impedes access of T cells to the survival factor IL-7 on the FRCn, and reduces the production of IL-7 within LTs, thereby causing increased apoptosis of naïve T cell populations. (c) As the infection progresses, depletion of T cells decreases LTβ, and this, compounded by collagen-impeded access to LTβ for the FRCn, results in further loss of FRCn and IL-7. Loss of the FRCn leads to further T cell depletion, particularly in naïve T cell populations. (d) This model summarizes the ‘vicious cycle’ model of T cell depletion, encompassing the key pathological events described in (a–c): chronic immune activation in HIV-1 and SIV infection elicits a T regulatory response that activates TGFβ1 signaling in fibroblasts, resulting in cumulative collagen deposition. Collagen deposition and loss of the FRCn together cause increased apoptosis, which, along with AICD, depletes T cells. Depletion of T cells decreases LTβ, which, along with collagen-impeded access to LTβ for the FRCn, results in loss of the FRCn and IL-7. This vicious cycle of survival interdependencies and collagen deposition cause progressive T cell depletion, particularly in naïve T cell populations. HAART can prevent the loss of CD4⁺ T cells due to the direct effects of infection and normalize immune activation, thereby reducing losses of CD4⁺ T cells due to AICD. However, pre-existing collagen deposition, loss of the FRCn and T cell depletion will limit immune reconstitution by the continued cyclical mechanisms. Abbreviations: FRCn, fibroblastic reticular cell network; LNs, lymph nodes; IL, interleukin; AICD, activation-induced cell death; TGF, transforming growth factor; LTs, lymphoid tissues; SIV, simian immunodeficiency virus; HAART, highly active antiretroviral therapy.

Impairment of thymic T cell production:

Destruction of thymic structure, thymocyte depletion and infiltration by activated T cells are observed in AIDS patients (Haynes et al., 2000; Zeng et al., 2012). HIV-1 directly kills thymocytes and dendritic cells required for normal development, and this could be a cause of CD4 T cell depletion (Haynes et al., 2000).

Immune activation and inflammation:

While the degree of CD4 T cell lymphopenia is an important predictor of HIV-related morbidity and mortality, persistent immune activation and inflammation are now recognized to be stronger and independent predictors of morbidity and mortality (Duprez et al., 2009; Kuller et al., 2008). A number of causative factors for sustained immune activation and inflammation have been identified, and are either directly or indirectly related to HIV replication. They include the innate and adaptive immune responses against HIV and associated pathogens, translocation of bacterial products through the compromised mucosal barrier, and potential bystander stimulation of lymphocytes and macrophages by HIV gene products (Appay and Sauce, 2008). However, the potential consequences of immune activation and its links to the CD4 T cell decline and immunodeficiency in HIV-1 infection remain a matter of debate.

ii) Immune regulation and fungi

(1) Defects in CD4 T lymphocytes

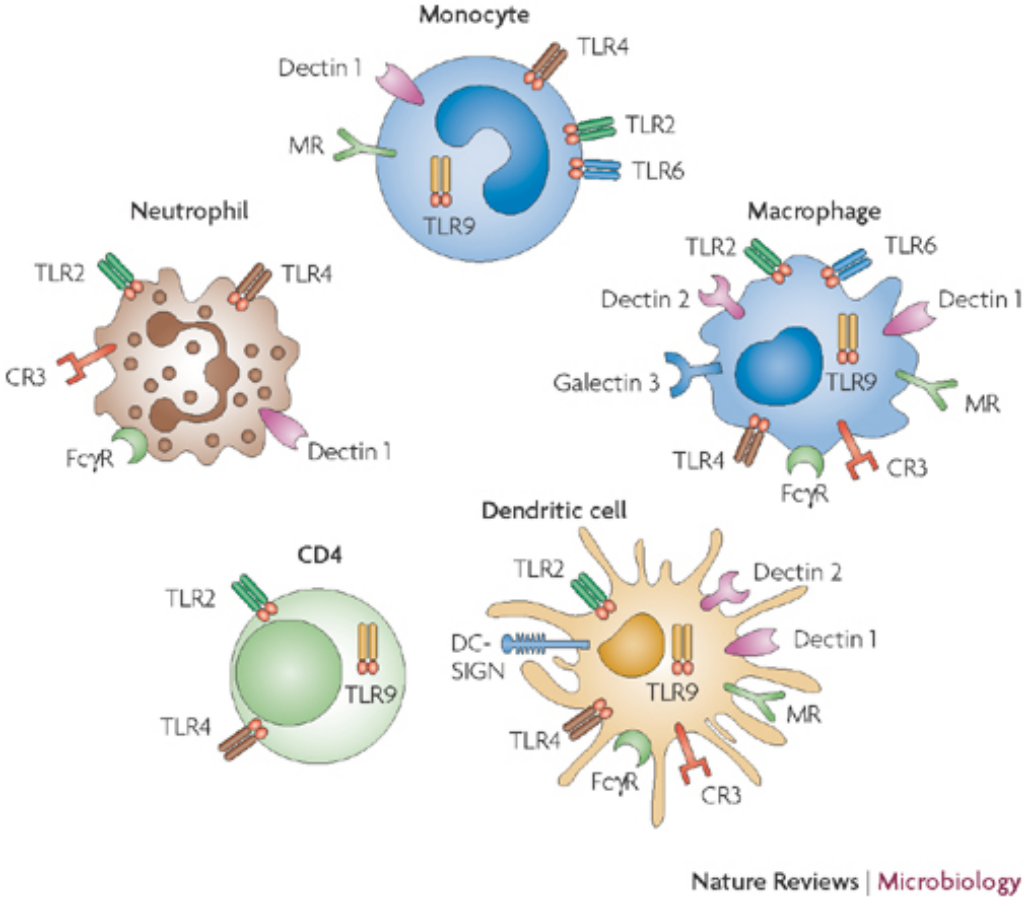
The risk of opportunistic fungal infections increases with the degree of immune deficiency. CD4⁺ lymphocyte defects are a primary risk factor for developing PCP, but the immune response to *Pneumocystis* is complex. Defects in B cells and antibody production may also predispose to PCP. CD8⁺ lymphocytes seem to be important in *Pneumocystis* clearance. A CD4 count <200 cells/ μ L was the leading pre-cART risk factor for PCP and is still an important risk factor in the cART era. The risk of PCP increases exponentially once the CD4 count falls below 200 cells/ μ L. When cART yields sustained CD4 cell recovery above 200/ μ L, the risk of PCP is sufficiently reduced to safely discontinue both primary and secondary prophylaxis.

(2) Other defects

Phagocytic cells (neutrophils, monocytes, macrophages and dendritic cells) are essential for protection against fungal pathogens. Loss of these cells, or defects in their antifungal effector functions, therefore results in susceptibility. While an innate response mediated primarily by neutrophils and macrophages is sufficient to control infections by some fungi, full protection against most pathogens also requires an adaptive response, initiated and directed by dendritic cells (DC) with contributions from other cells such as monocytes. This ultimately generates an immune response involving several fungal-specific components, but an augmentation of the antifungal activities of phagocytes (through the actions of IFN- γ for example) is required for protection in all cases. The IL-17 pathway regulates antifungal immunity through upregulation of proinflammatory cytokines, including IL-6, neutrophil-recruiting chemokines, and antimicrobial peptides, which act to limit fungal overgrowth, and this has been well demonstrated against *Candida*.

The figure below shows key cells involved in host defenses against fungi.

Key cells involved in host defenses against fungi:



(Netea et al., 2008)

(3) Immunosuppression and invasive aspergillosis

As stated above, the classical risk factors for invasive aspergillosis, namely neutropenia and corticosteroid therapy, are not always present in HIV-infected patients.

Even when present at normal levels, neutrophils and peripheral blood macrophages from HIV-infected individuals exhibit impaired activity against *A. fumigatus* in vitro (Pitrak et al., 1993; Roilides et al., 1993).

iii) Impact of cART on fungal infections

Chapter *Fungal infections in patients with AIDS* in Medical Mycology (B. Denis, et al) to be published soon

The advent of cART resulted in dramatic declines in morbidity and mortality among HIV-infected patients with advanced immune dysfunction (Palella et al., 1998) Successful cART is characterized by a marked reduction in viral load and a subsequent increase in the CD4 cell count. However, partial restoration of cell-mediated immunity and, possibly, other immune effectors may facilitate the development of an inflammatory reaction at former sites of infection, and this can mimic reactivation of the opportunistic disease itself. This “immune reconstitution inflammatory syndrome” (IRIS) has been reported in AIDS patients with cryptococcosis and histoplasmosis, PCP and penicilliosis (Boulware et al., 2014a; Breton et al., 2006; Lortholary et al., 2005; Shelburne et al., 2005; Sungkanuparph et al., 2009). Cases of IRIS reported during the course of histoplasmosis in AIDS patients were associated with uveitis, liver abscesses, arthritis, and necrotizing lymphadenopathy. In a prospective study of 101 AIDS patients who had cryptococcal meningitis, IRIS occurred in 13% of cases (Sungkanuparph et al., 2009). In the case of cryptococcosis, IRIS can present as aseptic meningitis with increased intracranial pressure. Other manifestations include necrotic lymphadenopathy, necrotizing pneumonia, and cerebral or medullary abscesses. Reported risk factors for IRIS include early initiation of cART after the diagnosis of cryptococcosis, a high CSF CrAg titer, fungemia, a low CD4 cell count, and high viral load.

The symptoms and signs of IRIS pose a diagnostic challenge for the clinician, who must decide whether the patient is experiencing relapse of an initial infection, a new infection, or IRIS. When an opportunistic infection occurs at a low CD4 cell count, studies have shown that starting cART early after treatment of the opportunistic infection improves overall survival, except in the case of cryptococcal meningitis. In a recent randomized study of 500 HIV-infected individuals with cryptococcal meningitis, deferral of cART for 5 weeks after the diagnosis of cryptococcal meningitis was associated with significantly better survival than cART initiation after 1 week (Boulware et al., 2014a). CART should be introduced cautiously in patients with cryptococcal meningitis, and it is advisable to wait at least until CSF cultures have become negative (Boulware et al., 2014a; Kaplan et al., 2009). If IRIS occurs, treatment with anti-inflammatory agents, including corticosteroids, may be effective, without necessitating a change in cART or in anti-infective treatment. In steroid-refractory cases, thalidomide might be indicated (Brunel et al., 2012)

Chapter 2. Methods

1) The French Hospital DataBase On HIV – the ANRS CO4 cohort

This part is inspired by an article from Murielle Mary Krause, *Int J Epidemiol* 2014.

a) Creation of the FHDH - ANRS CO4 cohort (FHDH)

The French Hospital Database on HIV is a multi-hospital open cohort in which inclusions have been ongoing since 1989, with the aim of collecting clinical information on HIV-infected patients managed in reference HIV treatment and information centers (CISIH: Centers d'Information et de Soins de l'Immunodeficiency Humaine) created by the French Ministry of Health in 1987. Patients are eligible if they are infected by HIV-1 or HIV-2; are managed in a participating center; and give their written informed consent. The French Ministry of Health funds technical research assistants who devote half their time to clinical research and the other half to FHDH data collection in the participating centers. The data thus collected are used to describe HIV-infected patients receiving hospital care in France, as well as HIV disease outcomes and their determinants, including antiretroviral treatment (ART).

The project was approved by the French computer watchdog authority (CNIL) on 27 November 1991 (*Journal Officiel*, 17 January 1992). Because of the need to return to the patient medical records to validate data for specific research projects, CNIL approval for a local correspondence list was obtained in 1999. The database includes data from 70 hospitals or wards distributed among 26 of the 28 COREVIH (Coordination régionale de la lutte contre le VIH). Since its creation, FHDH has been supported by INSERM (French National Institute of Health and Medical Research) and the French Ministry of Health. It became an ANRS (French National Agency for Research on AIDS and Viral Hepatitis) cohort (CO4) in 1996.

FHDH now includes data from 70 general or university hospitals located in 21 of the 26 French regions, including overseas departments (Aquitaine, Picardie, Poitou-Charentes, Limousin and Corse do not participate).

b) Objectives

The FHDH cohort is one of the largest of its type in the world, and the research projects draw on the biggest advantage of the database, namely its size. Care is taken not to encroach on other ANRS research topics. The areas of research include:

-Therapeutics strategies: mid- and long-term clinical outcomes, including causal inference models of observational data

-Severe AIDS and non AIDS morbidity/mortality: role of HIV infection and of exposure to antiretroviral treatments in impaired renal function, fractures, malignancies and cardiovascular diseases

-Public health issues surrounding HIV infection in France

-Open themes: Any participating center or researcher can submit a project to the scientific committee

In addition, 2 strategic groups have been created to develop specific projects on migrants and on HCV co-infection. A scientific committee with a maximum of 40 members, regularly renewed, debates FHDH research themes and selects projects according to their feasibility and scientific pertinence.

The French Hospital Database has numerous collaborations, including ART-CC, EUROCOORD (NOE FP7 2011-2015, CASCADE and COHERE) and HIV Causal. Besides research projects, the database allows the epidemiology of HIV infection in

patients in hospital care in France to be analyzed (in France, ARV treatments can only be initiated or changed in hospital). The completeness of the database was around 53% in 2009, and the database is representative of patients in care in France. The database has been used to evaluate the public health impact of antiretroviral drugs, with 13 contracts involving 3 partners (INSERM, INSERM Transfert, pharmaceutical companies) either ongoing or completed. The data can also be used to estimate the cost of care for HIV-infected individuals, represented mainly by antiretroviral drugs (62%) and hospitalization (31%).

c) Data collected

The database includes data from 70 hospitals or wards distributed among 26 of the 28 COREVIH (Coordination régionale de la lutte contre le VIH). The database includes data on over 128 000 patients aged at least 15 years and seen at least once between 1 January 1992 and 31 December 2012, with a mean follow-up of 88 months. Updates are ongoing, following a change in the software used for data collection. Since the creation of FHDH, collected baseline variables have included date of birth, gender, geographic origin, HIV transmission group, height, date of primary infection (when known) and dates of the last negative (when known) and first positive HIV tests. Variables collected at each follow-up visit include weight; standard biological markers such as the CD4 cell count and plasma HIV-RNA level; blood cell counts, lipids and liver enzymes; the date and type of AIDS and non-AIDS events, using the International Disease Classification; antiretroviral treatments and prophylaxis of opportunistic infections; and the dates and causes of death, as reported in the medical records. Since 2005, additional data have been collected on alcohol and tobacco use; serological and virological data on co-infection with hepatitis B or C virus; and liver biopsy results. Of note, FHDH linkage with the French deaths registry is not

authorized. Data are collected prospectively by trained research assistants, using either dedicated software provided by the French Ministry of Health, or computerized medical records. Standardized variables are collected at each outpatient visit or hospital admission during which a new clinical manifestation is diagnosed, a new treatment is prescribed, or a change in biological markers is noted, and/or at least every 6 months. Many validation procedures are implemented before inclusion of the data into the database, as well as local audits, to ensure the quality of the data.

d) Published results from FHDH

Since 1992, 192 publications based on FHDH data have been published in peer-reviewed journals, including 104 since 2010.

