Fungal Infections in AIDS

Andrew H. Limper[1], M.D., Antoine Adenis[2,3], M.D., MPH, Thuy Le[4,5], MD, DPhil, Thomas S. Harrison[6], FRCP*.

1. Mayo Clinic College of Medicine, Rochester Minnesota, 55905 USA
2. Inserm CIC 1424, Centre d'Investigation Clinique Antilles Guyane, Centre Hospitalier de Cayenne, Cayenne, France
3. Equipe EA 3593, Ecosystèmes Amazoniens et Pathologie Tropicale, Université de Guyane, Cayenne, France
4. Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam
5. Hawaii Centre for AIDS, University of Hawaii at Manoa, Honolulu, USA
6. Institute of Infection and Immunity, St Georges, University of London, London, UK

* corresponding author

currently 4134 words Dear Editors: please may we be allowed these few over 4000, as only 4 figures

100 refs with a supplementary reference list for online publication

Search Strategy and selection criteria

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

Key Points

- Although the incidence of systemic fungal infections in HIV-infected patients has decreased in many resource-rich areas following introduction of antiretroviral therapy and earlier diagnosis and treatment of HIV infection, in many resource-limited settings this incidence is not yet decreasing due to continued late diagnosis and challenges with retention in HIV care.
- New PCR-based assays can distinguish colonization from infection with Pneumocystis.
- Measurement of CSF pressure is essential in cryptococcal meningitis, and management of raised CSF pressure through careful therapeutic lumbar punctures reduces mortality
- In large parts of the world, HIV-related histoplasmosis is often neglected, undiagnosed, or misdiagnosed as tuberculosis, due to lack of access to current diagnostics.
- Talaromycosis (formerly penicilliosis) is the second most common bloodstream infection in AIDS patients in Vietnam. Patients present very late in the illness with significant on-treatment morbidity and mortality. A large randomized trial comparing amphotericin B and itraconazole as initial treatments will report soon.
- Novel, affordable, point-of-care diagnostics for pneumocystis, histoplasmosis, and talaromycosis, and wider access to effective antifungals are urgently needed

to reduce the burden of HIV-associated fungal infections in resource-limited settings.

Abstract 200 words

Fungi are major contributors to the opportunistic infections that threaten patients with AIDS. *Pneumocystis jirovecii* is the commonest respiratory infection and *Cryptococcus neoformans* the commonest central nervous system infection in AIDS patients across large parts of the world. *Histoplasma capsulatum* and *Talaromyces* (*Penicillium*) *marneffei* are thermally dimorphic fungi that cause disseminated infections, the former being especially common in parts of the Americas, and the latter endemic in SE Asia.

With widespread availability of ART and earlier testing for HIV, the incidence of systemic fungal infections has decreased in HIV-infected persons in the developed world. But, importantly, in many areas of high HIV prevalence, many patients continue to present late with a low CD4 cell count or re-present with persistent low CD4 cell counts due to problems of adherence and or ART resistance. For pneumocystosis, talaromycosis, and in particular histoplasmosis, affordable, rapid point-of-care diagnostic tests, as have been developed for cryptococcosis, are urgently needed. In addition, antifungal drugs, including amphotericin B, liposomal amphotericin B, and flucytosine, need to be made much more widely available. Such measures, together with continued international efforts in education and training in the management of fungal disease, have the potential to significantly improve patient outcomes.

Introduction

Fungi are major contributors to the opportunistic infections that threaten patients with late stage HIV infection. *Pneumocystis jirovecii* is the commonest respiratory infection and *Cryptococcus neoformans* the commonest central nervous system infection in AIDS patients across large parts of the world. *Histoplasma capsulatum* and *Talaromyces* (formerly *Penicillium*) *marneffei* are thermally dimorphic fungi that cause disseminated infections, the former being widespread, although especially common in the Americas, and the latter endemic in SE Asia. Herein, we review the epidemiology and progress in diagnosis and therapy for these major systemic fungal pathogens seen in HIV patients. In addition, *Coccidioides* sp. and *Emmonsia* sp. are significant pathogens in AIDS patients

in the Americas and South Africa respectively [1,2]. *Candida* species commonly cause mucosal, oral, vaginal and oesophageal infections in those with stage 3 and 4 HIV disease, and fungal skin and nail infections are major causes of morbidity in HIV-infected individuals. Fortunately, mucosal candida infections usually respond readily to azole therapy and immune reconstitution with antiretroviral therapy (ART), and, in the era of ART, recurrent azole resistant *Candida* infections are rare.

