S2.1d
Incidence of mixed fungal infections in post-COVID-19 outbreak of Mucormycosis

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S2.1 Update on Mucormycosis, September 21, 2022, 3:00 PM - 4:30 PM

Introduction: Post-COVID-19 rhino orbital mucormycosis has emerged as an important life-threatening complication adding to mortality. Fungal infections are a major health challenge, especially in the immunocompromised.

Mucormycosis is a severe, frequently fatal fungal infection that has a unique predisposition to infect patients with diabetes. The most probable reasons for the emergence of these cases could be the extensive use of steroids in the management of coronavirus disease 2019 (COVID-19) patients and extensive dysregulated immune response due to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. A prompt diagnosis is vital for the effective management of invasive rhino-orbital fungal infections due to their propensity for angioinvasion and destructive spread with brain involvement.

Materials and Methods: A total of 150 surgical pathology specimens received with a clinical suspicion of invasive fungal infection during the post-COVID-19 outbreak of mucormycosis were retrieved from the archives of the Department of histopathology. The cases were reviewed for the presence of *Aspergillus* fruiting bodies by senior pathologists and microbiologists on a multi-headed microscope. The morphological features of the fruiting bodies were noted and correlated with the fungal KOH and culture. The tissue reaction pattern, presence of oxalate crystals, and morphology of the fungal hyphae were also noted in each case showing *Aspergillus* fruiting bodies.

Results: A total of 8 out of 150 cases (5.3%) showed the presence of *Aspergillus* fruiting bodies. The histopathological diagnosis given in these 8 cases were—*Aspergillus* (1), combined *Aspergillus* and *Mucorales* (7). Two types of fungal hyphae were noted in all seven cases of combined infection. Granulomatous tissue reaction was noted in two out of seven cases of combined infection. Calcium oxalate crystals were noted in the single case of Aspergillosis and were absent in all cases of mixed infection.

Conclusion: To conclude *Aspergillus* fruiting bodies are found in a small but significant number of cases of post-COVID-19 Rhino-Orbital invasive mold infections so while reporting the surgical specimens with clinical suspicion of post-COVID-19 mucormycosis one should be aware of the possibility of mixed fungal infections and look for *Aspergillus* fruiting bodies as a tell-tale sign of mixed *Mucorales* and *Aspergillus* infection. However, their presence does not estimate the true incidence of mixed fungal infections for which immunohistochemistry and polymerase chain reaction are needed.

S2.2a
African histoplasmosis

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S2.2 Histoplasmosis and talaromycosis, September 21, 2022, 3:00 PM - 4:30 PM

African histoplasmosis caused by Histoplasma carpsulatum var dubosii is endemic in Africa with few cases reported from outside Africa usually attributed to travelling or visits to sub-Saharan Africa. The pathogenesis is yet unclear. Infection can be acquired via inhalation of microconidia or by direct inoculation. African histoplasmosis commonly presents with papules, nodules, ulcers, swellings, lymph node enlargement, eczematoid, or psoriosiform skin lesions. Subcutaneous abscesses may also develop with discharging sinuses containing yeast cells of the fungus. Although it is generally believed to be acquired through inhalation, the lungs are usually spared. Disseminated forms are usually characterized by the involvement of bones and other organs including the gastrointestinal tract. As a result of limited availability of diagnostics, data on its prevalence and epidemiology are scarce. As with classical histoplasmosis, African histoplasmosis also mimics other clinical entities including TB and neoplasms. More awareness and a high index of suspicion on the part of clinicians will lead to early diagnosis and invariably improve clinical outcomes. An extensive review of literature revealed 365 cases of African histoplasmosis reported globally; 236 cases from Africa and 38 cases from other geographical regions including an indigenous case report from India; the location of the remaining cases was not found, positive HIV status was found in 75 cases only. No statistically significant relationship was observed when comparing the relationship between positive HIV status and fatal outcomes ($P > .05$, Fisher's exact test). A case report from the UK mentioned 21 cases of African histoplasmosis previously identified in the region and all were associated with travelling to Africa. Out of the 365 identified cases, diagnostic modality was specified in 264 cases; histopathology (87.9%, $n = 232$), culture (20.8%, $n = 55$), microscopy (7.2%, $n = 19$), serology (2.7%, $n = 7$), cytology ($n = 1$, 0.4%), polymerase chain reaction ($n = 43$, 16.3%), and peripheral blood film ($n = 1$, 0.4%). Amphotericin B ($n = 53$), itraconazole ($n = 37$), and ketoconazole ($n = 26$) were the predominant antifungals used for treatment. More studies are required to ascertain the true burden and epidemiology of African histoplasmosis and to determine whether these cases reported outside Africa were autochthonous or imported from Africa. Diagnosis by culture, although the gold standard is also not routinely achieved due to lack of biosafety level 3 cabinet especially in resource-limited settings, leading to significant under diagnosis. It is imperative that capacity building and strengthening is instituted to aid the diagnosis and management of African histoplasmosis. There is a need for more studies to ascertain whether there are additional phylogenetic species within the African clade.