Regarding mid- and long-term clinical outcomes, research based on the FHDH showed that an immunological response at 6 months was predictive of better clinical outcome, regardless of the virologic response. Studies conducted within ART-CC and HIV Causal showed that treatment initiation at a CD4 cell count above $350/\text{mm}^3$ was beneficial in terms of AIDS events and mortality. This has just been confirmed by the START trial. Within COHERE, studies showed the public health benefit resulting from the arrival of new antiretroviral drugs in the period 2000-2009 among patients in whom the first three antiretroviral families had failed.

In the research theme “role of HIV infection and of exposure to antiretroviral treatments”, research from FHDH showed a doubling of the incidence of non AIDS-defining cancers among people living with HIV by comparison with the general population of the same age and gender, even in the era of combination antiretroviral therapy. It was also demonstrated that immunosuppression is a major risk factor both for AIDS-defining cancers (non-Hodgkin's lymphoma, Kaposi's sarcoma, cervical

cancer) and for non-AIDS cancers (lung, liver, anal canal, Hodgkin's disease). More recently, research from FHDH showed that patients whose viral load is controlled by treatment and whose CD4 cell count is restored to more than 500/mm³ for at least 2 years no longer have a higher risk of non-Hodgkin's lymphoma than the general population. A similar result was obtained for the risk of MI. A case-control study nested within the FHDH cohort showed that the risk of myocardial infarction was increased by exposure to first-generation protease inhibitors, confirming FHDH results published in 2003 (the first to show this association), and that the link to abacavir exposure could not be considered causal. HIV replication and immune status (CD4 nadir, current CD8) were also shown to influence the risk of myocardial infarction, independently of traditional risk factors and antiretroviral drug exposure.

Regarding public health issues surrounding HIV infection in France, FHDH results contributed to the discovery that a large proportion of HIV-infected patients enter care late in France. This work has recently been updated to reflect the new definition of late management, namely a CD4 cell count below 350/mm³ or AIDS status at the first hospital visit. FHDH studies showed that nearly one in two patients is managed late and that this late management increases the risk of death for up to 4 years after treatment initiation, by comparison with more timely management, even among individuals with only moderate immunosuppression (CD4 cell count between 200 and 350/mm³).

Since the creation of FHDH, we have been able to study epidemiological trends in opportunistic infections among HIV-infected individuals in France. For PCP, incidence rates in the FHDH were as high as 18.5/1000 PY [16.4-20.5] in 1992, falling to 4.7/1000 PY [4.0-5.4] in 1997 and 2.7/1000 PY [2.2-3.1] in 2006 (RICE data). With the availability of cART, a study from FHDH showed that PCP/toxoplasmosis

prophylaxis could be stopped when the CD4 cell count was steadily above 200/mm³ and 15% (Abgrall et al., 2001). Another study from FHDH showed that PCP survival at 3 years rose from 51% in the pre-cART era to 87% in 2001-2003 (Grabar et al., 2008).

Even if these opportunistic infections are now rare, the large size of the FHDH cohort allows us to continue to study their epidemiology. This is why we decided to study trends in fungal infections in the FHDH, focusing on two topics: PCP in the recent period 2004-2011, and invasive aspergillosis during the period 1992-2011.

2) Study of *Pneumocystis jirovecii* pneumonia (PCP) in the FHDH

a) Definition of cases

PCP diagnosis was considered definitive if there was histologic or cytologic evidence of *P. jirovecii* in bronchoalveolar lavage, sputum and/or lung biopsy specimens. The diagnosis was considered presumptive if there was a recent history of dyspnea or nonproductive cough in the absence of evidence of bacterial pneumonia, and a positive clinical response to PCP therapy.

A previous study of PCP in the FHDH cohort showed that recorded diagnoses of PCP were accurate (88.5% of 455 definitive diagnoses of first episodes of PCP were confirmed (Abgrall et al., 2001)).

b) Selection of patients

In order to study trends in PCP in France during the period 2004-2011, we selected HIV-infected individuals from the FHDH cohort who had a first diagnosis of PCP. Among 85 743 patients followed in the FHDH between 01/2004 and 12/2011, 1721 patients were diagnosed with PCP, of whom 1488 had a first episode of PCP during the study period. Furthermore, to analyze immunovirological outcomes after PCP, we excluded 169 patients with no available CD4 cell count within 3 months of PCP diagnosis, and 60 patients without available follow-up. Among the 1259 patients selected for the study, we compared baseline characteristics and outcomes between HIV-infected individuals previously enrolled in the FHDH and patients in whom PCP revealed their HIV infection. As a prior history of AIDS-defining illness (ADI) has been shown to influence the prognosis, previously enrolled patients were divided into 2 groups: those with a prior history of AIDS-defining illness (ADI) and those without

a prior history of ADI. ADI was defined as a category C illness in the 1993 CDC criteria.

3) Study of invasive aspergillosis (IA) in the FHDH

a) Selection of patients

Unlike PCP and toxoplasmosis, invasive aspergillosis is a rare and difficult diagnosis in HIV-infected individuals. Diagnosis often relies on clinical-radiological features, and specific biopsies are rarely done. Some biological data, such as the neutrophil count, are not systematically recorded in the database, and neither are specific microbial data on aspergillosis and radiological information. Thus, in order to study invasive aspergillosis in the FHDH, we first reviewed the medical charts of preselected patients. We first selected all HIV-infected individuals enrolled between 01/1992 and 12/2011, with ICD-9 or ICD-10 code B44 (aspergillosis): B440 (invasive pulmonary aspergillosis), B441 (other pulmonary aspergillosis), B442 (tonsillar aspergillosis), B447 (disseminated aspergillosis), B448 (other aspergillosis) or B449 (aspergillosis unspecified). This first selection was undertaken in 2012, when 515 patients were found to have corresponding ICD codes. Participating centers were contacted and authorization to retrieve the medical records was obtained from the heads of department.

b) Data collection: national retrieval of medical records

Four clinical research assistants working at INSERM UMRS 1136 retrieved medical records from the different clinical centers between May 2012 and May 2013. I first informed them of the specificities of aspergillosis, invasive aspergillosis and aspergillosis in HIV-infected individuals. In order to obtain all available information, a

specific CRF was prepared to record clinical, radiological, biological and microbiological criteria, treatments, outcomes, and causes of death. This CRF is available in the appendix.

Among the 515 patients with a corresponding ICD 9 or ICD10 code, 173 charts were not available for review (141 charts corresponding to 77, 44, and 20 patients, respectively, during the periods 1992-1995, 1996-2001 and 2002-2011) or had too many missing data (32 charts corresponding to 13, 14, and 5 medical records, respectively, during the periods 1992-1995, 1996-2001 and 2002-2011). 86% of the missing medical records and 84% of the incomplete medical records were for patients with an IA diagnosis in the period 1992-2001. Clinical, radiological, biological and therapeutic data relative to aspergillosis were retrieved. Finally, 342 charts were reviewed by a data review committee (DRC) which included 2 infectious diseases experts (BD and OL), in order to validate the cases.

c) Validation of IA cases and definition of “EORTC IA” and “HIV-related IA” among validated IA cases

Individuals without clinico-radiological signs of IA were excluded. Each EORTC criterion was checked. We added to the EORTC host factor "fewer than 500 neutrophil cells/mm³ for more than 10 days", the criterion "fewer than 800 neutrophil cells/mm³ with growth factor treatment for more than 10 days". Validated IA cases meeting the EORTC criteria were classified as proven, probable or possible. Biopsy, percutaneous aspiration or autopsy was required for proven cases.

When a case did not meet the EORTC host-factor criteria, the review committee examined radiological features, the results of microscopic examination and culture of bronchoalveolar lavage fluid (BAL), the results of GM antigen testing (GM), and associations with other pathogens, as in previous studies (Khoo SH et al, 1994). Patients who were infected both by an *Aspergillus* sp. and by a major respiratory copathogen that was detected in BAL fluid and might have explained the illness, namely *Mycobacterium tuberculosis*, *M. avium*, *Streptococcus pneumoniae*, *Histoplasma capsulatum*, or *Pneumocystis jirovecii*, were not considered as having IA. We also excluded patients considered to have transient *Aspergillus* colonization (patients without radiological abnormalities, bronchial endoscopy, or signs of necrotic tracheobronchitis on endoscopy), a typical aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), or a possibly false-positive galactomannan (GM) test. This concerned seven patients, one with disseminated histoplasmosis, three with a surgically treated aspergilloma and no histological signs of surrounding invasion, one with GM positivity at 0.5 plus pan-sinusitis and an aspergilloma of the maxillary sinus, and two ICU patients with septic shock on piperacillin-tazobactam and negative fungal culture of sputum. Cases not meeting the EORTC criteria were defined as

“HIV-related IA” and categorized into two groups: Group 1: no EORTC-defined host factor, a new radiological lesion compatible with the clinical manifestations, *Aspergillus* sp. recovered from BAL or a bronchial aspirate, no other identified pathogen, and/or galactomannan antigen >0.5 in serum or BAL, and no alternative diagnosis; Group 2: no host factor, new radiological lesion compatible with the clinical features, *Aspergillus* sp. detected in BAL or bronchial aspirate, and concomitant infection by a pathogen other than *M. tuberculosis*, *M. avium*, *S. pneumoniae*, *Histoplasma capsulatum* or *Pneumocystis jirovecii*. A total of 242 diagnoses were validated.

4) Statistical methods

In both studies, statistical methods were used to calculate prevalence/incidence rates. Survival was estimated with the Kaplan Meier method, and Cox proportional hazards models were used. Baseline characteristics of the patients at the time of fungal diagnosis were compared by using the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. All statistical analyses used the SAS software package (version 9.3; SAS Institute, Cary, NC, USA).

a) Estimation of prevalence and incidence in the FHDH:

i) PCP

The incidence of PCP among previously enrolled patients was calculated for each year during the study period by dividing the number of PCP cases among previously enrolled patients by the number of person-years of follow-up during the relevant year in the cohort. The frequency of inaugural PCP (revealing HIV infection) among newly enrolled patients was calculated for each year during the study period by dividing the number of PCP cases in the reference group by the number of new patients included in the cohort during the same year. Trends in the proportion of patients diagnosed with PCP during the study period among all previously enrolled patients was compared by using the Cochran Armitage test for trend.

ii) Invasive aspergillosis (IA)

The estimation of IA incidence rates took into account the difficulty of retrieving medical records (particularly for diagnoses made before 2002, which accounted for 85% of unretrieved medical records), especially for deceased patients. Furthermore, the IA validation rate was significantly higher when the CD4 cell count at diagnosis was below $100/\text{mm}^3$ than when it was above $100/\text{mm}^3$ (82% vs 53%; $p < 0.0001$). Thus,

the number of IA cases was first corrected for non-retrieval of medical records. After stratification for the study period (1992-1995/1996-2001/2002-2011) and the CD4 cell count ($CD4 < 100/mm^3$ or $CD4 \geq 100/mm^3$) at IA diagnosis, the rate of IA validation was applied to the 141 medical records that had not been reviewed. Incidence rates were then calculated for each period by dividing the estimated number of IA cases by the corresponding number of person-years (PY) of follow-up.

b) Survival estimates for PCP and invasive aspergillosis:

i) PCP

We compared immune reconstitution and survival after PCP diagnosis across the different patient groups. We wanted to determine if previously enrolled patients, who could have been on cART before PCP onset, had the same capacity for immune reconstitution as patients with inaugural PCP. Furthermore, we analyzed survival three years after PCP onset according to baseline characteristic at PCP diagnosis, adjusting for immune reconstitution and virological control after PCP. We defined immune reconstitution as a CD4 cell count above $200/mm^3$ after PCP diagnosis for patients with counts $< 200/mm^3$ at PCP diagnosis. We defined virological control as HIV viral load [VL] ≤ 50 copies/ml. The study of immune reconstitution after PCP onset involved the 1145 patients with CD4 cell counts $< 200/mm^3$ at PCP diagnosis. Data were censored three years after PCP diagnosis.

ii) Invasive aspergillosis

As survival has improved among HIV-infected individuals with the availability of cART, and among hematological patients with invasive aspergillosis (IA) with the availability of voriconazole, we studied three periods: pre-cART and pre-voriconazole

(<1996), post-cART and pre-voriconazole (1996-2001), and post-cART and post-
voriconazole. Kaplan-Meier estimates were used to study three-month survival rates
after IA diagnosis in HIV-infected individuals, and to compare them across the three
periods. Kaplan-Meier survival estimates were compared between patients with
“EORTC IA” and those with “HIV-related IA”.

c) Study of risk factors in Cox proportional hazards models

i) PCP

Cox proportional hazards models were used to identify factors associated with survival
or immune reconstitution, including the patient subgroup (HIV-infected individuals
with inaugural PCP vs previously enrolled patients), age (<30 years, >30 to <50, and ≥
50), region of origin (sub-Saharan Africa, others), the HIV exposure category (men
who have sex with men (MSM), intravenous drug users (IDU), heterosexuals), the
CD4 cell count at PCP diagnosis ($CD4 < 50/mm^3$, $50 \leq CD4 < 100$, $100 \leq CD4 < 200$,
 $CD4 \geq 200$), and HIV viral load at PCP diagnosis ($VL < 5 \log \text{ copies/ml}$, $VL \geq 5 \log$
 copies/ml). In the Cox model for immune reconstitution, virological control ($VL \leq 50$
 copies/ml) was included as a time-dependent variable. In the Cox model for the risk of
death within 3 years after PCP onset, virological control and immune reconstitution
($CD4 > 200/mm^3$) were included as time-dependent variables. In our analysis, patients
previously enrolled with a prior ADI had a poorer prognosis. As there were more IV
drug users in this group, and as death could have been related to other causes such as
overdose, we conducted sensitivity analyses after excluding IV drug users.

ii) Invasive aspergillosis

We chose a short period after IA diagnosis (3 months) to study risk factors for death after IA, because we wanted to study the direct impact of IA, as in previous studies (Herbrecht et al., 2002). We included in the Cox models the following baseline characteristics: age (<40, 40–59, or ≥ 60 years), the HIV transmission group (intravenous drug users vs others), CD4 cell count at diagnosis (log₂ CD4), the IA classification (EORTC or HIV-related), and the period of diagnosis. We introduced voriconazole exposure as a time-dependent covariable in the Cox model in the last period, when voriconazole was available.

Furthermore, in the EORTC definition, “possible IA” does not require microbiological criteria and the diagnosis is considered less robust than for EORTC “proven” and “probable” IA. Our definition of “HIV-related IA, group 2” could include concomitant infection by a pathogen other than *M. tuberculosis*, *M. avium*, *S. pneumoniae*, *Histoplasma capsulatum* and *Pneumocystis jirovecii*. To substantiate our results, two sensitivity analyses were undertaken: one after excluding EORTC “possible IA”, and the other after excluding both EORTC “possible” IA and “HIV-related IA, group 2”.

