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Science & Society

AIDS-Related Mycoses: Current Progress in the Field and Future Priorities

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Opportunistic fungal infections continue to take an unacceptably heavy toll on the most disadvantaged living with HIV-AIDS, and are a major driver for HIV-related deaths. At the second EMBO Workshop on AIDS-Related Mycoses, clinicians and scientists from around the world reported current progress and key priorities for improving outcomes from HIV-related mycoses.

Global Burden of HIV-Related Fungal Disease

Whilst many fungi cause low-grade superficial infections, these microorganisms are a major cause of high-mortality invasive infections in immunocompromised individuals [1]. Advanced HIV infection continues to be a major driver for invasive fungal diseases, despite the global scale-up of antiretroviral therapies (ARTs) [1]. Defining the global burden of fungal diseases presents a major challenge, as they are often insidious in nature, and there are intrinsic challenges in their diagnosis, as well as a global lack of capacity for fungal diagnostics [1]. Best estimates suggest that there are up to a million invasive fungal infections per annum related to HIV-AIDS [primarily cryptococcosis, pneumocystosis, histoplasmosis, and talaromycosis (formerly penicilliosis)], with a consequent mortality of up to 500 000 per annum [2]. This places HIV-related fungal disease at nearly the same level of mortality as other major infectious diseases such as malaria and tuberculosis [3,4]. Current case fatality rates for cryptococcal meningitis vary between 30% and 70% for patients diagnosed and treated in sub-Saharan Africa [5,6]. Recent data from the Amazon region for HIV-associated histoplasmosis indicates a 50% overall mortality rate at 1 year [7]. Studies in Uganda indicate on overall mortality of around 20% for HIV-related *Pneumocystis* pneumonia [8], and a mortality rate of 28% for HIV-associated talaromycosis in Viet Nam [9]. In addition, oral candidiasis is very common and is associated with a high degree of morbidity if untreated [1].

The Joint United Nations Programme on HIV/AIDS (UNAIDS) has established an ambitious treatment target known as 90-90-90 to control the AIDS epidemic[i]. But even if these targets are reached, there will still be a substantial burden of fungal disease in patients who present for care late or disengage from or fail ART. Despite this, fungal disease has not had the same level of focus from the global community, although as a result of recent efforts from the medical mycology community, we hope this is now changing.

At the inaugural meeting of the EMBO Workshop on AIDS-Related Mycoses in Cape Town, South Africa, in July 2013, five key goals were identified to improve outcomes from these deadly diseases:

(i) Better epidemiological surveillance for HIV-related fungal diseases.
(ii) Better laboratory and point-of-care testing.
(iii) Improved access to existing drugs.
(iv) Expansion of capacity for medical mycology training.
(v) Increased funding for development of diagnosis, treatment, and implementation programmes, especially in resource-poor settings.

Recent Progress in the Field

As part of ongoing efforts to stem the tide of fungal disease, over 100 researchers spearheading the battle against HIV-related fungal diseases in Africa, Asia, North and South America, Europe, and Australasia met at the 2nd EMBO AIDS-Mycoses Workshop for 3 days in July, 2016, in Cape Town, South Africa, to discuss current progress in the field and future priorities.

The workshop was notable for the geographic breadth of participants, as well as excellent coverage for all the major HIV-related fungal diseases. The major topics discussed were epidemiology and public health, diagnostics, host–pathogen interactions, immunology, drug resistance, treatment strategies, new antifungal drugs, and vaccines. Detailed updates were given on epidemiology and public health aspects of the major AIDS-related mycoses, including cryptococcosis, histoplasmosis, talaromycosis, and pneumocystosis. In addition, newly recognized HIV-associated invasive fungal infections due to *Emmonsia* spp. (recently renamed *Emergomyces*) were highlighted. These infections appear to be unique to Southern Africa, and the clinical presentation mimics disseminated histoplasmosis. A notable observation was that whilst antiretroviral roll out appears to have had some impact on the incidence of cryptococcal meningitis in recent years, half of all cases occur after ART has been established [10]. When taken in the context of the global failure to reduce new HIV infections, emerging HIV drug resistance, and challenges in retaining patients in ART care in resource-limited settings, it is clear that the ongoing epidemic of cryptococcal meningitis will be sustained. The high rates of HIV-associated infection and mortality from histoplasmosis in Latin America and talaromycosis in South and South East Asia were also highlighted [9,11]. Key highlights in fundamental research in AIDS-related mycoses included genomics-based studies of cryptococcal evolution and resistance in the host, a number of studies detailing aspects of metabolic adaptation of *Cryptococcus neoformans* and *Talaromyces marneffei* in the host, and descriptions of the latest discoveries in understanding the innate and adaptive immune responses to *Pneumocystis* spp.