With widespread availability of ART and earlier testing and treatment for HIV, the incidence of systemic fungal infections has decreased in persons living with HIV in the developed world, although room for improvement still remains[3S]. However, importantly, in many areas of high HIV prevalence, particularly in Sub-Saharan Africa, there is as yet little evidence for a significant decline in cases [4S] . Many patients continue to present late with a low CD4 cell count [5S] . In addition, as enrolment data for recent cryptococcal meningitis trials shows, while there is no decline in the total number of cases, half or more of cryptococcal patients are now ART-experienced [6S] but with persistent low CD4 cell counts due to problems of retention in care and or ART resistance. Thus, further efforts to address the problem of fungal infections through rapid point-of-care diagnostics for these major fungal pathogens and global access to antifungal drugs are needed, as an integral part of an effective response to the HIV pandemic.

Pneumocystis **Pneumonia**

Pneumocystis pneumonia has emerged as a major cause of infection in those with HIV/AIDS, with an estimate of >400,000 cases worldwide annually.[7] Many of these patients are unfortunately undiagnosed or diagnosed late particularly in resource-limited settings. The mortality of *Pneumocystis* pneumonia ranges from 10 to 30% or higher, depending on the patient population, comorbidities, and whether the diagnosis is made early enough.[8,9] While the incidence has been reduced by implementation of ART, *Pneumocystis* continues to be a problem in patients who are unaware that they are HIV-infected, and in those that fail or default from ART.[10]

Pathogenesis: *Pneumocystis* species are members of the *Ascomycetous* fungi[S] . Each mammalian species may harbor at least one unique species of the *Pneumocystis* genus,[11] for example, *Pneumocystis jirovecii* infecting humans and *Pneumocystis carinii* infecting rats. Serological and epidemiological data indicate that most humans are exposed and transiently infected with *Pneumocystis* early in life.[12] With normal immune responses, this early infection is effectively cleared. However, during periods of immune suppression such as in HIV-infected patients with CD4 cell counts <200/μL, the organism proliferates leading to life-threatening pneumonia. CD4 immunity is absolutely essential for long-term control and memory responses to this fungus, with contributing immunity provided by innate immune responses, CD8 cells, and B-lymphocytes[S]. In the absence of effective CD4-based immunity, innate inflammatory responses promote the accumulation of inflammatory cells including neutrophils and CD8 lymphocytes, which strongly contribute to lung injury. Appreciation of this exuberant innate immune response has led to the use of adjunctive anti-inflammatory corticosteroids in moderate to severe *Pneumocystis* pneumonia.[13] Current evidence suggests that *Pneumocystis* can be acquired from other infected individuals. Therefore, whenever feasible, immunosuppressed patients should not be directly exposed to individuals with active *Pneumocystis* pneumonia.[14,15]

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

Over the past decade, a number of laboratories have switched to identification using PCR. Nested PCR has been useful to identify colonization as well as clinical infection,

whereas, single copy real-time PCR assays have been devised to rapidly identify patients with clinical infection, rather than colonization.[17,18] There remains a pressing need for inexpensive point-of-care diagnostic strategies, which are technically less demanding, that can be applied in regions with limited resources,[7] and work is ongoing to develop relevant *Pneumocystis* antigen detection systems and thermocycler independent amplification strategies that may be utilized in such settings to facilitate diagnosis.

Beta D-glucan assays in the serum can be useful as a screening tool and adjunct to diagnosis, since it has good sensitivity in patients with HIV and *Pneumocystis* pneumonia. Although Beta D-glucan testing does cross react with the glucans released from other fungi, high levels of Beta-D Glucan (>100 pg/ml) in HIV infected patients with the appropriate clinical scenario does provide evidence to support initiating therapy.[19]

Vast regions of the world including sub-Saharan Africa, Asia, and South America lack ready access to laboratory facilities equipped for the diagnosis of *Pneumocystis* pneumonia, rendering the true burden and impact of this infection under-recognized. For instance, unsuspected *Pneumocystis* infection was found to be the cause of up to 7% of all severe pneumonia in children under age five in Mozambique, when modern diagnostic methods were applied.[20] In regions with limited laboratory facilities, the diagnosis of *Pneumocystis* is often made on clinical grounds. While such an approach is pragmatic, it lacks specificity, which is necessary to rapidly focus the appropriate antibiotic therapy towards *Pneumocystis* when it is present.

Therapy: The mainstay of therapy has long been intravenous sulfamethoxazole-trimethoprim (TMP-SMX) with the trimethoprim component dosed at 15-20 mg/kg/day and sulfamethoxazole at 75-100 mg/kg/day. This is given in four equally divided doses for 21 days. For patients with milder disease, and once the disease is under control, therapy can be safely switched from IV to oral administration. It is useful to monitor therapeutic trough levels to avoid toxicity and ensure benefit, though infrequently done.[13] Often, clinical improvement is not observed for up to one week. Patients who cannot

tolerate TMP-SMX are usually treated with the combination of primaquine and clindamycin or pentamidine. Atovaquone is generally reserved for those with milder disease.[13] Of note, efavirenz significantly reduces atovaquone concentrations[S]. Only TMP-SMX is currently present on the WHO Essential Medicine List for *Pneumocystis* pneumonia, with the alternatives appearing on the complementary list and for other indications.