Chapter 3. Trends in PCP among HIV-infected individuals in France in the cART era

The first part of our work consisted of an epidemiological study of HIV-infected individuals who had a first diagnosis of PCP nowadays in France. As PCP is a good marker of severe immunosuppression and as 82% of HIV-infected individuals enrolled in the FHDH cohort had been on cART for at least 6 months at the time of this study, our first hypothesis was that most PCP cases would be diagnosed among late presenters, in whom PCP revealed their HIV infection.

To our surprise, PCP revealed HIV infection in only half the 1259 patients with a first diagnosis of PCP during the period 2004-2011. The other half were previously enrolled patients (median 8 years since enrolment), 68% of whom had received more than one year of cART before PCP diagnosis, and two-thirds of whom had a history of CD4 cell counts above 350/mm³. At PCP diagnosis, their median CD4 cell count was 38/mm³ and their VL was 5.2 log₁₀ copies/ml, values similar to those of patients with inaugural PCP (53/mm³ and 5.1 log₁₀ copies/ml).

This led us to examine retention in care in the FHDH cohort. We defined adequate adherence to care as having a CD4 cell count at least every 6 months. The rate of adherence to care before PCP diagnosis was estimated and compared with the rate of adherence to care among all patients enrolled in the FHDH. The median proportion of time spent with adequate adherence to care was 85% (IQR, 66–96) for all FHDH enrollees, but only 45% (IQR, 1–81) during the 2 years before PCP diagnosis among previously enrolled patients. Therefore, some patients' adherence had diminished over time, increasing the risk of developing an AIDS-defining clinical illness.

We found that previously enrolled patients, especially those with a prior history of AIDS-defining clinical illness, had slower immune reconstitution after PCP than patients with inaugural PCP (by comparison with patients with inaugural PCP, risk factor of achieving a CD4 cell count above 200/mm³: HR 0.7 (0.6-0.9) for previously enrolled patients without a prior ADI, and 0.6 (0.4-0.8) for previously enrolled patients with a prior ADI). In addition, the 3-year mortality rate after PCP diagnosis was 25% among previously enrolled patients with a prior ADI, compared to only 8% among previously enrolled patients without a prior ADI and 9% among patients with inaugural PCP. Previously enrolled patients with a prior ADI had a poorer prognosis than patients with inaugural PCP, even after adjustment for virological control and immune reconstitution (HR, 2.4 [95%CI, 1.5–3.7]), demonstrating persistent immune system dysfunction after an ADI that is not only reflected by the CD4 cell count.

Thus, in order to reduce the incidence of PCP and to improve its prognosis, we need to maintain patients in care in the long term, concentrating first on patients who have been in care for many years, and to design interventions to maintain adherence over time, especially among patients with a history of ADI. We are now working on indicators of adherence to care in order to help clinicians identify patients at risk. Also, as half the cases of PCP diagnosed during the study period could have been avoided by early screening for HIV, more efficient screening strategies must be developed in conjunction with the different public health stakeholders.

Critical Importance of Long-Term Adherence to Care in HIV Infected Patients in the cART Era: New Insights from *Pneumocystis jirovecii* Pneumonia Cases over 2004–2011 in the FHDH-ANRS CO4 Cohort

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Abstract

Objective: To describe characteristics and outcomes of HIV-infected patients with *Pneumocystis jirovecii* pneumonia (PCP) over 2004–2011 in France, in particular in those previously enrolled (PE) in the French Hospital Database on HIV (FHDH).

Methods: PE patients with an incident PCP were compared with patients with an inaugural PCP revealing HIV infection (reference). Adequate adherence to care was defined as a CD4 measurement at least every 6 months. Immune reconstitution (CD4 \geq 200/mm³) and risk of death were studied using Kaplan-Meier estimates and multivariable Cox proportional hazards models.

Results: In a context of a decreasing incidence of PCP, 1259 HIV-infected patients had a PCP diagnosis, and 593 (47%) were PE patients of whom 161 (27%) have had a prior history of AIDS-defining clinical illness (prior ADI). Median time since enrolment was 8 years for PE patients; 74% had received cART. Median proportion of time with adequate adherence to care was 85% (IQR, 66–96) for all FHDH enrollees, but only 45% (IQR, 1–81) for PE patients during the 2 years before PCP. Median CD4 cell count (38/mm³) and HIV viral load (5.2 log₁₀ copies/ml) at PCP diagnosis did not differ between PE patients and the reference group. Three year mortality rate of 25% was observed for PE prior ADI group, higher than in PE non-prior ADI group (8%) and the reference group (9%) (p<0.0001). In the PE prior ADI group, poor prognosis remained even after adjustment for virological control and immune reconstitution (HR, 2.4 [95%CI, 1.5–3.7]).

Conclusion: Almost 50% of PCP diagnoses in HIV-infected patients occurred presently in patients already in care, mainly with a previous cART prescription but with waning adherence to care. Having repeated ADI is contributing to the risk of death beyond its impact on immune reconstitution and viral suppression: special efforts must be undertaken to maintain those patients in care.

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Introduction

Pneumocystis jirovecii pneumonia (PCP) is a well-recognized major opportunistic infection and one of the most common AIDS-defining illnesses (ADI) [1–3]. Before the introduction of *Pneumocystis* prophylaxis and combination antiretroviral therapy (cART), it was estimated that 75% of HIV infected patients developed PCP during their lifetime [3,4]. After 1996, with the availability of cART, a dramatic decline in PCP incidence was observed in HIV infected patients [5]. In previous studies, the

proportion of PCP diagnoses that revealed HIV infection ranged from 23% to 55%, while none or few patients with a previously known HIV diagnosis were under cART [6–8].

In industrialized countries, fewer than 10% of patients taking cART experience virological failure [9]. In 2011, 82% of HIV-infected patients enrolled in the FHDH cohort had been on cART for at least 6 months, and 89% of them had a viral load (VL) below 50 copies/ml [10]. Therefore, the risk of presenting a PCP should be limited to basically late presenters. However, retention in care is an emerging problem: a meta-analysis conducted between 1995

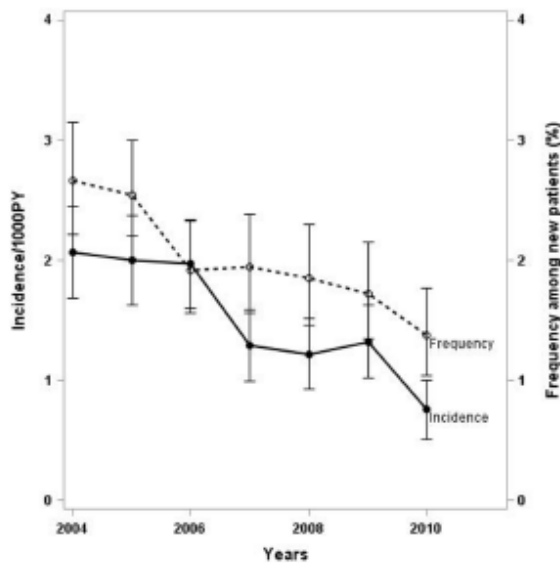


Figure 1. Trends in the risk of PCP overtime: incidence of first PCP for previously enrolled patients (bolt line) and frequency of inaugural PCP in newly enrolled patients (dotted line).
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and 2009 in the United States showed that only 59% of patients regularly attended medical appointments [11]. In 2013, some authors reported that half of the patients presenting with an ADI [12,13] and specifically PCP [12,13] were already in care. This raises concerns about long-term adherence to care and its repercussion on morbidity and mortality. Here we studied the characteristics and outcome of patients who presented a PCP diagnosis while already in care with or without a prior ADI and compared them with late presenter patients with an inaugural PCP during 2004–2011 in France. In particular the role of repetitive ADI on risk of mortality was investigated.

Patients and Methods

Patients

The French Hospital Database on HIV (FHDH) is a nationwide hospital-based cohort [14]. This epidemiological network was created in 1989. Seventy participating hospitals across France provide data on HIV-infected patients, who represent 50–60% of all HIV-seropositive patients receiving care in France. The only FHDH inclusion criteria are HIV-1 or HIV-2 infection and written informed consent. Trained research assistants prospectively collect clinical, biological and therapeutic data and causes of death from medical records, using specialized software. For this analysis, we considered all HIV-1-infected patients enrolled in the FHDH who had a first diagnosis of PCP. PCP diagnosis was considered definitive if there was a histologic or cytologic evidence of *P. jirovecii* on bronchoalveolar lavage, sputum and/or lung biopsy specimens. Diagnosis was considered only presumptive if there was a recent history of dyspnea or nonproductive cough in the absence of evidence of bacterial pneumonia, and a positive clinical response to appropriate therapy for PCP. We excluded patients with no CD4 cell count within 3 months of PCP diagnosis and surviving patients with no CD4 cell count after PCP. Patients previously enrolled (PE) in the FHDH with an incident PCP were

compared to patients with a PCP revealing HIV infection (taken as the reference group). The group of PE patients was divided into patients with a prior history of AIDS-defining illness (ADI) (PE prior ADI) and those without prior ADI (PE non prior ADI). ADI was defined as a C category according to the 1993 CDC criteria.

Definition of adequate adherence to care

Adherence to care of patients enrolled in FHDH was considered adequate if they had a CD4 cell count at least every 6 months. The duration of adequate adherence to care was calculated as the sum of the number of days between two CD4 cell counts, after excluding days exceeding the time window of 6 months. For patients with no CD4 cell counts prior to PCP, the duration of adequate adherence to care were set at 0. The proportion of time spent with adequate adherence to care was determined by dividing the number of days with adequate adherence to care by the total length of follow up. This indicator of adherence to care was calculated for each patient with at least a visit in the period 2004–2011 and who were followed up for at least 3 years in the FHDH cohort. For PE with an incident PCP, adherence to care was first calculated from the first visit (enrolment in the FHDH) until PCP diagnosis; then adherence to care was calculated during the last 2 years before PCP. In the whole cohort, adherence to care was calculated from the first visit (enrolment in the FHDH) until the last one in the FHDH cohort.

Statistical analysis

Incidence of PCP among PE patients was calculated for each year during the study period by dividing the number of PE PCP cases by the number of person-years followed during that year in the cohort. Frequency of inaugural PCP among newly enrolled patients was calculated for each year during the study period by dividing the number of PCP cases in the reference group by the number of new patients included in the cohort during the same year. Evolution of the proportion of PCP diagnosis in PE patients during the study period was compared by a Cochran Armitage test for trend.

Baseline characteristics were compared across the 3 groups, PE prior ADI, PE non-prior ADI and patients with a PCP revealing HIV infection (reference group) by using the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. Kaplan-Meier estimates of the proportion of patients with virological control (HIV viral load [VL] ≤ 50 copies/ml), the proportion of patients with immune reconstitution ($CD4 > 200/mm^3$), and survival were compared between the three groups of patients after right censoring the data 3 years after PCP diagnosis.

Cox proportional hazards models were used to identify factors associated with outcome, including age (<30 years, ≥ 30 to <50, and ≥ 50), country of origin (sub-Saharan Africa, others), the HIV exposure category (men who have sex with men (MSM), intravenous drug users (IDU), heterosexual), CD4 cell counts at PCP diagnosis ($CD4 < 50/mm^3$, $50 \leq CD4 < 100$, $100 \leq CD4 < 200$, $CD4 \geq 200$), and HIV viral load at PCP diagnosis ($VL < 5$ log copies/ml, $VL \geq 5$ log copies/ml). In the Cox model for immune reconstitution, virological control was included as time-dependent variable. In the Cox model for mortality, virological control and immune reconstitution were included as time-dependent variables.

All tests were two-sided, and P values below 0.05 were considered to denote statistical significance. All statistical analyses used the SAS software package (version 9.3; SAS Institute, Cary, NC, USA).

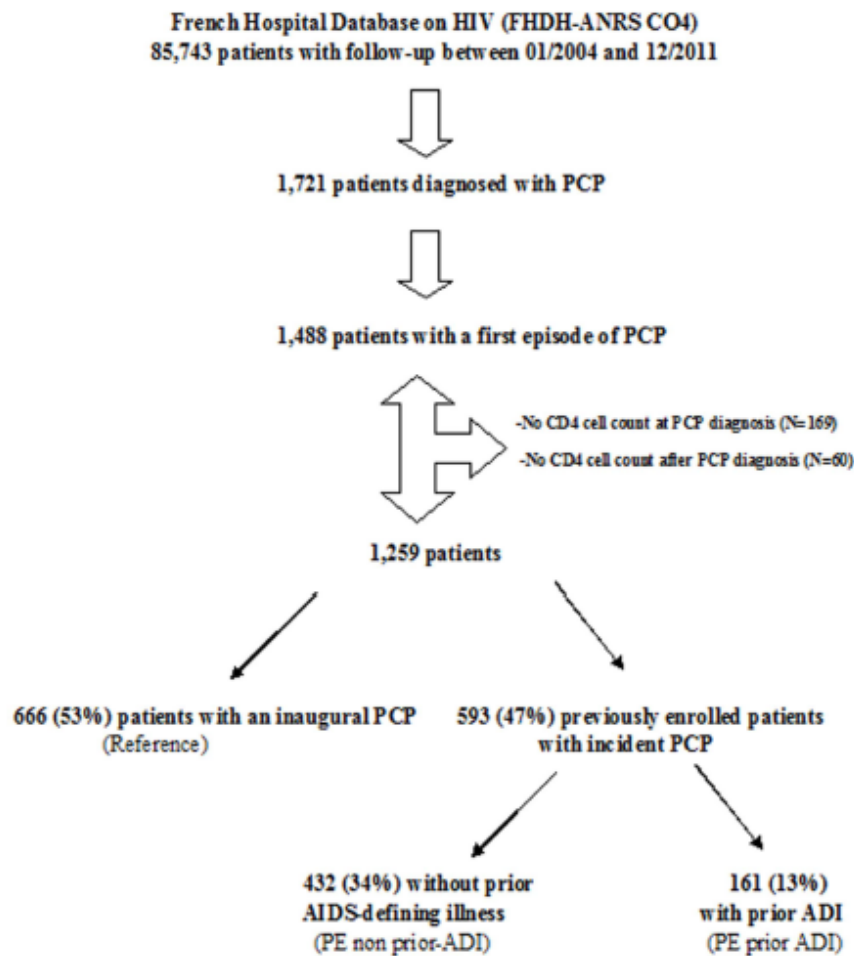


Figure 2. Patient selection flow diagram.
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Results

Trends of PCP over time

Over the period 2004–2011, 85,743 patients had a follow-up in the FHDH and 35,985 new patients were included in the FHDH cohort. There was a decreasing frequency of PCP in the group of patients with an inaugural PCP and a decreasing incidence of PCP for previously enrolled patients (figure 1). In addition, the proportion of PCP diagnosed in PE group among all PCP diagnosis has remained stable during the study period (46% in 2004 vs. 40% in 2010, Cochran Armitage test for trend $p=0.77$).