S2.2c
Talaromycosis in HIV-negative patients: challenges and counter-measures

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S2.2 Histoplasmosis and talaromycosis, September 21, 2022, 3:00 PM - 4:30 PM

Talaromycosis is an infection caused by the thermally dimorphic fungus *Talaromyces marneffei* (*T. marneffei*). It is endemic in tropical countries of Asia and Southeast Asia and has recently been recognized far beyond the traditional endemic areas. Talaromycosis was thought to be exclusively associated with HIV infection. However, an increasing number of *T. marneffei* infections have been reported in non-HIV-infected patients. Although the incidence of talaromycosis in non-HIV-infected patients is < 10%, the atypical presentation of this disease can lead to misdiagnosis, inappropriate treatment, and poor outcomes, in addition to tremendous burden and suffering for patients and their families. Screening for immunodeficiencies in patients with talaromycosis is crucial in clinically suspicious cases. Our cohort studies identified three major immunodeficiencies in non-HIV patients with talaromycosis: (1) 85.9% anti-interferon-γ autoantibodies, (2) primary immunodeficiency disorders, and (3) tumors. In disseminated talaromycosis, most patients present with fever, anemia, weight loss, respiratory symptoms, hepatosplenomegaly, lymphadenopathy, and osteolytic destruction and are often misdiagnosed as tuberculosis and other diseases. Localized talaromycosis, on the other hand, is usually manifested by ulcers or masses in the mouth, throat, and external genitalia. Although antigen screening is an effective approach for diagnosing talaromycosis in patients with advanced HIV disease, pathogen-based detection is still limited by atypical clinical presentation. Direct microscopic examination, mycological culture, and histopathology are the standard traditional diagnostic methods used to isolate *T. marneffei* from clinical specimens, however, due to time-consuming, it might lead to delay diagnosis. Metagenomic next-generation sequencing (mNGS) technology has shown promising results as a rapid, convenient method for detecting *T. marneffei* from various types of specimens, leading to correct diagnosis and appropriate treatment. Based on our current retrospective study, mNGS has been shown to be equivalent and possibly superior to conventional fungal culture in terms of speed and specificity in the diagnosis of talaromycosis. In terms of final clinical diagnosis, mNGS showed a high sensitivity of 97.22% compared with conventional culture (61.11%). Correction of underlying immunodeficiencies and early use of antifungal agents are important treatment strategies for talaromycosis. Currently, there is no optimal therapeutic regimen for the treatment of talaromycosis in this specific group of patients. Amphotericin B is the first line to initial antifungal treatment, other antifungal agents such as voriconazole have shown good efficacy against talaromycosis. We investigated the efficacy of voriconazole in the treatment of talaromycosis using population pharmacokinetics. C-reactive protein (CRP) was found to significantly affect voriconazole plasma concentrations. Optimization of initial dosing based on CRP levels may be useful to guide voriconazole dosing in clinical practice. The mortality rate in non-HIV talaromycosis is higher than in the HIV population, which may be due to the nonspecific and complex clinical manifestations. Failure to initiate antifungal treatment in a timely manner often results in poor prognosis and even death. The course of treatment is protracted, unclear, and depends on the immune status of the patient. Diagnosis and treatment of talaromycosis remain a challenge. Optimization of diagnostic tools and treatment regimens to ensure early detection and prompt antifungal treatment should be considered.