Adjunctive corticosteroid therapy is given to patients with moderate to severe *Pneumocystis* pneumonia, as evidenced by a room air PaO2 < 70 mmHg or alveolar-arterial oxygen gradient of >35 mmHg. For these individuals, prednisone is provided at 40 mg twice daily on days 1-5, 40 mg once daily on days 6-10, then 20 mg once daily to day 21.[21] In paediatric populations, prednisone may be given at 1 mg/kg twice daily on days 1-5, 0.5 mg/kg twice a day on days 6-10; and finally 0.5 mg/kg once daily on days 11-21.[22]

Prevention: Appropriate *Pneumocystis* prophylaxis is essential for preventing this often-lethal infection. HIV-infected patients who have CD4 counts <200/ microliter, or with a history of oropharyngeal candidiasis, should receive prophylaxis with one double strength TMP-SMX tablet thrice weekly, or one single strength TMP-SMX tablet once a day.[23] TMP/SMX in the developing world has additional benefits, reducing early mortality from malaria, reducing anemia and improving growth in children.[24] Alternatives for patients that cannot tolerate TMP/SMX include dapsone, atovaquone, or dapsone with pyrimethamine and leucovorin.[13] Dapsone should be use cautiously in individuals with G6PD deficiency.[13] In general, prophylaxis can be discontinued when CD4 counts are consistently >200 cells/microliter for greater than three months. An effective *Pneumocystis* vaccine is not yet available, but pre-clinical primate studies support the potential efficacy of such an approach for HIV-infected patients.[25]

Cryptococcosis

Latest estimates suggest that HIV-associated cryptococcal meningitis accounts for 150,000-200,000 deaths per year mostly in Sub-Saharan Africa, where the associated mortality remains around 70% at 3 months[S]. Most HIV-associated infections are caused by *C. neoformans* although in Botswana, up to 30% are *C. gattii*[S]. Patients present with headache and fever, with a median duration of 2 weeks [26]. Many patients develop nausea, vomiting, diplopia due to 6th nerve palsies, and reduced visual acuity related to raised cerebrospinal fluid (CSF) pressure. Untreated, symptoms progress to abnormal mental status, reduced conscious level, seizures, and finally coma.

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

Antifungal therapy. Gold standard antifungal therapy remains the combination of amphotericin B deoxycholate (D-AmB), 0.7-1 mg/kg/d, plus flucytosine (100 mg/kg/d in 4 divided doses), for the initial 2 weeks, followed by fluconazole 400-800 mg/d for 8 weeks, and 200 mg/d thereafter for a minimum of one year and until immune reconstitution [29-31]. Addition of flucytosine was associated with a 40% reduction in mortality compared with D-AmB alone [32]. Pre-hydration with Normal saline and pre-emptive replacement of potassium and magnesium is recommended to mitigate the toxicities of D-AmB [30], but anaemia remains a significant problem where transfusion

capacity is limited. Liposomal amphotericin B (Ambisome. L-AmB) at 3-6 mg/kg/d is as effective and better tolerated than D-AmB [33]. Studies are ongoing to determine if it could be used in intermittent high doses, as in leishmaniasis[S], to provide a convenient and cost effective induction treatment. Of note, with care to adjust doses in case of renal impairment, flucytosine is generally well-tolerated in this patient population for 2 weeks [34].

Complications. Raised CSF pressure caused by a blockage of CSF reabsorption at the level of the arachnoid granulations is common with around a quarter of patients having a pressure of >35 cm [35]. Untreated, high pressure is associated with increased mortality [35], but increasing evidence points to the effectiveness of careful therapeutic lumbar punctures [26,36,37]. Only the most severe cases may require a temporary lumbar drain or ventricular shunt [38,39]. Importantly, high CSF pressure may develop in the second and third weeks of treatment, despite effective sterilization of the CSF, meaning that an LP should be repeated if symptoms persist or recur.

Current recommendations are to start ART between 4 and 6 weeks after starting antifungal therapy, based on studies suggesting 3 days and 8 days is too soon [40,41], and 6 weeks or later, probably unnecessarily late[S]. Of importance given that in many centres half of cryptococcal meningitis cases now occur in ART-experienced patients, it is prudent to also only switch to second line ART in those thought to have ART resistance, or re-start ART in those who have discontinued taking ART, after 4 weeks of antifungal therapy. Of note, these cases of cryptococcal meningitis in patients failing ART need to be distinguished from unmasking cryptococcal immune reconstitution inflammatory syndrome (CM-IRIS) cases [42S] whose presentation is precipitated by starting ART, and in whom ART should be continued. Frequent clinical review is needed given some overlapping toxicities of antifungal and antiretroviral drugs.