Characteristics of the patients

Over our study period a first episode of PCP was diagnosed in 1488 patients, of whom 1259 patients with at least a CD4 cell count within 3 months of PCP diagnosis and at least one CD4 cell count during follow-up were retained for analysis (figure 2).

The patients' characteristics at PCP diagnosis are shown in table 1. Overall, 593 (47%) patients were previously enrolled in the FHDH. The majority of patients were males (73%). Median age was 42 years (IQR, 36–49). Migrants represented 12% of the

overall population, and sub-Saharan patients were more numerous among patients with a PCP revealing HIV infection. The IDU HIV transmission group was more numerous in the PE group. The median CD4 cell count and median viral load at PCP diagnosis were $38/\text{mm}^3$ (IQR, 15–92) and 5.2 \log_{10} copies/ml (IQR, 4.6–5.7), respectively, with no difference of clinical importance between PE patients, and patients in the reference group.

For PE patients, median time since enrolment was 8.2 years (IQR, 3.7–11.3) and 27% of them had had a prior ADI. Although 439 (74%) of these patients had already had a prescription for cART, all but 18 of them had detectable VL at PCP diagnosis (median, 5 \log_{10} copies/ml [IQR, 4.1–5.6]). Among PE patients with at least one year of cART prescription before PCP, 277 (69%) patients have had at least once high CD4 cell count (CD4 cell count $>350/\text{mm}^3$), and 318 (80%) patients a virological response (HIV viral load <500 copies/ml). Median proportion of time with adequate adherence to care was 60% (IQR, 33–84) since FHDH enrolment for PE patients until PCP diagnosis, and 45% (IQR, 1–81) during the 2 years before PCP diagnosis, compared to a median proportion of 85% (IQR, 66–96) for all FHDH enrollees.

Table 1. Patients with a first episode of PCP in 2004–2011: characteristics at PCP diagnosis.

	Previously Enrolled patients with an incident PCP (all PE)	Previously Enrolled patients with an incident PCP without prior ADI (PE non-prior ADI)	Previously Enrolled patients with an incident PCP with prior ADI (PE prior ADI)	Patients with an inaugural PCP revealing HIV infection (reference)	P value ^a
Total	593 (100%)	432 (100%)	161 (100%)	666 (100%)	
Male gender	417 (70%)	293 (68%)	124 (77%)	502 (75%)	0.001
Age (years)	42 (37–47)	42 (37–47)	42 (38–48)	42 (36–50)	0.55
Sub-Saharan origin	50 (8%)	32 (7%)	18 (11%)	103 (15%)	0.0003
Transmission group					<0.0001
MSM	154 (26%)	116 (27%)	38 (24%)	189 (28%)	
IDU	109 (18%)	68 (16%)	41 (25%)	20 (3%)	
Heterosexual	330 (56%)	248 (57%)	82 (51%)	457(69%)	
CD4/mm³	38 (15, 92)	53 (18,140)	33 (10,99)	34 (13,70)	<0.0001
HIV viral load, log₁₀ copies/ml (N = 1169)	5.2 (4.6,5.7)	5.1 (4.1, 5.6)	5.0 (3.3, 5.6)	5.3 (4.8, 5.7)	<0.0001
Time since enrolment (years)	8.2 (3.7, 11.3)	8 (3.7,11.1)	8.7 (3.8,11.7)	NA	
cART >1 year	400 (68%)	262 (61%)	138 (86%)	NA	
Patients with cART >1 year					
Duration of treatment (years)	7 (4.8–9)	6.7 (4.6, 8.8)	7.8 (5.1, 8.8)	NA	
CD4 nadir up to cART initiation	180 (81–300)	204 (106, 324)	119 (36, 220)	NA	
CD4>350/mm ³ *	277 (69%)	191 (73%)	86 (62%)	NA	
HIV viral load <500 copies/ml **	318 (80%)	203 (77%)	115 (83%)	NA	

Figures are medians (Interquartile Range) or N (%); ^aP value: χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables, comparison between PE non-prior ADI, PE prior ADI, and reference groups.

* Percentage of patients with CD4 cell count >350/mm³ after cART initiation;

**percentage of patients with HIV viral load <500 copies/ml after cART initiation ADI: AIDS-defining illness, MSM: Men who have Sex with Men, IDU: Intravenous Drug User.

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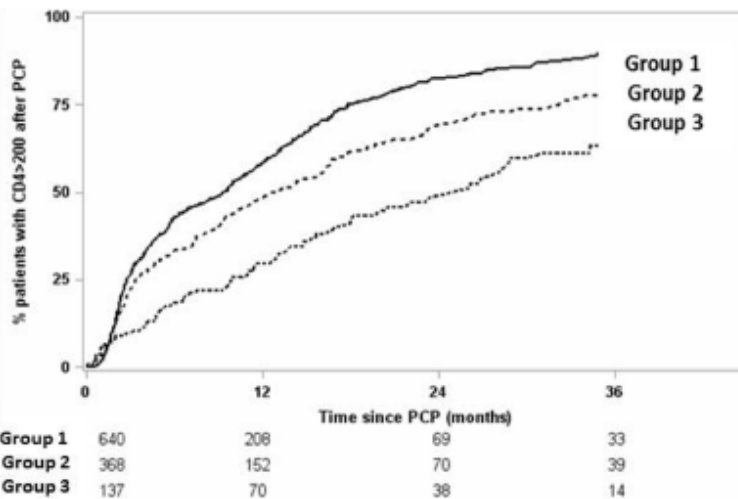


Figure 3. Kaplan-Meier estimates of immune reconstitution (CD4 cell count $\geq 200/\text{mm}^3$) probability, and number of patients at risk, after PCP diagnosis for patients with an inaugural PCP revealing HIV infection (reference, group 1), previously enrolled patients with incident PCP without prior AIDS-defining clinical illness (ADI) (PE non-prior ADI, group 2) and previously enrolled patients with incident PCP with prior ADI (PE prior ADI, group 3).

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Table 2. Factors associated with immune reconstitution (CD4>200/mm³) within 3 years after PCP.

	Total	Nb of events within 3 years after PCP	Univariate analysis HR* (95%CI)	Adjusted analysis HR* (95%CI)	P value
Patient group					<0.0001
Reference group with inaugural PCP	640	468	1.0	1.0	
PE non-prior ADI patients	368	236	0.7 [0.6–0.8]	0.7 [0.6–0.9]	
PE prior ADI patients	137	63	0.4 [0.3–0.6]	0.6 [0.4–0.8]	
Age [years]					0.62
<30	93	60	1.0 [0.8–1.3]	1.1 [0.8–1.4]	
[30–49]	802	540	1	1	
≥50	250	167	1.2 [1.0–1.4]	1.1 [0.9–1.3]	
Transmission group					0.001
MSM	308	230	1.3 [1.2–1.6]	1.1 [0.9–1.3]	
Heterosexual	724	481	1	1	
IDU	113	56	0.6 [0.5–0.8]	0.6 [0.5–0.8]	
Origin					0.0002
Sub-Saharan	146	78	1	1	
Other	999	689	1.7 [1.3–2.1]	1.6 [1.2–2.1]	
CD4 at PCP diagnosis (mm³)					<0.0001
[0–49]	718	439	1	1	
[50–99]	245	181	2.0 [1.7–2.4]	2.2 [1.8–2.6]	
[100–199]	182	767	2.6 [2.1–3.1]	3.1 [2.6–3.9]	
HIV viral load (VL) at PCP diagnosis					<0.0001
<5 log ₁₀ copies/ml	405	256	1	1	
≥5 log ₁₀ copies/ml	662	468	1.3 [1.1–1.5]	1.4 [1.2–1.7]	
HIV VL≤50 copies/ml after PCP (time-dependent covariable)					<0.0001
No	NA	NA	1	1	
Yes	NA	NA	2.3 [1.9–2.9]	2.3 [1.8–2.8]	

*Adjusted HR>1: in favor of achieving a CD4 cell count ≥200/mm³; PE non prior ADI: previously enrolled patients without prior AIDS-defining illness, PE prior ADI: previously enrolled patients with prior AIDS-defining illness, MSM: Men who have Sex with Men, IDU: Intravenous Drug User.
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Virological, immunological and clinical outcomes

One year after PCP diagnosis, VL was ≤50 copies/ml in 64% of PE non-prior ADI patients, 49% of PE prior ADI patients and 76% of patients in the reference group ($p<0.0001$). Among the 1145 patients (91%) with CD4 cell counts below 200/mm³ at PCP diagnosis, 52% had CD4 cell counts ≥200/mm³ one year after PCP diagnosis, and 84% at three years. At 1 and 3 years, respectively, CD4 cell counts ≥200/mm³ were achieved in 48% and 78% of PE non-prior ADI patients, and 29% and 68% of PE prior ADI patients and 58% and 90% of patients in the reference group (figure 3). PE non-prior ADI patients (HR, 0.7 [95%CI, 0.6–0.8]) and PE prior ADI patients (HR, 0.6 [95%CI, 0.4–0.8]) had slower immune reconstitution than patients in the reference group (table 2).

Mortality at 1, and 3, years after PCP diagnosis was 7%, and 11%, respectively. In subgroup analyses, mortality at 1 and 3 years was respectively 5% and 8% among PE non-prior ADI patients, 16% and 25% among PE prior ADI patients and 6% and 9% among patients in the reference group ($p<0.0001$). After adjustment on baseline characteristics, PE prior ADI patients were at increased risk of mortality (HR, 2.7 [95%CI, 1.7–4.2]) compared to patients in the reference group (Table 3). This higher

mortality risk for PE prior ADI patients remained after adjustment on viral suppression and immune reconstitution (HR, 2.4 [95%CI, 1.5–3.7]).

Discussion

In a context of a decreasing risk of PCP, 47% of patients diagnosed with PCP have already been in care but presented a poor adherence to care during the 2 years prior to PCP diagnosis. In addition, the 3 year mortality rate after PCP diagnosis was very high (25%) for previously enrolled patients with prior ADI, higher than in previously enrolled patients without prior ADI and the reference group of patients with an inaugural PCP revealing HIV infection.

This is the largest observational study of PCP in the recent cART era; however several limitations must be discussed. First, we used PCP diagnoses and deaths reported in the FHDH cohort. A previous analysis of this cohort showed that 93% of PCP diagnoses were confirmed on examining the patients' clinical records [15]. Second, among 1488 patients with a first episode of PCP during the study period, 229 patients were excluded from this analysis because of a lack of CD4 cell counts at PCP diagnosis or during

Table 3. Factors associated with risk of death after PCP within 3 years after PCP.

Patient group	Total	Nb of events within 3 years after PCP	Univariate analysis HR* (95%CI)	Model I: Adjusted analysis HR* (95%CI)	P value	Model II: Adjusted analysis HR* (95%CI)	P value
Reference group with inaugural PCP	666	52	1	1	<0.0001	1	<0.0001
PE non-prior ADI patients	432	29	0.8 [0.5–1.3]	0.8 [0.5–1.3]		0.8 [0.5–1.3]	
PE prior ADI patients	161	35	2.9 [1.9–4.4]	2.7 [1.7–4.2]		2.4 [1.5–3.7]	
Age (years)					0.002		0.0009
<30	98	9	1.3 [0.6–2.6]	1.4 [0.7–2.9]		1.3 [0.6–2.7]	
[30–49]	888	68	1	1		1	
≥50	273	39	2.0 [1.3–2.9]	2.1 [1.4–3.1]	0.008	2.2 [1.4–3.3]	0.12
Transmission group							
MSM	343	24	0.8 [0.5–1.2]	0.8 [0.5–1.2]		0.8 [0.5–1.3]	
Heritosexual	787	72	1	1		1	
IDU	129	20	1.7 [1.1–2.8]	1.6 [0.9–2.7]	0.45	1.5 [0.9–2.6]	0.24
Origin							
Sub-Saharan	153	12	1	1		1	
Other	1106	104	1.2 [0.6–2.1]	1.3 [0.7–2.4]		1.5 [0.8–2.7]	
HIV VL>50 copies/ml after PCP (time-dependent covariable)							0.0009
No	NA	NA	1	1		1	
Yes	NA	NA	0.3 [0.2–0.6]	0.4 [0.2–0.7]		0.4 [0.2–0.7]	
CD4>200/mm³ after PCP (time-dependent covariable)							0.0008
No	NA	NA	1	1		1	
Yes	NA	NA	0.3 [0.2–0.5]	0.4 [0.3–0.7]		0.4 [0.3–0.7]	

*Adjusted HR>1: in favor of an increased risk of death; PE non prior ADI: previously enrolled patients without prior AIDS-defining illness, PE prior ADI: previously enrolled patients with prior AIDS-defining illness, MSM: Men who have Sex with Men, IDU: Intravenous Drug Use.
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subsequent follow-up. No excess mortality was observed in this patient subgroup (data not shown). Third, the rate of loss to follow up at 3 years after PCP was 16%. But owing to the French law that prevents nominative or indirectly nominative data collection in FHDH; vital status could not be checked against the national death registry. However no difference in rate of loss of follow-up was observed between the 3 groups (Log Rank test $p = 0.74$).

In previous studies of PCP few patients had been prescribed cART before PCP onset [6–8] but an increase of the proportion of ADI and specifically of PCP have been recently reported in pretreated HIV infected patients followed up in industrialized countries despite free access to care [12,13]. In our study, among previously enrolled patients, 2/3 had had at least a cART prescription for more than one year before PCP. Before PCP, 67% of patients with at least one year of cART prescription have had an immunological response and 80% a virological response; theoretically those patients should not have presented a PCP episode. Since all PE patients had very low CD4 cell count and high VL at PCP diagnosis, it could be hypothesized that for some patients, adherence had diminished over time with a risk of developing ADI. Patients are now facing the challenge of life-long adherence to medications and care and our policy must evolve. Efforts have been focused on adherence at the beginning of cART. We need now to concentrate on patients followed up for many years and design interventions to lower the risk of decreasing adherence over time.

It was striking to note that PE patients (with and without prior ADI) had a worse immune reconstitution than the reference group. CD4-nadir before cART initiation has been shown as the strongest predictor of the immune response [16,17]. The extent of prior CD4 T cell depletion determined the size of the viral reservoir after prolonged suppressive antiretroviral therapy [18]. In our study, PE prior ADI patients have had a lower CD4 nadir at cART initiation (median of CD4: 119/mm³ (IQR, 36–220)) compared to PE non-prior ADI patients, (median of CD4: 204/mm³ (IQR, 106–324)). This could explain the difference of immune reconstitution after PCP between these two groups of patients. The role of antiretroviral drug resistance could also be hypothesized to explain that previously enrolled patients had a lower rate of immune reconstitution than patients with PCP revealing HIV. The time required to reach a CD4 cell count above 300/mm³ has been shown to increase with the number of failed regimens [19].