S2.2d
Evaluation of new tools for the diagnosis of histoplasmosis

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S2.2 Histoplasmosis and talaromycosis, September 21, 2022, 3:00 PM - 4:30 PM

In sub-Saharan Africa (SSA) and West African countries, histoplasmosis is rarely diagnosed probably due to lack of epidemiological information, insufficient training and awareness of frontline healthcare workers, and clinical features very similar to those of tuberculosis that can be misleading. This fungal infection mainly affects immunocompromised patients and particularly advanced HIV patients, with a high case-fatality rate in the absence of treatment (from <10% to >40%). The classical diagnostic methods are microscopic observation of yeasts with suggestive morphology and a positive culture from a biological sample. However, direct examination requires regular practice, and the yeasts can be confused with other pathogens and culture takes prolonged incubation (often 2-6 weeks) and involves, when positive, handling in a level 3 security laboratory.

Implementing non-invasive diagnostic tools will allow us to improve histoplasmosis diagnosis for the most exposed patients and also to evaluate the prevalence of this fungal infection in countries where data are still lacking. Rapid diagnostic tests (RDTs) such as the TB Lam for the diagnosis of tuberculosis or the Cryptococcal antigen (CrAg) lateral flow assay (LFA) for cryptococcosis have demonstrated their usefulness for the management of advanced HIV patients in similar contexts.

Recently, two RDTs have been made commercially available for the diagnosis of histoplasmosis, based on urinary monoclonal antigen detection: (1) Histoplasma Capsulatum Urinary Antigen Rapid Test from Optimium Imaging Diagnostics (OIDx) and (2) Histoplasma Urine Antigen Lateral Flow Assay from MiraVista Diagnostics (MV).

Objectives and Methods: Our objective was to evaluate these new tools, by experimenting with their feasibility in low- and middle-income countries (LMICs) and by studying their diagnostic performances using different sample collections recovered from patients with disseminated histoplasmosis (culture proven), other HIV-related infections, and proven negative urines (culture and other Histoplasma antigen detections).

Results: Preliminary results were obtained using the EDIRAPHIS study frozen samples from hospitalized patients diagnosed with proven positive and negative histoplasmosis from French Guiana and Suriname ($n = 43$) tested with OIDx and MV tests. We calculated a Se = 74.2% and a Sp = 83.3% for OIDx and a Se = 77.4% and a Sp = 91,7% for MV. A low number of false positives for both tests, <17% for OIDx and <9% for MV were observed. We have a perfect correlation between the observers with a Kappa coefficient of 100% for both tests. Overall, the probabilities that the patient had histoplasmosis with a positive test were 92% and 96% for OIDx and MV respectively.

Conclusion: These first results are very promising and will be completed with two other specimen collections to increase the total numbers of our sampling and get a whole picture of the performances of these two RDTs. The next step will be to implement these new tools at the bedside or in laboratories together with other tests in different settings across SSA.

Diffusion of RDTs together with appropriate training of clinical and laboratory teams and accessibility to treatment may help reduce the burden of histoplasmosis in endemic areas of SSA where the prevalence of the advanced-HIV disease is high.

S2.3c
High-resolution digital DNA melting: a breakthrough in diagnosing IMI?