Paradoxical CM-IRIS occurs in 15-20% of cases. These patients respond to treatment but later have a recurrence of symptoms, at a median of around one month after starting ART [43S]. In patients representing with a recurrence of symptoms, CSF pressure should be

measured and managed, as raised pressure is common in CM-IRIS. Re-introduction of induction antifungal therapy may be considered pending CSF culture results, and while alternative diagnoses are actively pursued. If CM-IRIS remains the likely diagnosis, and the patient is deteriorating, then short courses of corticosteroids in this particular situation have been used successfully. In contrast, corticosteroids with initial antifungal therapy have been shown to be harmful [6]. While CM-IRIS may be life-threatening, related mortality should be less than in earlier series, with increased awareness and prudent timing of ART.

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

Histoplasmosis

Histoplasmosis is caused by *Histoplasma capsulatum* (Hc) a thermally dimorphic ascomycete [49]. The mold form is distributed worldwide in moist and enriched soils containing bird or bat guano [50]. Autochthonous cases of HIV-associated histoplasmosis have been described on five continents, the Americas accounting for most cases, notably the Central Eastern USA and Latin America [51]. However, HIV-associated histoplasmosis is more widespread than previously thought and probably neglected, undiagnosed or mainly misdiagnosed as tuberculosis [52S]. In endemic areas, histoplasmosis incidence ranges between 2-25% and represents the first AIDS-defining infection in up to 50-75% of HIV patients [53]. Lethality rates range between 10-60% depending on whether the diagnosis is made by experienced physicians with adequate infrastructure and access to antifungals other than fluconazole [54,55].

Pathogenesis: Soil disruption aerosolizes microconidia or mycelial fragments which are inhaled and, at body temperature, convert into yeasts in the lungs [50]. Infection may also develop when, years after the primary infection, quiescent organisms are reactivated during immunosuppression.[56S] Once in the lungs, Hc survives phagocytosis in macrophages, facilitating its dissemination throughout the mononuclear phagocyte system. An early robust proinflammatory response (TH1/TH17) is required to control Hc growth [57]. Hence, people living with HIV are at higher risk of disseminated histoplasmosis, an AIDS-defining condition, which is lethal if left untreated [50S].

Environmental exposures to bird or bat guano and history of exposure to chicken coops are associated with an increased risk of histoplasmosis [58]. Host factors such as low CD4 count <200/mm3, nadir CD4 count <50/mm3, CD8 count <650/mm3, absence of ART or systemic antifungal therapy, the first 6 months of ART, history of *Herpes simplex* infection and male gender are independently associated with histoplasmosis in those with HIV infection [59].

Clinical Features and Diagnosis: In AIDS, histoplasmosis usually presents as a disseminated disease (>95%). All organs and tissues may be involved. Fever, fatigue and

weight loss are almost universal. Cough and dyspnea are the most frequent localizing symptoms, in association with diffuse radiologic infiltrates usually with a miliary reticulonodular pattern (Figure 2A) [53]. Abdominal pain and diarrhea are frequent and reflective of colonic ulcerations (Figure 2C)[S]. Lymph node enlargement, hepatosplenomegaly, and muco-cutaneous manifestations are diagnostic clues [53]. LDH, liver enzymes (TGO>TGP) and ferritin elevation with or without pancytopenia and/or hemophagocytosis syndrome should lead to further investigations [51]. Classically subacute (1 to 2 months), the disease evolution varies from latency to 10-20% fulminant severe forms ("septic shock" with multiorgan failure), mainly in late presenters, with fatality rates reaching 50-70% [51,53].

Direct examination with special staining (May-Grünwald Giemsa, PAS, and Grocott-Gömöri methenamine-silver) and culture of all tissues or body fluids at room temperature are the Gold Standard methods for diagnosis [60]. Bone marrow aspiration, blood culture and tissue biopsies are all useful [51]. Direct examination is rapid but culture, which requires a BSL-3 laboratory, takes a median of 2 weeks, and up to 6 weeks. For both, sensitivity varies with sample type, disease severity and operator experience [51]. Antibody detection is of most interest in CSF for the diagnosis of neuro-meningeal forms [61]. Although useful molecular tools are being developed, their place in care and treatment is still evolving [51]. Detection of Hc antigen is among the most sensitive and rapid means to diagnose disseminated histoplasmosis in AIDS [62]. To date, the noninvasive reference method in the USA has been a polyclonal quantitative *Histoplasma* antigen radioimmunoassay in urine, blood and BAL, but it is unavailable in other endemic areas [51]. However, a new commercially available monoclonal EIA detecting galactomannan antigen in urine is being made widely available with promising results [62,63]. It is noteworthy that the highest yield is achieved by combining several diagnostic methods.