Despite cART, having an ADI like PCP is still associated with a poor vital prognosis. Overall, in the last fifteen years, the 3-year post-PCP mortality rate have not changed in the FHDH cohort from 12% in 1998–2000 [1] to 11% in the present study. However, a special group of patients with very high mortality (25%, PE prior ADI patients), was identified in our study, probably explaining why the overall mortality rate has not fallen. Since IDU were more numerous in PE prior ADI patients, a sensitivity analysis was undertaken in order to see if part of the poor prognosis could be explained by non-AIDS cause of death such as

overdose. In this sensitivity analysis excluding the IDU group, an increase of risk of death was still observed for PE prior ADI patients (HR = 2.6 [1.6–4.3]).

Patients in the PE prior ADI group who presented with another ADI, here PCP, had a poorer prognosis that persisted after adjusting on baseline characteristics and on immune reconstitution and viral suppression. HIV viral load and CD4 cell counts are strong predictors of the risk of death but having repeated ADI is contributing to the risk of death beyond its impact on immune reconstitution and viral suppression. This is in line with the results of a large collaborative European study that showed a persistent role of prior AIDS on the risk of death, even in patients with CD4 cell counts $\geq 500/\text{mm}^3$ [20]. One hypothesis may be an immune system dysfunction not exclusively translated in the CD4 cell count which could deepen at each ADI. Clinicians must be aware of the pejorative effect of an ADI even at a time where powerful treatments are available. Warning message on importance of maintaining viral suppression and immune reconstitution to prevent any new ADI must be regularly reinforced and especially for patients with a previous ADI.

Conclusion

In a context of diminishing risk of PCP, half of the patients diagnosed with PCP in the recent cART period were already in care; the majority of them had already started cART but with vanishing adherence to care. Efforts need to be stressed on those patients with a history of previous ADI in order to prevent further event which will increase mortality. The consequences of having had an ADI need to be explained to patients as well as the necessity of maintaining an excellent observance and never stop cART.

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Author Contributions

Conceived and designed the experiments: BD MG OL DC. Analyzed the data: BD MG DC. Wrote the paper: BD MG NdC FM MR AM GMG OL DC. Has full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis: BD. Drafted the article: BD. Made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data: BD MG DC. Made substantial contributions to conception and design, and interpretation of data: OL. Made substantial contributions to acquisition and interpretation of data: NdC FM MR AM GMG. Critically revised the article for important intellectual content: BD MG NdC FM MR AM GMG OL DC. Provided final approval of the version to be published: BD MG NdC FM MR AM GMG OL DC.

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Chapter 4

The second part of our work was an analysis of invasive aspergillosis (IA) among HIV-infected individuals over a 20-year period in the FHDH cohort. Work published before the advent of cART had shown that invasive aspergillosis was a rare but dreadful event in HIV-infected individuals, with a median survival time of only 3 months after diagnosis of invasive aspergillosis. Invasive aspergillosis occurred in patients with low CD4 cell counts, half of whom did not have classical predisposing host factors (neutropenia, corticosteroid use). Since these publications, cancer diagnoses have increased among HIV-infected individuals, leading to more chemotherapy-induced neutropenia and corticosteroid use, and new host-related risk factors for invasive aspergillosis.

In that context, the evolution of the incidence of invasive aspergillosis in HIV infected individuals was unknown, and we wanted to analyze who would have an invasive aspergillosis diagnosis nowadays. We were also interested to know how these diagnoses were made, as EORTC criteria were first proposed in 2002 (and modified in 2008) for invasive aspergillosis in hemato-oncological settings. The FHDH medical records were reviewed to collect all the information needed to validate cases of invasive aspergillosis. We used strict criteria looking if invasive aspergillosis cases corresponded to the EORTC definition (noted as “EORTC IA”), otherwise invasive aspergillosis validated on clinical, microbial and radiological factors was defined as “HIV related IA”. We thus validated a total of 242 cases recorded in the FHDH cohort over a 20-year period.

The incidence of invasive aspergillosis fell over time, from 19 per 10 000 PY (95% CI, 16.0-23.0) in the period 1992-1995 to 2.2 per 10 000 PY (95% CI, 1.8-2.6) in 2002-2011. The ratio of EORTC IA cases to all validated IA cases remained stable (50.4% in 1992-1995, 48.1% in 1996-2001, and 54.4% in 2002-2011 ($p=0.7$)). Surprisingly, half the

patients with validated IA did not have the classical predisposing host factors selected as criteria for the EORTC IA definition. The median CD4 cell count at IA diagnosis increased slightly over time but remained low (median 9/mm³ (IQR, 4-26) in 1992-1995, 18/mm³ (IQR, 9-80) in 1996-2001 and 82/mm³ (IQR, 12-327) in 2002-2011 (p< 0.0001)), while the median neutrophil count was above 1500/mm³ in the last 2 periods (1263/mm³ (760–2535) in 1992-1995, 1500/mm³ (700–3004) in 1996-2001, 1810/mm³ (500–3920) in 2002-2011 (p=0.5)). In order to facilitate the diagnosis of invasive aspergillosis in HIV-infected individuals, we propose to include the parameter “CD4 cell count below 100/mm³” as a predisposing host factor, although this should first be validated in a prospective study.

With a median survival time of only 3 months after diagnosis of invasive aspergillosis in the pre-cART era, we assumed that survival would have improved after cART became available in 1996. The prognosis of HIV-infected individuals improved dramatically after 1996, while that of hematological patients with invasive aspergillosis improved with the advent of liposomal amphotericin in 1997 and voriconazole in 2002. We defined three periods to study time trends in the outcome of invasive aspergillosis: pre-cART and pre-voriconazole (1992-1995), post-cART and pre-voriconazole (1996-2001), and post-cART and post-voriconazole (2002-2011). We confirmed that median survival after invasive aspergillosis improved with the advent of cART, from 1.9 months (IQR, 0.9-3.1 months) during the first period, to 10.3 months (IQR, 2->100 months) and 29 months (IQR, 2.2-98.2 months) during the following two periods. After 2002, patients who received voriconazole had better 3-month post-IA survival rates (HR= 0.1 (95% CI, 0.01-0.80)). These results support first-line voriconazole therapy for invasive aspergillosis in HIV-infected individuals.

IA remains a difficult diagnosis in the context of HIV infection, and it might help to add “a low CD4 cell count” as a risk factor. A threshold of $100/\text{mm}^3$ seems appropriate, but further studies are needed to validate this proposition.

Relevance of EORTC Criteria for the Diagnosis of Invasive Aspergillosis in HIV-Infected Patients, and Survival Trends Over a 20-Year Period in France

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Background. Before the advent of combination antiretroviral therapy (cART), roughly 50% of cases of invasive aspergillosis (IA) associated with human immunodeficiency virus (HIV) infection involved individuals without classical predisposing host factors, and the median survival time was <4 months after diagnosis. We examined if the situation evolved over time using the revised European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC) definition and analyzed survival trends after diagnosis over 20 years.

Methods. A data review committee evaluated 342 medical records that mentioned IA in the French Hospital Database on HIV. Validated cases were classified as fulfilling the EORTC criteria or otherwise as "HIV-related IA." Three periods were analyzed: pre-cART (before 1996), cART era prevoriconazole (1996–2001), and 2002–2011.

Results. Among 242 validated cases of IA, 124 (51%) fulfilled the EORTC criteria (EORTC-IA) and 118 (49%) were classified as "HIV-related," with similarly low CD4 cell counts in both groups. The proportion of EORTC-IA cases remained stable across the 3 periods (50%, 48%, and 54%, respectively). The 3-month survival rate improved after the advent of cART (38% vs 69%), with no difference between EORTC-IA and HIV-related IA (hazard ratio [HR], 1.2 95% confidence interval [CI] [0.7–1.8]). Voriconazole exposure decreased mortality in 2002–2011 (HR, 0.1 95% CI [0.01–0.8]).

Conclusions. In the cART era, EORTC criteria, developed for use in hematology/oncology, still applied to only half the cases diagnosed among HIV-infected patients. A rapid diagnosis of IA is paramount to improve survival. For patients who do not fulfill the EORTC definition, we suggest that the addition of "HIV infected with a CD4 count <100 cells/μL" to the EORTC host criteria be validated.

Keywords. AIDS; aspergillosis; incidence; EORTC; outcome.

Before the advent of combination antiretroviral therapy (cART), invasive aspergillosis (IA) in human immunodeficiency virus (HIV)-infected individuals tended to

occur at CD4 counts <100 cells/μL, often in patients with prior or concomitant opportunistic infections [1–4]. Up to half of these individuals did not have neutropenia or prolonged steroid therapy, 2 known risk factors for IA [1, 5]. The lungs were involved in >70% of cases, and serum galactomannan (GM) assay was considered unreliable. Median survival after diagnosis was <4 months [5, 6]. IA was removed from the Centers for Disease Control and Prevention's list of AIDS-defining opportunistic infections in 1984.

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In 2008, revised European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG; hereafter "EORTC") criteria were proposed for IA in hematologic-oncologic settings, based on host factors and clinical and mycologic features [7]. The main host factors were prolonged neutropenia, immunosuppressive treatments, allogeneic stem cell transplantation, and inherited severe immunodeficiency; HIV infection was not included [7]. In the pre-cART era, previous studies have reported that EORTC host criteria were not present in the majority of HIV-infected patients with IA [1, 5]. Here, in a large sample of HIV-infected patients with IA, we studied the proportion of patients fulfilling EORTC criteria for IA (EORTC-IA) over time, and examined trends in IA incidence and survival over the past 20 years.

PATIENTS AND METHODS

Patients

Individuals were selected from the French Hospital Database on HIV (FHDH), a nationwide hospital-based cohort created in 1989 [8]. Seventy participating hospitals across France provide data on HIV-infected individuals, representing 50%–60% of all HIV-seropositive individuals receiving care in France. The only inclusion criteria are HIV type 1 or 2 (HIV-1 or HIV-2) infection and written informed consent. Trained research assistants prospectively collect clinical, biological, and therapeutic data and causes of death from the medical records, using specialized software. For this analysis, we considered all HIV-1-infected individuals enrolled in the database from 1992 to 2011.

Validation of IA Cases

Medical records were screened for diagnoses of aspergillosis, using codes from the *International Classification of Diseases, Ninth Revision (ICD-9; 117.3)* or *Tenth Revision (ICD-10; B440–B442, B447–B449)*. Clinical, radiologic, biological, and therapeutic data relating to aspergillosis were retrieved by trained research assistants in all participating hospitals across France from May 2012 until May 2013. The cases were then validated by a data review committee that included 2 experts in infectious diseases (B. D. and O. L.). Individuals without radiologic signs of IA were excluded. Each case was checked for the EORTC criteria [7]. In addition to the EORTC host factor "<500 neutrophils/ μ L for >10 days," we considered "<800 neutrophils/ μ L with growth factor treatment for >10 days." Validated IA cases meeting the EORTC criteria were classified as proven, probable, or possible. For proven cases, patients had biopsies or percutaneous aspiration or postmortem autopsies.

When a case did not meet the EORTC host factor criteria, the review committee examined radiologic features, results of microscopic examination, and culture of bronchoalveolar lavage

(BAL) fluid, results of GM antigen testing, and associations with other pathogens, as in previous studies [1]. Patients who were infected by both an *Aspergillus* species and a major respiratory copathogen detected in BAL fluid that might have explained the illness—namely, *Mycobacterium tuberculosis*, *Mycobacterium avium*, *Streptococcus pneumoniae*, *Histoplasma capsulatum*, or *Pneumocystis jirovecii*—were not considered as IA. We also excluded cases considered to have transient *Aspergillus* colonization (patients without radiologic abnormalities and without bronchial endoscopy or without signs of necrotic tracheobronchitis at endoscopy), a typical aspergilloma, allergic bronchopulmonary aspergillosis, or a possibly false-positive GM test (7 patients: 1 case of disseminated histoplasmosis, 3 cases of surgically treated aspergilloma with no histologic signs of surrounding invasion, 1 case of GM positivity at 0.5 with pan-sinusitis and an aspergilloma of the maxillary sinus, and 2 intensive care unit patients with septic shock on piperacillin-tazobactam and a negative fungal culture of sputum). Cases not meeting EORTC criteria were defined as "HIV-related IA" and categorized into 2 groups: (1) group 1: no EORTC-defined host factor, a new radiologic lesion compatible with the clinical manifestations, *Aspergillus* recovered from BAL fluid or a bronchial aspirate, no other identified pathogen, and/or GM antigen >0.5 in serum or BAL fluid, and no alternative diagnosis; (2) group 2: no host factor, new radiologic lesion compatible with the clinical features, *Aspergillus* species detected in BAL fluid or bronchial aspirate, and a concomitant pathogen other than *M. tuberculosis*, *M. avium*, *S. pneumoniae*, *H. capsulatum*, or *P. jirovecii*.

Statistical Analysis

To evaluate the relevance of the EORTC criteria for diagnosis of IA in HIV-infected individuals, we compared clinical features of IA and baseline characteristics between EORTC-IA and HIV-related IA by using the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. Three periods were compared: pre-cART (<1996), cART era prevoriconazole (1996–2001), and the cART and voriconazole era (2002–2011).

Estimation of IA incidence rates took into account the difficulty of retrieving medical records in the oldest period, in particular for diagnosis before 2001, especially for deceased patients. The number of IA cases was first corrected for nonretrieval of medical records. After stratification for the period and the CD4 count (<100 or \geq 100 cells/ μ L) at IA diagnosis, the rate of IA validation on chart review was applied to unretrieved records. Incidence rates were then calculated for each period by dividing the estimated number of IA cases by the corresponding number of person-years (PY) of follow-up. Survival comparisons over time were based on Kaplan-Meier estimates, with data censoring occurring 3 months after IA diagnosis. The impact of the study period on survival trends was studied by using multivariable Cox proportional hazards models adjusted for the IA classification (EORTC-IA or HIV-related IA), age

(<40, 40–59, or ≥60 years), the HIV transmission group (intravenous drug users vs others), and the CD4 cell count at diagnosis (log₂ CD4). A separate analysis was undertaken for the period after 2001, in which voriconazole treatment was entered as a time-dependent variable in the Cox model. Sensitivity analysis was performed after exclusion of cases classified as possible EORTC-IA, and after exclusion of cases classified as either possible EORTC-IA or HIV-related IA group 2. All tests were 2-sided, and *P* values <.05 were considered to denote statistical significance. All statistical analyses used the SAS software package (version 9.3; SAS Institute, Cary, North Carolina).

RESULTS

Relevance of the EORTC Classification and Clinical Features of Validated IA

During the study period, 515 HIV-infected individuals were reported with an aspergillus ICD-9 or ICD-10 code, and 342 complete medical records were retrieved for analysis, of which 242 (70%) were validated by the data review committee (Figure 1).