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S2.3 Novel diagnostic tools for invasive mold infection, September 21, 2022, 3:00 PM - 4:30 PM

Invasive mold infections (IMIs) such as Aspergillosis, Mucormycosis, Fusariosis, and Lomentosporiosis have emerged as important pathogens in immunocompromised patients, with mortality rates as high as 50% to nearly 80% for these infections. Outcomes can be substantially improved with early initiation of appropriate antifungal therapy, yet early diagnosis remains difficult to establish and often requires multidisciplinary teams evaluating clinical and radiological findings plus supportive microbiologic findings. Conventional fungal culture and PCR techniques are limited by low sensitivity, long turn-around time, and are insufficient for differentiating between infection and colonization. Other non-culture-based tests, such as the Galactomannan test, are available only for invasive aspergillosis and are limited by imperfect test performance. Better and more rapid diagnostics are needed to enable early diagnosis and targeted treatment of IMIs and improve survival.

We have developed a new technology called digital high resolution melting analysis (dHRM) to enable a rapid and robust diagnosis of IMI. This technology accomplishes fast genotyping of fungal genomic sequences in clinical samples by: (1) conducting broad-based amplification of fungal genes in a digital PCR format, and (2) conducting high-resolution melting of the DNA amplicons in each digital reaction. High resolution melting measures the fluorescence of a saturating intercalating dye as dPCR-amplified fungal DNA fragments are rapidly heated and disassociated, producing sequence-defined melt curves in a closed reaction format. These melt curves serve as unique fungal 'fingerprints', which allows us to correctly identify disease-causing pathogen(s) with an accuracy of 99%-100% through the use of machine learning methods trained to tolerate variations in reaction conditions. This approach provides a simple, low-cost, fast, and robust method for pathogen identification.

Here, we will present the performance of this new technology on clinical bronchoalveolar lavage (BAL) samples from patients with and without IMI. A total of 75 patient BAL samples were tested, coming from 30 patients with proven ($n = 1$), probable ($n = 25$), or putative ($n = 4$) invasive pulmonary aspergillosis (IPA) infections, and from 45 patients classified as not having IPA ($n = 4$ possible IPA and $n = 41$ no IPA). Our results show that dHRM was able to reliably differentiate between different *Aspergillus* spp. and was also able to identify *Aspergillus* spp. in BAL samples from patients with probable IPA where qPCR had resulted in negative. In a few instances, it also detected rare molds in culture-negative samples and multiple *Aspergillus* spp. within one BAL sample, a phenomenon that has been previously described in cultures. Interestingly, there was no clear correlation between dHRM results and BAL galactomannan levels, suggesting that dHRM may serve as an independent method allowing for a combined diagnostic approach. While further validation and optimization are needed, these results suggest that dHRM is a promising new diagnostic technology capable of providing clinically relevant information within 2-3 h.

S2.3d
Mixed biofilm of *Aspergillus fumigatus* and *Stenotrophomonas maltophilia*: Microscopic visualization of galactosaminogalactan and galactomannan polysaccharides in the extracellular matrix

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S2.3 Novel diagnostic tools for invasive mold infection, September 21, 2022, 3:00 PM - 4:30 PM

Introduction: *Aspergillus fumigatus* (Af) and *Stenotrophomonas maltophilia* (Sm) are commonly co-isolated from the airways of cystic fibrosis patients, especially in mixed biofilms. A mixed Af-Sm biofilm model, developed by our lab, demonstrated that Sm exhibits an antibiosis effect on Af by (1) inhibiting fungal growth, (2) rendering the fungal mycelia highly branched, and (3) increasing the fungal cell wall thickness.[1] The presence of three Af cell wall polysaccharides, galactomannan (GM), galactosaminogalactan (GAG), and α-1,3-glucan, have been described in the extracellular matrix (ECM) of Af biofilms.[2] GM and GAG are known to be released out from the cell wall of Af.[3] GAG contains N-acetylgalactosamines (GalNAc) which undergo partial deacetylation extracellularly, to be converted to GalN, and thus transform this polysaccharide into a polycation with strong surface binding properties.[4]

Aim of the study: Analyze the structure of fungal cell wall polysaccharides in the formation of Af biofilm alone or mixed with Sm.