Therapy: In moderately severe to severe cases, i/v liposomal amphotericin B (3-4 mg/kg/day) is recommended for two weeks or until clinical improvement[64]. An alternative lipid formulation of amphotericin B at the same dosage may be preferred over D-AmB (0.7mg/kg daily) if liposomal amphotericin is unavailable. Continued treatment

with oral itraconazole (200mg three times daily for 3 days, followed by 200mg twice daily) is given for at least one year.[64] Non-severe cases can be treated with itraconazole alone.[64] Prevention is based on recommendations for workers at risk and long term suppressive therapy with itraconazole 200mg daily as primary (only in USA) or secondary prophylaxis [65S]. ART should be started promptly, within the month following antifungal therapy initiation [64]. Nevirapine and efavirenz are moderate inducers of the CYP3A4 enzyme, and have been shown to reduce the level of itraconazole [66]. However, both itraconazole and its major metabolite hydroxyitraconazole are equally active antifungal agents[S], and it remains unclear whether itraconazole dose adjustment is needed. In view of these interactions, a randomly obtained serum level of at least >1.0 µg/mL of itraconazole is recommended after 2 weeks of therapy [64,67].

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

Talaromycosis (formerly penicilliosis)

Epidemiology. *Talaromyces marneffei* causes a life-threatening mycosis affecting primarily immunocompromised residents and travelers in Southeast Asia, southern China, and northeastern India (Figure 3).[71,72] The intersection with HIV has transformed *T. marneffei* from a rare human pathogen to a major cause of HIV-associated death, trailing only tuberculosis and cryptococcosis or *Pneumocystis* pneumonia in Thailand and Hong Kong.[73,74] In Vietnam, talaromycosis makes up 4-11% of AIDS-related admissions[75,76] and is the second most common cause of bloodstream infections after cryptococcosis.[77] ART has led to a decline in incidence, but talaromycosis remains a major problem in undiagnosed and/or untreated HIV infection. Increasingly

talaromycosis occurs outside endemic regions due to increased migration and international travel,[78S] and a rise in use of chemotherapy and immunosuppression.[79S]

Ecology and transmission. Pathogen reservoirs and disease acquisition are being elucidated. The bamboo rat is the only non-human host of *T. marneffei* with a high frequency of asymptomatic infection.[80,81] The isolates from bamboo rat and human have similar or identical genotypes.[80,81] However, there is no evidence of direct bamboo-rat-to-human transmission; instead occupational exposure to plants and animals has been associated with human infection.[82] This association is recently confirmed in a large case-control study from Vietnam, which additionally reveals that residents from or travelers to highland areas are at increased risk.[83] Incidence increases 30%-50% during the rainy months,[76,84] and can be predicted by humidity levels.[85,86] *T. marneffei* has been isolated from bamboo-rat feces and soil samples within bamboo-rat burrows,[87,88S] and *T. marneffei* DNA detected in elephant-associated soil.[89] Collectively, these epi-ecological data suggest an interplay amongst multiple environmental reservoirs involving soil, plants, and animals, in which bamboo rat may be exploited by *T. marneffei* to expand its biomass and biogeography. Human infections likely occur through inhalation of *T. marneffei* conidia. The incubation period is estimated to be 1-3 weeks in acute disease, whilst reactivation disease can occur many years after exposure in immunocompromised hosts.

Clinical features and outcomes. The majority of infections occur in patients with CD4 T-cell counts <100 cells/mm^3. Patients typically develop disseminated disease with fever, weight loss, hepatosplenomegaly, lymphadenopathy, respiratory and gastrointestinal abnormalities. Papulonecrotic skin lesions are present in 60-70% of patients (Figure 4A). Common laboratory findings include anaemia, thrombocytopenia, and elevated transaminases. [71,75,76] Concomitant opportunistic infections are common, particularly tuberculosis and *Salmonella* infections.[75,76S]

Case fatality rates in treated patients vary, from 10% in Thailand and Hong Kong[71,90] up to 33% in China and Vietnam,[76,85,91] reflecting differences in time to diagnosis and access

to ART. Some clinical and laboratory predictors of mortality include older age, shorter duration of symptoms, dyspnea, absence of fever or skin lesions, thrombocytopenia, and elevated lactate dehydrogenase levels.[75,76]