A number of advances in the development and implementation of point-of-care-based testing (POCT) strategies were highlighted during the meeting. In particular, there has been enormous progress with the implementation of cryptococcal lateral flow device-based screening programmes in sub-Saharan Africa. South Africa has recently implemented national reflex laboratory screening with a projected 250 000 persons estimated to screened per annum [12]. The strategy of treating antigen-positive patients reduces the likelihood of future cryptococcal meningitis [13]. Further progress was also reported with the development of rapid tests for *Talaromyces marneffei* and *Histoplasma capsulatum* infection.

Access to currently available antifungal medicines as well as the development of novel antifungal drugs is an urgent priority, due to the limited drug classes and emerging resistance to triazoles. Planned recruitment to the AMBITION study was outlined (intermittent high-dose AmBisome [liposomal amphotericin B] on a high-dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa), as well as current Phase 1/2 studies for VT-1129, a new oral agent for cryptococcal disease, progress of the ACTA trial (oral fluconazole plus flucytosine or one week amphotericin B-based therapy versus 2 weeks amphotericin B-based therapy), and the ASTRO trial (Adjunctive Sertraline for the Treatment of HIV-associated cryptococcal meningitis). There continues to be inadequate access to flucytosine, amphotericin B, and itraconazole in countries with major burdens of either cryptococcal meningitis or endemic mycoses. The emergence of triazole resistance in both *Cryptococcus neoformans* and *Aspergillus fumigatus* was also discussed [14,15]. Notable progress has been made in understanding the genetic basis for the emergence of cryptococcal heteroresistance during therapy, and combination antifungal strategies to limit this.

Advocacy for fungal diseases continues to gather pace, through the Global Action Fund for Fungal Infections(GAFFI)[ii], the Cryptococcal Meningitis Advocacy Group(CryptoMAG)[iii], and the planned establishment of new groups focused on pneumocystosis and histoplasmosis. Whilst CryptoMAG successfully proposed the addition of flucytosine and amphotericin B to the essential medicines list, recent attempts to include itraconazole, a crucial drug for histoplasmosis, talaromycosis, and aspergillosis, on the WHO's (World Health Organisation's) essential medicines list have been unsuccessful. Furthermore, none of these AIDS-related mycoses are currently classified as neglected tropical diseases by the WHO.

Key Priorities for the Future

During the meeting, there was a major focus on discussing the key priorities to move the field forward, and five priorities were identified:

(i) Better collaborative working structures for basic scientists and clinical researchers to accelerate translational medicine. The meeting itself acted as an environment in which basic scientists and clinical

researchers intensively interacted. Promoting greater interaction in the future will lead to accelerated translation. The 3rd AIDS-related Mycoses Conference is planned in 3 years' time.

(ii) Better diagnostics and improved surveillance. It is particularly apparent that, without POCTs for the major AIDS-related mycoses, these infections will remain difficult to diagnose, and treat, and their true global burden will remain very difficult to ascertain.

(iii) Access to established medicines, as well as development of new medicines and vaccines. Access, in particular to flucytosine, amphotericin B, and itraconazole, is particularly patchy, and liposomal amphotericin B (Ambisome) remains very expensive in many countries. Acceleration of vaccination programmes should be a key priority, but will be challenging due to the difficulties in eliciting immune responses in immunocompromised people.

(iv) Consolidation and extension of consortia for the delivery of multicentre clinical trials. Whilst there are major groups working in the area of cryptococcal meningitis, better cohesion and extension to other AIDS-related mycoses will enable more rapid progress in this area.

(v) Extension of current advocacy groups and public engagement. New advocacy groups are being established, based on CryptoMAG, covering histoplasmosis and pneumocystis. GAFFI is currently supporting the Kenyan government in an ambitious 5-year medical mycology infrastructure developement programme.

Concluding Remarks

Invasive fungal infections continue to be a major cause of mortality in the context of advanced HIV infection globally. The medical mycology community made significant recent progress in delivering novel diagnostic and therapeutic strategies to limit mortality from these infections, and there are some encouraging novel therapies on the horizon. However, engagement of major funding bodies and governmental and nongovernmental organizations is urgently needed to enable substantial reductions in the unacceptably high morbidity and mortality from the AIDS-related mycoses.

Resources

[i] www.unaids.org/en/resources/documents/2014/90-90-90

[ii] www.gaffi.org

[iii] http://preventcrypto.org/about-us/

Supplemental Information

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Spotlight

Finally, Archaea Get Their CRISPR-Cas Toolbox

Uri Gophna,[1] Thorsten Allers,[2] and Anita Marchfelder[3,*]

The majority of archaea encode CRISPR-Cas systems but only a few CRISPR-Cas-based genetic tools have been developed for organisms from this domain. Nayak and Metcalf have harnessed a bacterial Cas9 protein for genome editing in *Methanosarcina acetivorans*, enabling efficient gene deletion and replacement.

Soon after their discovery as prokaryotic defence mechanisms, CRISPR (clustered regularly interspaced short palindromic repeats)-Cas systems were harnessed as molecular tools [1,2]. This development exploded when the potential of the CRISPR-Cas type II system was realized and the use of Cas9 as a genome-editing and gene-silencing tool was established [3]. The wide publicity that CRISPR-Cas9 applications gained has come mainly from their application in