The validation rate did not differ significantly across the 3 periods (80%, 70%, and 65%; *P* = .06), but was significantly higher when the CD4 count was <100 cells/μL (82% vs 53%; *P* < .0001). Among the 242 validated cases, 124 (51%) met the EORTC-IA criteria, and 118 (49%) were classified as HIV-related IA (Figure 1). The numbers of proven, probable, and possible cases of EORTC-IA and of group 1 and group 2 HIV-related IA are shown in Figure 1. Overall, 93 individuals (39%) had at least 1 EORTC host factor. Among the 52 individuals with proven EORTC-IA, 33 (64%) did not have a host factor. Among the 56 individuals with hematologic disorders, an EORTC host factor was present in 48 (86%) cases (21 non-Hodgkin lymphoma [NHL], 7 Hodgkin disease, 20 others), whereas no EORTC host factors were present in 8 cases (2 NHL and 1 Hodgkin disease without chemotherapy at IA diagnosis, 5 cases of myelodysplasia). An EORTC host factor was present in 25 of the 42 (60%) individuals with other malignancies. A predisposing treatment (chemotherapy or corticosteroids) was recorded in 69 cases (29%), and 28 of the 36 individuals receiving corticosteroids had an EORTC host factor.

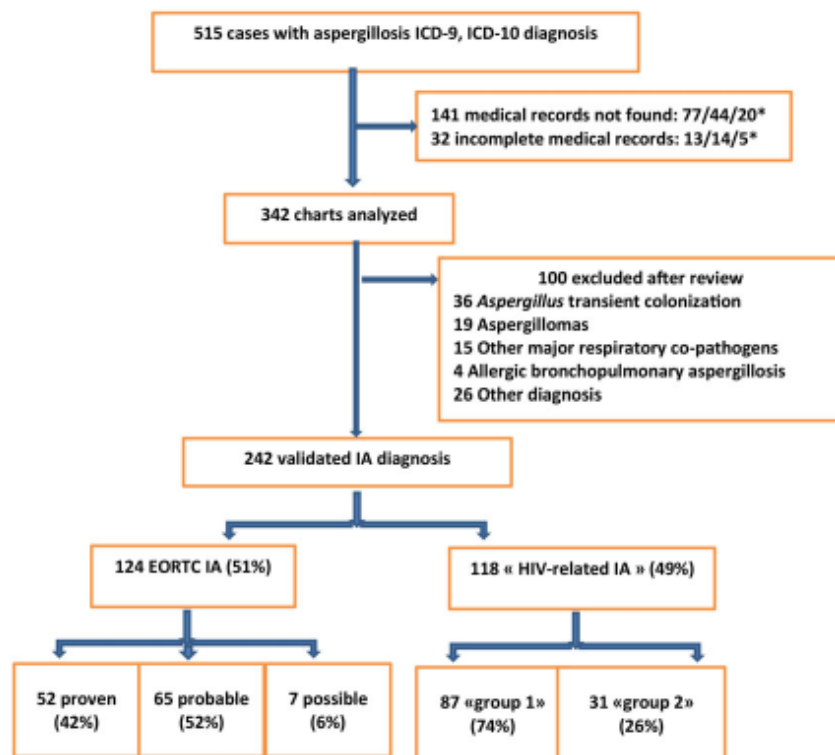


Figure 1. Validation of invasive aspergillosis (IA) recorded in the French Hospital Database on human immunodeficiency virus (HIV) Agence Nationale de Recherche sur le SIDA et les hépatites virales, France CD4 cohort among HIV-infected individuals between 1992 and 2011. *in 1992–1995/1996–2001/2002–2011 respectively. Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group; ICD, International Classification of Diseases.

Table 1. Characteristics of Human Immunodeficiency Virus (HIV)-Infected Individuals at Presentation of Invasive Aspergillosis (IA): 242 Validated IA Cases Recorded in the French Hospital Database on HIV Agence Nationale de Recherche sur le SIDA et les hépatites virales, France CO4 Cohort, 1992–2011

Characteristic	All (N = 242)	EORTC (n = 124)	HIV-Related (n = 118)	P Value
Age	41 (35–47)	41 (35–47)	42 (35–49)	.24
Male sex	198 (81.6)	101 (81.5%)	97 (82.2)	.88
Sub-Saharan African origin	16 (6.5)	9 (7.3)	7 (5.9)	.68
HIV risk group				.59
MSM	95 (39.3)	51 (41.1)	44 (37.3)	
IDU	52 (21.5)	29 (23.4)	23 (19.5)	
Others	95 (39.2)	44 (35.5)	51 (43)	
CD4 count, cells/ μ L	20 (7–124)	16 (7–110)	26 (8–168)	.1
CD4 category				.28
<100	174 (71.9)	92 (74.2)	82 (69.5)	
100–199	24 (9.9)	14 (11.3)	10 (8.5)	
\geq 200	44 (18.2)	18 (14.5)	26 (22.0)	
Neutrophil count, cells/ μ L	1580 (671–3021)	705 (306–1810)	2205 (1500–3240)	<.0001
AIDS before IA	147 (61.3)	87 (70.7)	60 (51.3)	.002
cART before IA	131 (54.1)	71 (57.3)	60 (50.9)	.31
IA clinical presentation: pulmonary				
Cough	166 (69)	74 (60)	92 (78)	
Chest pain	47 (19)	20 (16)	27 (23)	
Dyspnea	131 (54)	63 (51)	68 (58)	
Hemoptysis	25 (10)	9 (7)	16 (14)	
Sinus				
Sinusitis	31 (13)	25 (20)	6 (5)	
Intranasal lesion	13 (5)	13 (10)	0	
CNS				
Seizures	6 (2)	5 (4)	1 (1)	
Meningitis	0	0	0	
Hemiparesis	5 (2)	2 (2)	3 (3)	
Cranial nerve paralysis	4 (2)	4 (3)	0	
Location	N = 289 sites ^a	n = 159 sites ^a	n = 130 sites ^a	
Pulmonary	214 (74)	101 (64)	113 (87)	
Sinus	31 (11)	25 (16)	6 (4.5)	
CNS	22 (7.5)	16 (10)	6 (4.5)	
Other ^b	29 (10)	24 (15)	5 (4)	
Disseminated	28 (10)	15 (9)	13 (10)	

Data are presented as median (IQR) or No. (%).

Abbreviations: cART, combined antiretroviral therapy; CNS, central nervous system; EORTC, European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group; HIV, human immunodeficiency virus; IA, invasive aspergillosis; IDU, intravenous drug users; IQR, interquartile range; MSM, men who have sex with men.

^a One or >1 site possible.

^b Among EORTC-proven cases: orbit (n = 4), mastoid (n = 2), adrenal glands/kidney (n = 4), spleen (n = 1), myocardium (n = 1), breast (n = 1), vertebra (n = 1), bronchial tree (n = 8), pleural space (n = 1). Among HIV-related cases: invasive otitis (n = 2), orbit (n = 1), tracheobronchitis (n = 1), kidney (n = 1).

As shown in Table 1, IA tended to be diagnosed among patients with advanced immunosuppression: the median CD4 count was 20 cells/ μ L (interquartile range [IQR], 7–124 cells/ μ L), and the median neutrophil count was 1580 cells/ μ L (IQR, 671–3021 cells/ μ L). Individuals with EORTC-IA differed from individuals with HIV-related IA in terms of their neutrophil count at IA diagnosis, while CD4 cell counts were low in both groups. For patients with HIV-

related IA, the median neutrophil count was 2205 cells/ μ L; only 2 patients had a neutrophil count <500 cells/ μ L, but for <10 days, whereas the median CD4 was 26 cells/ μ L and 70% had a CD4 count <100 cells/ μ L. The majority of patients (74%) had pulmonary involvement, and 28 patients (10%) had disseminated disease (at least 2 noncontiguous sites), with no major differences between EORTC-IA and HIV-related IA (Table 1).

Table 2. Diagnostic Tests Performed to Diagnose Invasive Aspergillosis Among 242 Validated Cases Recorded in the French Hospital Database on Human Immunodeficiency Virus Agence Nationale de Recherche sur le SIDA et les hépatites virales, France CO4 Cohort, 1992–2011

Test	All (N = 242)	EORTC-IA (n = 124)	HIV-Related IA (n = 118)
<i>Aspergillus</i> species isolation ^a	210/241 (87%)	99/123 (80%)	111/118 (94%)
Direct microscopy	83/167 (50%)	43/79 (54%)	40/88 (45%)
Culture ^b	206/226 (91%)	98/112 (88%)	108/114 (95%)
<i>A. fumigatus</i>	92%	89%	94%
Histology	42/56 (75%)	42/49 (86%)	0/7
Galactomannan assay			
Serum	50/127 (39%)	27/70 (39%)	23/57 (40%)
BAL fluid	18/36 (50%)	10/20 (50%)	8/16 (50%)

Data are presented as positive tests/No. of tests performed (%).

Abbreviations: BAL, bronchoalveolar lavage; EORTC, European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group; HIV, human immunodeficiency virus; IA, invasive aspergillosis.

^a As based on medical records.

^b As based on the microbial records.

When performed, direct microscopy was positive in 50% of cases. *Aspergillus fumigatus* was the main species found in culture. Serum GM was assayed in half of the patients with suspected IA: 39% of tested patients were GM positive in serum and 50% in BAL fluid (Table 2). Serum GM was positive in 31 of 90 (34%) tested patients who were still alive at 3 months, and in 19 of 36 (53%) patients who died within 3 months ($P = .0012$). Biopsy was performed in 56 cases, and histopathologic results were compatible with IA in 42 cases (75%). EORTC-IA and

HIV-related IA did not differ according to the positivity rates of direct microscopy, culture, or GM assay.

Time Trends

After correction for unretrieved records, the estimated numbers of IA cases diagnosed among HIV-infected individuals followed in the FHDH Agence Nationale de Recherche sur le SIDA et les hépatites virales, France CO4 cohort were 145 before 1996, 124 in 1996–2001, and 107 after 2001 (Table 3). The incidence of IA fell over time, with rates of 19.0, 5.6, and 2.2 cases per 10 000 PY, respectively. The same trends were observed for 2 other pathogens (cryptococcal meningitis and *P. jirovecii* pneumonia) with a 10-fold decrease of incidence between the pre-cART and the recent periods. The ratio between EORTC-IA and HIV-related IA was stable across the 3 periods ($P = .7$; Table 4). No differences in the proportion of proven, probable, or possible EORTC-IA, or in the proportion of HIV-related IA group 1 and group 2, were observed (Supplementary Table 1). As shown in Table 4, the CD4 cdl count at IA diagnosis increased significantly over time ($P < .0001$). Overall, 61% of patients ($n = 147$) had AIDS at IA diagnosis, with no significant difference across the 3 periods ($P = .27$).

Among patients tested for GM in plasma or serum, 9% were positive in 1992–1995, 30% in 1996–2001, and 54% after 2001 ($P < .0001$). Among patients tested for GM in BAL, 33% were positive in 1992–1995, 33% in 1996–2001, and 67% after 2001 ($P = .0075$).

Trends in Overall Mortality 3 Months After IA Diagnosis

Survival after IA diagnosis improved during the study period, from a median of 1.9 months (IQR, 0.9–3.1 months) in 1992–1995, to 10.3 months (IQR 2 to >100 months) in 1996–2001 and 29 months (IQR, 2.2–98.2 months) in 2002–2011 ($P < .0001$). The difference between patients diagnosed in 1992–1995 and those diagnosed in the last 2 periods was

Table 3. Incidence of Invasive Aspergillosis Among Human Immunodeficiency Virus (HIV)-Infected Individuals Followed in the French Hospital Database on HIV Agence Nationale de Recherche sur le SIDA et les hépatites virales, France CO4 Cohort, 1992–2011

Characteristic	1992–1995, No.	1996–2001, No.	2002–2011, No.
Recorded IA diagnoses	179	173	163
Medical records not retrieved	90	58	25
Reviewed medical records	89	115	138
Validated IA	71	81	90
IA corrected ^a	145	124	107
PY of follow-up	76 425	220 759	489 583
Incidence for 10 000 PY (95% CI)			
IA	19.0 (16.0–22.3)	5.6 (4.7–6.7)	2.2 (1.8–2.6)
Cryptococcal meningitis	47.2 (42.5–52.3)	12.3 (10.8–13.8)	4.5 (3.9–5.2)
<i>Pneumocystis jirovecii</i> pneumonia	211.8 (201.7–222.3)	52.7 (49.7–55.8)	25.3 (23.9–26.8)

Abbreviations: CI, confidence interval; IA, invasive aspergillosis; PY, person-years.

^a Accounted for unretrieved medical records. After stratification by CD4 count (<100 vs ≥ 100 cells/ μ L) and period at IA diagnosis, the ratio of IA validation among reviewed charts was applied to records not retrieved.

Table 4 Characteristics of Validated Invasive Aspergillosis in the Pre-Combination Antiretroviral Therapy (cART) Era (<1996), cART Era Prevoriconazole (1996–2001), and the cART and Voriconazole Era (2002–2011)

Characteristic	1992–1995	1996–2001	2002–2011	P Value
Validated IA	71	81	90	
EORTC-IA	36 (50.7)	39 (48.1)	49 (54.4)	.7
HIV-related IA	35 (49.3)	42 (51.9)	41 (45.6)	
Age	38 (32–46)	39 (35–46)	46 (39–52)	<.0001
Male sex	61 (84.7)	68 (82.9)	71 (78)	.51
Sub-Saharan African origin	4 (5.6)	4 (4.9)	8 (8.8)	.54
HIV transmission group				.0007
MSM	42 (58.3)	31 (37.8)	23 (25.3)	
IDU	11 (15.3)	16 (19.5)	26 (28.6)	
Others	19 (26.4)	35 (42.7)	42 (46.2)	
AIDS before IA	39 (57)	47 (57)	61 (67)	.27
cART before IA	1 (1.4)	47 (57.3)	83 (91)	<.0001
CD4 count at IA diagnosis, cells/ μ L	9 (4–26)	18 (9–80)	82 (12–327)	<.0001
CD4 category at IA diagnosis, cells/ μ L				<.0001
<100	64 (90.2)	64 (79.0)	46 (51.1)	
100–199	3 (4.2)	7 (8.6)	14 (15.6)	
\geq 200	4 (5.6)	10 (12.4)	30 (33.3)	
Neutrophil count at IA diagnosis, cells/ μ L	1263 (760–2535)	1500 (700–3004)	1810 (500–3920)	.52

Data are presented as median (IQR) or No. (%).