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

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

Antifungal therapy and prevention. D-AmB and itraconazole are effective drugs, whilst *T. marneffei* is resistant to fluconazole,[75,76,90,91]. A multi-centre clinical trial comparing D-AmB and itraconazole induction therapy reported mortality after 6 months of 11.3% and 21.0%, respectively (absolute risk difference, 9.7%; 95% CI, 2.8% to 16.6%; P=0.006) (IVAP trial, registration ISRCTN97524945) [new citation at the end of the references]. Liposomal amphotericin B and voriconazole are also effective; however they are not available in resource-limited settings. International guidelines recommend D-AmB (0.6-1.0mg/kg/day) for 2 weeks, followed by itraconazole 400mg/day for 10 weeks,

with itraconazole 200mg/day continued until CD4 counts are >100 cells/mm^3 for at least 6 months.[98] Primary prophylaxis with itraconazole has been shown to reduce invasive fungal infections in patients with CD4 counts <200 cells/mm^3,[99] however the strategy has not been widely adopted in Asia due to concerns of costs, toxicity, drug resistance and interactions.

Conclusions

Serious fungal infections continue to contribute very significantly to HIV-related mortality world-wide. For pneumocystosis, talaromycosis, and in particular histoplasmosis, affordable, rapid point-of-care diagnostic tests, as have been developed for cryptococcosis, are urgently needed. In addition, antifungal drugs, including D-AmB, liposomal amphotericin B, and flucytosine, need to be made much more widely available [100]. D-AmB and flucytosine have been recently added to the WHO list of essential medicines. Such measures, together with continued international efforts in education and training in the management of fungal disease, have the potential to significantly improve patient outcomes.

CONTRIBUTORS

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

CONFLICTS OF INTEREST

TH has received an investigator award from Gilead Sciences, diagnostic tests for research from ImmunoMycologics, honoraria from Pfizer, and serves on the advisory board for Viamet.

ACKNOWLEDGEMENTS

TL is supported by the Hawaii Center for AIDS in the US and by the Joint Global Health Trial Grant funded by the Wellcome Trust, the Medical Research Council, and the Department for International Development in the UK.
AA is supported by an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-0025) and by the European Union (PO FEDER 2007-2013, N° Présage : 31362, Guyane française).

Figure 1. A. CT imaging of a 41 year old female with HIV infection presenting with cough and dyspnea. The imaging demonstrated mixed alveolar and interstitial infiltrates that proved on BAL to be due to *Pneumocystis jirovecii* pneumonia. **B**. Methenamine silver staining demonstrates typical *Pneumocystis* organisms clustered in alveolar exudates

Figure 2: A. Chest-X ray with a diffuse interstitial reticulonodular pattern in a HIV-infected patient with histoplasmosis; B. Photograph of a lymph node enlargement in a HIV-infected patient with histoplasmosis; C. Photographs of colonic ulcerations in a HIV-infected patient with histoplasmosis; D. Giemsa-stained bone marrow smear showing the yeast phase of *Histoplasma capsulatum*; E. Lactophenol blue-stained bone marrow culture showing the mold form of *Histoplasma capsulatum* (Sources: [A & B] Pr P. Couppié, [C] Dr D. Louvel, [D&E] Dr C. Aznar from Cayenne General Hospital, France)

Figure 3. Geographical distribution of *T. marneffei* infection

Figure 4. A) Photograph of skin lesions in a HIV-infected patient with talaromycosis, used with written consent of the patient; B) Giemsa-stained touch skin smear showing oval-shape yeast organisms inside and outside of a ruptured macrophage. The arrowhead highlights the midline septum in a dividing yeast cell characteristic of *T. marneffei;* C) Morphology of *T. marneffei* colonies and *T. marneffei* cells grew at 25^{o}C and at 37^{o}C on Sabouraud agar medium