Abbreviations: cART, combined antiretroviral therapy; EORTC, European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group; HIV, human immunodeficiency virus; IA, invasive aspergillosis; IDU, intravenous drug user; IQR, interquartile range; MSM, men who have sex with men.

particularly noteworthy, with 3-month survival rates of 38%, 68%, and 69%, respectively (Figure 2). After adjustment for IA classification, age, HIV transmission group, and CD4 cell

count at diagnosis, patients diagnosed in the cART era were more likely to be alive at 3 months than those diagnosed in the pre-cART period (hazard ratio [HR], 0.4 95% CI [0.3–

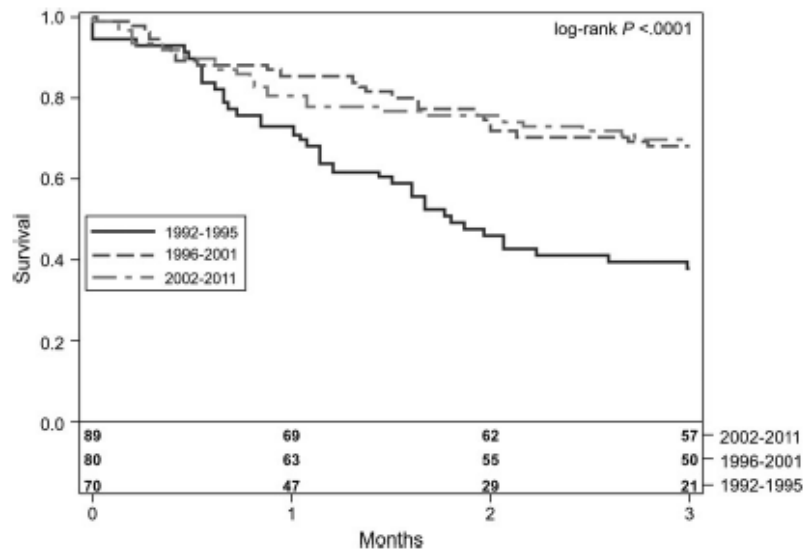


Figure 2. Three months' survival after invasive aspergillosis diagnosis in 3 periods: pre-combination antiretroviral therapy (cART) (<1996); cART era prevoriconazole (1996–2001); and the cART and voriconazole era (2002–2011).

0.7]; Supplementary Table 2). No difference in 3-month mortality was found between EORTC-IA and HIV-related IA (HR, 1.2 95% CI [0.7–1.8]; Supplementary Table 2). Among the 90 patients diagnosed in the most recent period (2002–2011), survival was markedly better among the 59 patients who received voriconazole (HR for death, 0.1 95% CI [0.01–0.8]; Supplementary Table 3). Results were confirmed in sensitivity analyses performed after excluding possible EORTC-IA or both possible EORTC-IA and HIV-related IA group 2 (data not shown).

DISCUSSION

In this large national observational study of invasive aspergillosis in HIV-infected individuals over a 20-year period, we examined 242 validated cases to assess the relevance of the EORTC criteria, which were developed for use in hematology/oncology. We found that only half the cases diagnosed among HIV-infected individuals met these criteria. The ratio between EORTC-IA and HIV-related IA was similar in the pre-cART period (1992–1995), the cART prevoriconazole period (1996–2001), and the cART and voriconazole era (2002–2011). The 3-month survival rate after IA diagnosis improved after the advent of cART. A protective role of voriconazole was observed in the period after 2001.

One limitation of this study was the difficulty of retrieving medical records before 2000, especially for deceased individuals. However, we used a conservative approach when calculating incidence rates, by applying the rates of IA validation to unretrieved medical records by strata of study period and CD4 cell count. In addition, we excluded individuals with major respiratory copathogens that could have explained their clinical manifestations, even though IA can occur at the same time as other opportunistic infections, especially *P. jirovecii* pneumonia [1]. Finally, we excluded cases in which radiologic information was lacking. Thus, our estimation of incidence was conservative.

Our findings illustrate the difficulties of diagnosing IA in HIV-infected individuals, even though early diagnosis and antifungal treatment are important for survival. Despite strict case validation, half the cases of IA in HIV-infected patients did not meet the EORTC criteria. Studies published before the cART era also showed that about half of HIV-infected patients with IA lacked predisposing host factors [5]. Here, patients with EORTC-IA differed from patients with HIV-related IA in terms of the neutrophil cell count at IA diagnosis, while other baseline characteristics were similar, including low CD4 cell count. EORTC criteria place emphasis on the neutrophil count, a factor that may not be relevant in all cases of IA in HIV-infected individuals.

The main features of IA were also similar in the 2 categories of IA, apart from the fact that extrapulmonary involvement was, by definition, more frequent in EORTC-IA (ie, biopsy of extrapulmonary lesions led to diagnosis of EORTC-proven IA even in the absence of host factor).

The sensitivity of GM tests seems to have improved over time, likely because only agglutination tests were available during the earliest period of this study, whereas enzyme-linked immunosorbent assays were used in the latter 2 periods. The increase in the rate of positive GM tests when the test was performed may also be explained by the fact that in the most recent period, the GM test was performed when IA suspicion was very high. Interestingly, positivity of GM tests in serum or plasma was predictive of 3-month survival.

As for cryptococcosis and *P. jirovecii* pneumonia, the incidence of IA fell across the 3 study periods, probably owing to a better CD4 cell count level over time in the HIV population. A major improvement in 3-month survival was observed after the advent of cART, with no significant difference between EORTC-IA and HIV-related IA in univariable or multivariable analyses. This further suggests that the validated cases were representative of IA in HIV-infected individuals.

The first antifungal treatments used were amphotericin B deoxycholate, which was highly nephrotoxic, and itraconazole. In November 1995 and November 1997, respectively, the FDA approved Abelcet, a lipid complex amphotericin B formulation, and ambisome, a liposomal amphotericin B formulation, both of which were better tolerated. In addition to cART, availability of these treatments (Supplementary Table 4) could explain the survival improvement observed in 1996–2001. However since 2002, voriconazole has been considered the first-choice treatment for IA in the hematologic/oncologic population, improving 3-month survival from 58% to 71% [9, 10]. Voriconazole interacts with antiretroviral drugs, and especially with protease inhibitors, complicating the choice of antifungal treatment. To study the effect of voriconazole among patients who survived long enough to receive this treatment, we entered voriconazole as a time-dependent covariable in the period 2002–2011. Our study is the first to confirm the positive impact of voriconazole on survival in HIV-infected individuals with IA. However, our inability to determine the precise delay between the first symptoms and voriconazole prescription implies that the true effect is probably not as high as a 90% reduction in the risk of death.

Previous studies have shown that even with normal levels, neutrophils and peripheral blood macrophages from HIV-infected individuals exhibit impaired activity against *A. fumigatus* in vitro, emphasizing the smaller relevance of EORTC criteria in these patients [11, 12]. In 2002–2011, in HIV-infected individuals, IA was still frequently diagnosed at a very low CD4 cell count, regardless of the neutrophil count. To be conservative, and to help clinicians make a diagnosis when IA does not fulfill EORTC criteria, we propose to add a host criteria of profound immunodepression, “HIV-infected individual with a CD4 count <100 cells/ μ L,” to the current EORTC definition. However, validation of a threshold will need a specific prospective study of patients with suspected IA.

In conclusion, half of the confirmed cases of IA in HIV-infected individuals in our study did not meet the EORTC criteria. Clinicians must be aware of the possibility of IA when an HIV-infected patient presents with a new pulmonary lesion that is not explained by bacterial or viral infection, especially when the CD4 count is <100 cells/ μ L. IA remains an important menace, with a 3-month mortality rate of 30% in the most recent period of this study. Our findings confirm the positive impact of voriconazole on decreasing mortality and suggest that this drug should be the first-line treatment for IA in HIV-infected individuals.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. O. L. has received fees for board membership from Pfizer, Astellas, and Merck, and consultancy fees from Novartis and Gilead Sciences. M. R. has received research grants from Novartis and lecture fees from Pfizer. D. C. has received institutional grant funding from Bristol-Myers Squibb (BMS), Janssen-Cilag, Merck Sharp & Dohme, Chibret, and ViiV; has received fees for lectures from BMS, Janssen-Cilag, and ViiV; and has received fees for board membership of Gilead France. B. D. has received lecture fees from Gilead and travel expenses from Janssen. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Supplementary table 1: Classification of validated cases of invasive aspergillosis (IA) in the pre-cART era (<1996), the post-cART, pre-voriconazole era (1996-2001), and the post-cART, post-voriconazole era (2002-2011)

	1992-1995 N=71	1996-2001 N=81	2002-2011 N=90
EORTC IA	36 (51%)	39 (48%)	49 (54%)
Proven	18 (50%)	18 (46%)	16 (33%)
Probable	17 (47%)	19 (49%)	29 (59%)
Possible	1 (3%)	2 (5%)	4 (8%)
HIV-related IA *	35 (49%)	42 (52%)	41 (46%)
Group 1	26 (74%)	28 (67%)	33 (80%)
Group 2	9 (26%)	14 (33%)	8 (20%)

*“HIV-related IA”

Group 1: no EORTC-defined host factor, a new radiological lesion compatible with the clinical manifestations, *Aspergillus* recovered from BAL fluid or a bronchial aspirate, no other identified pathogen, and/or galactomannan antigen >0.5 in serum or BAL fluid, and no alternative diagnosis;

Group 2: no host factor, a new radiological lesion compatible with the clinical features, *Aspergillus sp.* detected in BAL fluid or bronchial aspirate, and a concomitant pathogen other than *M. tuberculosis*, *M. avium*, *S. pneumoniae*, *Histoplasma capsulatum* or *Pneumocystis jirovecii*.

Supplementary table 2: Risk factors for death within 3 months of diagnosis of invasive aspergillosis (IA)

	Crude HR (95%CI)	p value	Adjusted HR (95%CI)	p value
Period of diagnosis		<.0001		.0006
Pre-cART (1992-1995)	1.0		1.0	
cART (1996-2011)	0.41 (0.27-0.64)		0.44 (0.27-0.70)	
IA classification "HIV-related"		0.26		0.48
	1.0		1.0	
EORTC IA	1.27 (0.84 -1.93)		1.17 (0.74-1.82)	
Age, years		0.93		0.74
<40	1.0		1.0	
40-60	0.92 (0.60-1.41)		1.14 (0.73-1.80)	
60+	1.03 (0.41-2.60)		1.44 (0.49-4.22)	
HIV risk group, IDU	1.03 (0.62 – 1.71)	0.90	1.27 (0.74-2.19)	0.39
CD4 at IA diagnosis (per Log2)	0.92 (0.85 – 1.01)	0.07	0.96 (0.87 -1.05)	0.34

Supplementary table 3: Risk factors for death within 3 months of diagnosis of invasive aspergillosis (IA) in the period 2002-2011

	Crude (95%CI)	HR	p value	Adjusted (95%CI)	HR	p value
Voriconazole exposure*	0.10 (0.01-0.73)		0.024	0.10 (0.01-0.80)		0.029
IA classification						
"HIV-related"	1.0		0.30	1.0		0.34
EORTC	0.66 (0.31-1.44)			0.67 (0.30-1.52)		
Age, years						
<40	1.0		0.48	1.0		0.54
40-60	0.64 (0.28-1.45)			0.72 (0.30-1.70)		
60+	1.13 (0.24-5.26)			1.50 (0.30-7.57)		
HIV risk group, IDU	1.18 (0.51-2.72)		0.69	1.63 (0.68-3.92)		0.27
CD4 at IA diagnosis (per Log2)	1.00 (0.87-1.15)		0.99	0.97 (0.83 -1.13)		0.69

*Voriconazole exposure as a time-dependent covariable

Supplementary table 4: Treatments for “EORTC IA” and “HIV-related IA” between 1992 and 2011:

	EORTC IA* N=124	HIV-related IA* N=118	All *
Ampho B			
Deoxycholate	50	39	89
Liposomal	12	8	20
Lipid	5	2	7
complex			
Itraconazole	57	72	129
Voriconazole	36	26	62
Caspofungin	15	4	19

*patients could receive more than one treatment.

Chapter 5. Discussion and general perspectives

I would first like to explain why I chose this subject area. Indeed, HIV-related fungal infections are no longer a hot topic in resource-rich settings, having been superseded by cancer, long-term complications, HIV-related aging, and HIV reservoirs, etc. Many studies have shown a dramatic decline in PCP since the advent of cART, and we now have access to powerful and much better-tolerated antiretroviral regimens. More than 82% of patients enrolled in the FHDH cohort in 2011 have been on cART for at least 6 months, and 89% of them have a viral load below 50 copies/ml. However, the burden of fungal illness remains high in low-income countries. In addition, few recent studies of opportunistic infections in resource-rich settings have been published. In (“Opportunistic infections and AIDS malignancies early after initiating combination antiretroviral therapy in high-income countries,” 2014), one study focused on the impact of cART on AIDS-defining events associated with IRIS, but PCP was not included, because of the low associated risk of IRIS. In this latter study, the considered AIDS-defining events were rare after cART initiation, with an estimated incidence of 2.3 per 1000 PY in the period 1996-2013 for tuberculosis, the most frequent event, and 0.35 per 1000 PY for cryptococcosis. Studies recently published in resource-rich settings have focused on tuberculosis and CNS opportunistic infections. Yet PCP remains one of the most common AIDS-defining events in resource-rich settings; for example, it was the second most frequent AIDS-defining event in France in 2001-2003 (Grabar et al., 2008). Furthermore, we were struck by the large number of PCP cases still declared by some FHDH participating centers.

This is why I decided to launch an epidemiologic study of fungal infections among HIV-infected individuals, using data from the FHDH cohort. I chose to study two very different diseases: PCP, a very well characterized and frequent AIDS-defining event, and invasive aspergillosis, a very rare but life-threatening fungal infection in HIV-infected individuals.

Because of its very low incidence, invasive aspergillosis was removed from the Centers for Disease Control and Prevention's list of AIDS-defining opportunistic infections in 1984. Fungal infections are reliable markers of severe immunosuppression: PCP is extremely rare when the CD4 cell count is above $200/\text{mm}^3$ (or 15%), while invasive aspergillosis (like cryptococcosis, histoplasmosis and penicilliosis) occurs at a median CD4 cell count below $50/\text{mm}^3$.

I had different objectives for the two fungal infections:

As there were no publications on PCP trends in the late cART period, I analyzed the recent incidence of PCP in HIV-infected individuals, as well as the patients' baseline characteristics and outcomes.

22 years ago my PhD co-director had published a study of 33 HIV-infected individuals with invasive aspergillosis. At that time, before the advent of cART and active antifungals, the short-term prognosis was grim. As no recent publications were available, we thought the FHDH cohort, with its very large size (data on more than 130 000 HIV-infected individuals), would yield interesting data on invasive aspergillosis among HIV-infected individuals. We decided to study incidence trends over time, how the diagnosis was made, the nature of the population at risk, how patients were treated, and how the prognosis of invasive aspergillosis changed over a 20-year period. We also wanted to see if the EORTC diagnostic criteria for IA in the hematological setting were adapted to the context of HIV infection.

In France, as special efforts have been made to increase HIV screening uptake, and as French experts have recommended cART even for patients with CD4 cell counts $>350/\text{mm}^3$ since 2008, most cases of PCP among HIV-infected individuals should theoretically have been related to HIV screening failure. In the FHDH, 1259 HIV-infected individuals had a first diagnosis of PCP during the period 2004-2011. Thus, PCP still exists, and, with a 3-year

mortality rate of 25% among patients who developed PCP after a previous AIDS-defining event, we show that it is still a major issue, especially for patients who are not retained in care, among whom the mortality rate is particularly high.

Djave et al (Djave et al., 2015) obtained similar results in San Francisco for HIV-infected individuals with a first diagnosis of AIDS-defining illness: the five-year mortality rate was 35% during the period 1996-2012. As H. Masur stated in his editorial relative to the previous study (Masur and Read, 2015), “we still have room for improvement”.

So how could we improve the prognosis of patients with PCP?

-First of all by better diagnosis and early treatment:

PCP is easier to diagnose in a context of HIV infection. First, fungal load is higher in induced sputum and BAL fluid than in HIV-seronegative individuals. Second, when an HIV-infected individual presents with progressive dyspnea to a service used to managing HIV-related opportunistic infections, the most likely diagnosis will be PCP, and treatment with trimethoprim-sulfamethoxazole can be prescribed without delay. The diagnosis will be confirmed 48 h later on BAL fluid or induced sputum. However, all physicians, and particularly general practitioners who are not used to dealing with HIV-infected individuals, must be fully informed of opportunistic infections. Indeed, most patients have already seen a doctor before receiving the correct diagnosis, and have been prescribed various ineffective antibiotics. They finally arrive at the hospital with severe PCP revealing their HIV infection.

New diagnostic tools such as qPCR and B D glucan are available, but immunofluorescence on BAL fluid or induced sputum remains the gold standard. It is still difficult to distinguish colonization from infection by means of qPCR, and false-positive results are an issue with B D glucan. However, those tests have good negative predictive values. High serum B D glucan is useful for diagnosis especially when induced sputum or BAL fluid cannot be obtained.

Better standardization of qPCR could improve PCP diagnosis in future. The detection of serum β D glucan and *P. jirovecii* DNA by qPCR could usefully be combined as serum markers for the diagnosis of PCP in patients with severe diseases or other conditions where BAL fluid cannot be obtained.

Regarding treatment, trimethoprim-sulfamethoxazole is effective and usually well tolerated, but treatment can still be hindered by toxicity, allergic reactions and G6PD deficiency. Alternatives include intravenous pentamidine (effective for severe PCP, but rather toxic) and atovaquone (not validated for severe PCP). Resistance to trimethoprim-sulfamethoxazole (due to dihydropteroate synthetase mutation) is very rare, and this treatment is cheap and effective, meaning that further therapeutic research on PCP is unlikely. CART should be started early after PCP diagnosis.

-Another approach is to prevent PCP occurring in the first place:

- By improving HIV screening and giving PCP prophylaxis to immunosuppressed patients.

HIV screening is still inadequate in France, as reflected by the fact that half of all patients with PCP are diagnosed very late, with PCP revealing their HIV infection. A study based on the French national surveillance program showed that 30% of individuals with newly diagnosed HIV infection already had AIDS or a CD4 cell count below $200/\text{mm}^3$ (Cazein et al., 2011). CART should be introduced rapidly after HIV diagnosis in order to limit the risk of opportunistic infections, and PCP prophylaxis should be prescribed until the CD4 cell count recovers to at least $200/\text{mm}^3$.

- By maintaining patients in care:

We found that almost 50% of PCP diagnoses among HIV-infected individuals involved patients already in care who had received cART prescription but whose adherence to care was

waning. A small number of patients are lost to follow-up and return to care with severe opportunistic infections. This has also been observed in other studies conducted in resource-rich settings (Lee et al., 2013; Llibre et al., 2013). We thus need to understand the determinants of poor long-term adherence, such as the challenge of life-long medication and examinations. Efforts have mainly focused on adherence at the beginning of cART. We now need to concentrate on interventions designed to lower the risk of poor adherence among patients who have been in care for many years. Indicators are also needed to alert clinicians when patients start to attend consultations less regularly. It would certainly be interesting to study the possible role of social factors which, unfortunately, are not available in the FHDH database. Better collaboration between HIV specialists and general practitioners might help to maintain patients in care. Some French infectious diseases departments have already taken measures. For example, in the infectious diseases department of one Paris hospital, a reminder letter is sent first if a patient does not attend a consultation. Then the patient is called by telephone, and the general practitioner is informed if they do not answer. HIV specialists now need to see HIV-infected individuals on cART less frequently, although the ideal timing between two consultations is a matter of debate. A meta-analysis of text-message interventions designed to promote adherence to cART concluded that this method could be useful and could be optimized by 1) asking the recipient to reply and 2) by not sending the messages too frequently (Finitisis et al., 2014). Another study showed that a reminder by a member of the clinical staff was significantly more effective than an automated system for lowering the “no-show” rate (Parikh et al., 2010). Various options for maintaining patients in care (see below) are listed in the IAPAC guidelines for optimizing the HIV care continuum (International Advisory Panel on HIV Care Continuum Optimization, 2015), and we are now also working on indicators of adherence to care in the FHDH cohort.

Table: Recommendations for increasing retention in care, ART adherence, and viral suppression (*Journal of the International Association of Providers of AIDS Care* 2015; 14 (Supplement 1))

23. Systematic monitoring of retention in HIV care is recommended for all patients. (A II)
 - 23a. Retention in HIV care should be considered as a quality indicator. (B III)
 - 23b. Measuring retention in HIV care using electronic health record and other health system data is recommended. (BII)
 - 23c. Use of clinic databases/surveillance systems for HIV clinical monitoring and population-level tracking is recommended. (B II)
24. Routine ART adherence monitoring is recommended in all patients. (A II)
 - 24a. Viral suppression is recommended as the primary adherence monitoring metric. (B II)
 - 24b. Routine collection of self-reported adherence data from patients is recommended. (A II)
 - 24c. Pharmacy refill data are recommended for adherence monitoring. (B II)
25. Information and communication technologies aimed at supporting patient self-care are recommended. (B II)
 - 25a. Mobile health technology using weekly interactive components (eg, 2-way SMS) is recommended. (B I)
 - 25b. Alarm devices are recommended as reminders for PLHIV with memory impairment. (A I)
26. Patient education about and offering support for medication adherence and keeping clinic appointments are recommended. (A I)
 - 26a. Pillbox organizers are recommended, particularly for HIV-infected adults with lifestyle-related barriers to adherence. (B II)
27. Neither directly administered nor directly observed ART is recommended for routine clinical care settings. (A I)
 - 27a. Directly administered ART is recommended for people who inject drugs and released prisoners at high risk of ART nonadherence. (B I)
28. Proactive engagement and reengagement of patients who miss clinic appointments and/or are lost to follow-up, including intensive outreach for those not engaged in care within 1 month of a new HIV diagnosis, is recommended. (B II)
 - 28a. Case management to retain PLHIV in care and to locate and reengage patients lost to follow-up is recommended. (B II)
 - 28b. Transportation support for PLHIV to attend their clinic visits is recommended. (B II)

- Third, our study illustrates the poor immune recovery among patients with PCP who had previously been diagnosed with AIDS, and their high 3-year mortality rate. Chronic immune activation certainly plays an important role, and it could be interesting to look at immunological differences between patients with PCP revealing their HIV infection and patients who develop PCP after being diagnosed with another ADI. In the first chapter, pages 50-57, we discuss immune dysregulation during HIV infection with persistent immune activation, inflammation and coagulation processes. Immune failure and inflammation are linked, and are probably the consequence of lymph node fibrosis and sustained damage to the gut. Lederman M summarized different lines of research, and the results of previous and ongoing studies (Lederman et al., 2013). They include:

- targeting residual viral replication, although most studies have shown no benefit of treatment intensification (Dinosa et al., 2009; Gandhi et al., 2010; Hatano et al., 2011; Hunt et al., 2013)
- targeting chronic viral coinfections: asymptomatic CMV replication could contribute to persistent immune activation in HIV-infected individuals on cART
- targeting microbial translocation (ongoing studies of prebiotic interventions with prebiotic oligosaccharide mixture to improve the gut microbiota composition, rifaximin (antibiotic that is not systemically absorbed) and sevelamer (sevelamer binds bacterial lipopolysaccharide in the gut lumen))
- interventions to improve CD4 T cell recovery. Trials of IL-2 therapy failed (INSIGHT-ESPRIT Study Group et al., 2009). IL-7 could be a good candidate but provides only a transient survival benefit for naïve CD4 and CD8 T cells
- targeting innate immune responses with drugs that have anti-inflammatory properties, but the efficacy of this approach needs to be confirmed in larger clinical trials.

Interventions to inhibit fibrosis and the disrupted stromal fibroblastic reticular cell in secondary lymphoid tissues are also interesting approaches to immune reconstitution, but currently the best route to immune reconstitution remains early diagnosis and cART initiation. For patients with a history of ADI before developing PCP, who had slower immune reconstitution in our study, switching to integrase inhibitor-containing cART could be an option. Immune reconstitution seemed to be improved in cART-naïve patients on a regimen that included an integrase inhibitor (Costagliola et al., 2014) and this approach could also be explored in previously treated patients.

Our second study focused on invasive aspergillosis in HIV-infected individuals. Using strict criteria, we validated 242 cases. However, half these cases did not meet the EORTC definition, confirming that it is not appropriate for the HIV/AIDS setting. As early diagnosis and treatment of invasive aspergillosis is vital, accurate diagnostic criteria are crucial. Furthermore, as invasive aspergillosis is rare in HIV-infected individuals (2.2 cases per 10000 PY (1.8-2.6) in the 2002-2011 period of our study), this diagnosis is rarely evoked and treatment delays can therefore occur. In order to hasten diagnosis, we tried to find clinical criteria for validated cases that did not meet the EORTC definition. We found that the polynuclear cell count could not be used as a risk indicator for invasive aspergillosis in this setting. However, the median CD4 cell count was below $100/\text{mm}^3$ at diagnosis of invasive aspergillosis, even in the last period studied here, and we therefore propose to add “HIV-infected individual with a CD4 cell count below $100/\text{mm}^3$ ” to the current EORTC definition, after validation. To further improve our case definition, we plan to review all CT scans recorded since 2002. In 2008, the term “new radiological image” used in the 2002 EORTC definition was replaced by the presence of at least one of the following three signs: dense, well-circumscribed lesions(s) with or without a halo sign, air-crescent sign, and cavity. We

will see if we confirm these signs in the context of HIV infection, by comparing the radiological aspects of “EORTC IA” and “HIV-related IA”. As we have seen, we cannot rely on Galactomannan antigen for the diagnosis of invasive aspergillosis. *Aspergillus* PCR is now better standardized for faster diagnosis. Predisposing genetic factors also need to be investigated. For example, genetic polymorphisms such as PTX3 have been shown to predispose allograft and solid organ transplant recipients to invasive aspergillosis (Cunha et al., 2014, p. 3; Wójtowicz et al., 2015).

In conclusion, we demonstrate that fungal infections among HIV-infected individuals are still a major issue in 2016, even in resource-rich settings. We are now planning to study cryptococcosis in the FHDH cohort, as this is another fungal infection occurring at very low CD4 cell counts. It is a frequent opportunistic infection in low income countries, with a ~50% mortality rate in the first 2 weeks, but it is now rare resource-rich settings. We examined trends in the incidence of cryptococcosis over time and found it was 10 times lower in the period 2002-2011 (4.5 (3.9-5.2) /10 000 PY) than in 1992-1995 (47.5 (42.5-52.5) /10 000 PY). We will examine who is most at risk nowadays, as well as changes in mortality over time, and the time interval between antifungal therapy and cART initiation.

Beyond epidemiological trends in these fungal infections, our results underline the need for more efficient HIV screening and better long-term retention in care, which should be considered as a quality indicator of patient management.

-Traitement prédisposant à l'AI: Non Oui NSP

Si oui, préciser :

Corticoïdes (≤ 2 mois avant l'AI) Dose journalière (en mg) :.....

Type de corticoïdes : solumédrol , cortancyl , solupred ,
soludecadron

Chimiothérapie (≤ 1 mois avant l'AI)

(cure de chimio pour tumeur ou pathologie hémato)

CRITERES CLINIQUES ET RADIOLOGIQUES DE L'AI

-Infection pulmonaire Non Oui NSP

Si oui, préciser :

Signes cliniques : toux douleur thoracique dyspnée hémoptysie

Scanner thoracique : Fait Non fait

Joindre une photocopie anonymisée de la conclusion du scanner

- Infection des sinus Non Oui NSP

Si oui, préciser :

Signes cliniques : sinusite atteinte de la muqueuse nasale atteinte du palais

Scanner des sinus : Fait Non fait

Joindre une photocopie anonymisée de la conclusion du scanner

-Infection du système nerveux central Non Oui NSP

Si oui, préciser :

Signes cliniques : méningite convulsions paralysie d'1 nerf crânien hémiparésie

Scanner ou IRM cérébrale : Fait Non fait

Joindre une photocopie anonymisée de la conclusion du scanner ou de l'IRM

- Autres sites éventuels Non Oui NSP

Si oui, préciser la localisation :

cutané endocardite infection du site opératoire autre :

CRITERES MYCOLOGIQUES

Remarque : *Prendre les résultats positifs les plus proches de la date de l'AI.*

Antigène aspergillaire : sérique (dans le sang) LBA (Lavage BronchoAlvéolaire) non fait

Si fait, résultats : -sérique : positif (>0,5) négatif (<0,5) douteux

- LBA : positif (>0,5) négatif (<0,5) douteux

Sérologie aspergillaire (Anticorps (=Ac) aspergillaire): fait non fait

Si fait, résultats : positif négatif

Autres (pour les cas>2007):

PCR aspergillus : fait non fait NSP **βD glucane** : fait non fait NSP

Si fait : positif négatif

Si fait: positif négatif

Isolement d'une souche d'aspergillus : Non Oui NSP

Examen direct : positif (présence de filaments) négatif non fait
NSP

Histologie : positif (joindre photocopie) négatif non fait
NSP

Culture : positif (présence d'aspergillus) négatif non fait
NSP

Si aspergillus + en culture :

Site où l'aspergillus a été isolé : voies respiratoires autres
préciser :.....

Genre/espèce d'aspergillus : A.fumigatus A.flavus A.nidulans
Autre

TRAITEMENT INITIAL DE L'ASPERGILLOSE INVASIVE

<i>MEDICAMENTEUX</i>	<i>Date de début</i>	<i>Date de fin</i>
1. AMPHOTERICINE B DEOXYCHOLATE (AMB) (FUNGIZONE®) <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
2. AMBISOME® <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
3. ABELCET® <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
4. ITRACONAZOLE (SPORANOX®) <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
5. VORICONAZOLE (VFEND®) <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
6. CASPOFUNGINE (CANCIDAS®) <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
7. AUTRE : <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _
8. PAS DE TRAITEMENT <input type="checkbox"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _

EXERESE CHIRURGICALE (de la lésion aspergillaire): Non Oui
 NSP

EVOLUTION

Le patient est-il décédé ?

Non Oui NSP

Si oui :

Date du décès : |_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

Cause du décès :

Pathologies ayant contribué au décès :

-
-

Merci de joindre une copie anonymisée du CR de décès

CONCLUSION (à remplir par Blandine) : Aspergillose possible probable prouvée exclue

Critères utilisés pour la conclusion : Hôte Clinique Radio Bio Microbio

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