Figure 1A

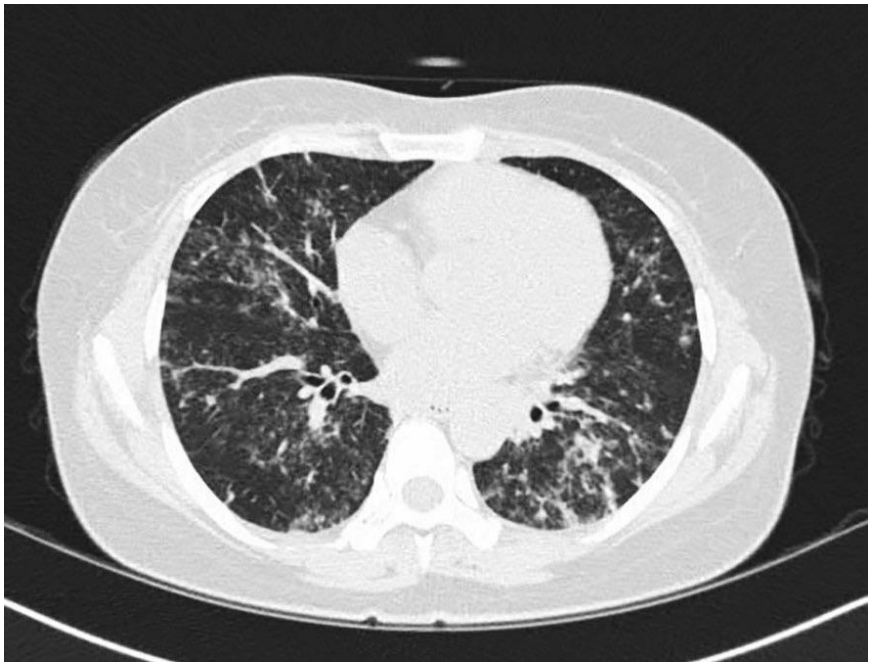


Figure 1B

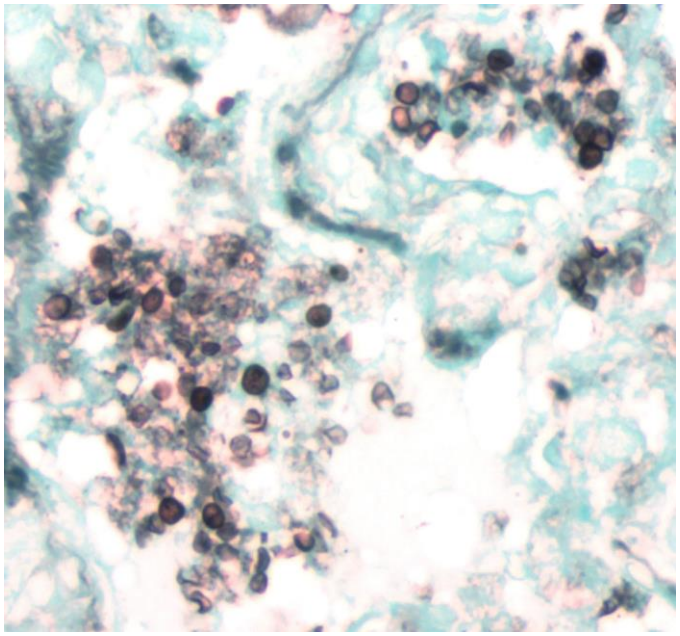
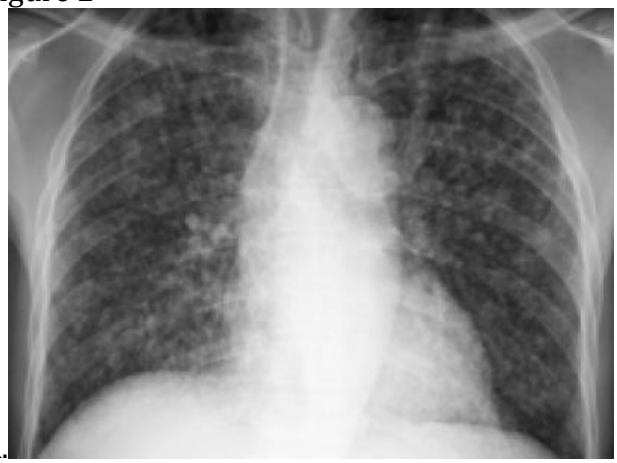
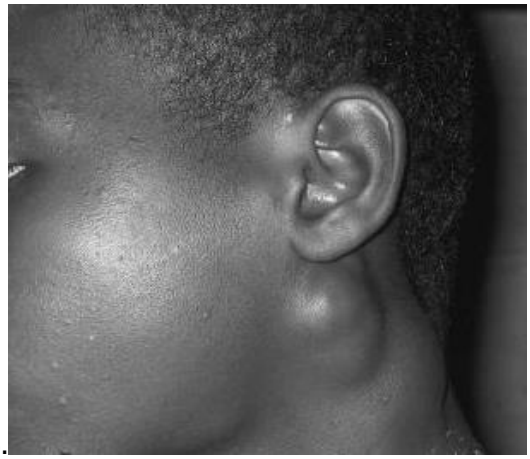
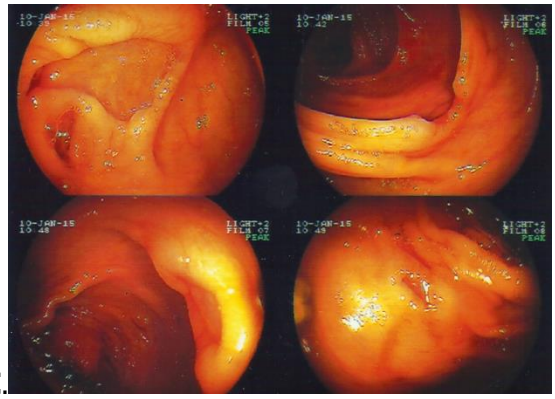
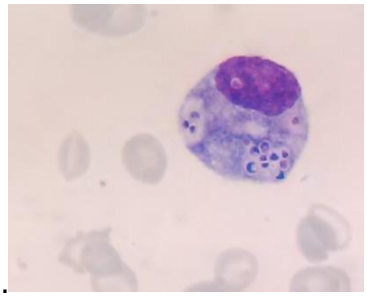


Figure 2

A. 

B. 

C. 

D. 

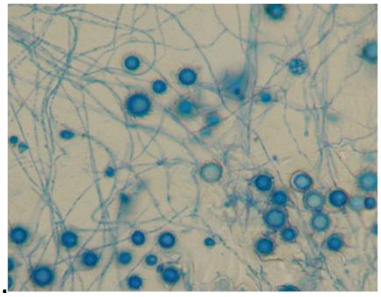
E. 

Figure 3

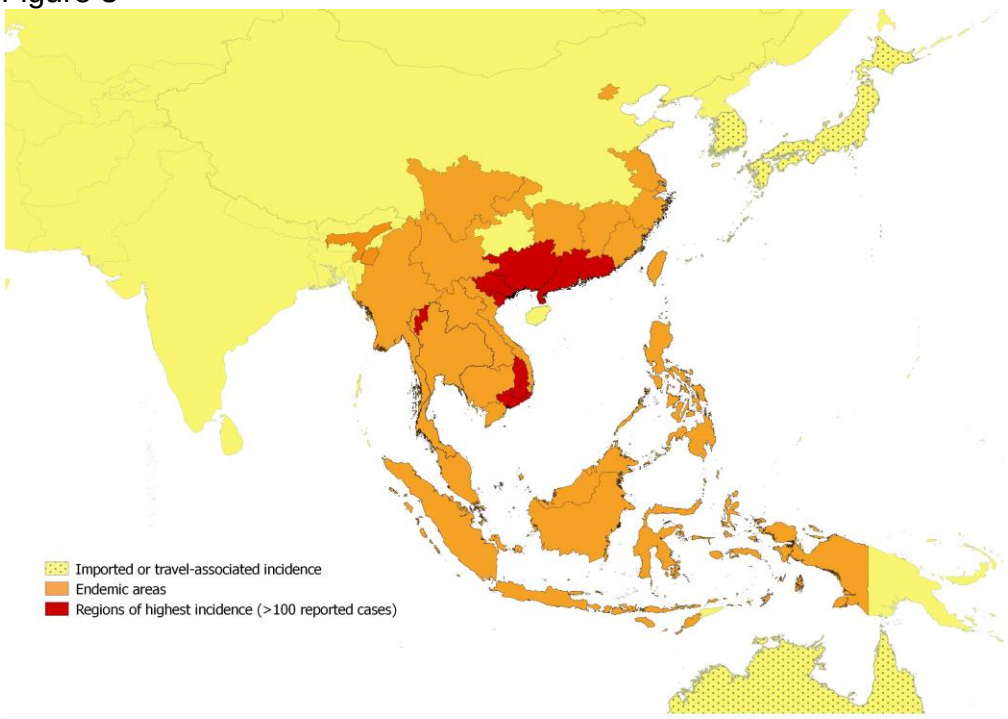
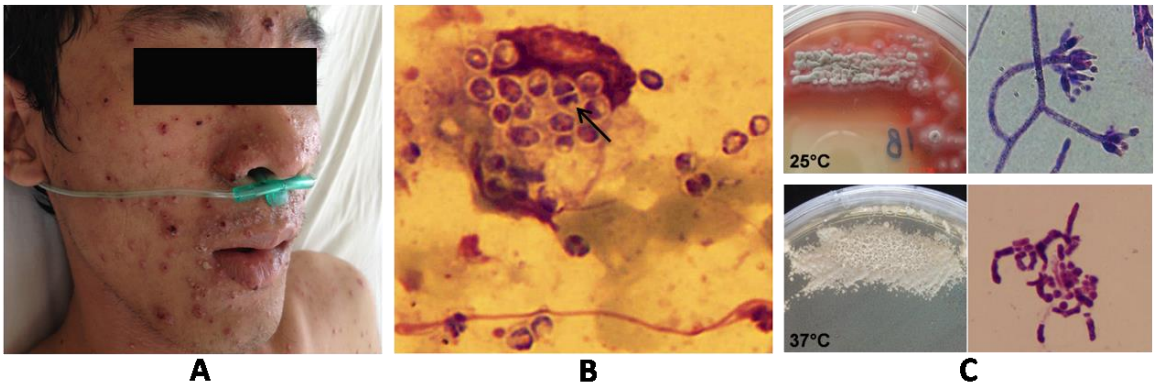


Figure 4



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