

skin, further highlighting the importance of considering the contextual relevance when evaluating the functions of secreted microbial enzymes.

S1.4b

Challenges in diagnosing and management of invasive fungal infections during the pandemic

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S1.4 Fungal infections in Asia, bringing it out of the dark, September 21, 2022, 11:00 AM - 12:30 PM

Invasive fungal diseases have been increasing in Asian countries. Recent advances in novel medical care such as solid organ and stem cell transplantations, chemotherapy for cancer treatment, and corticosteroid therapy, resulted in the increased prevalence of invasive mycoses. Invasive aspergillosis, mucormycosis, and endemic mycoses are among the most common mold infections in Asia. Non-classical and novel risk factors of invasive fungal diseases have been increasingly recognized in Asia. In contrast to the classical neutropenic patients, most of the patients with invasive mycoses who had non-classical risk factors are mostly non-neutropenic and may present with an atypical clinical manifestation. These novel risk factors include biological agents or small molecule kinase inhibitors used for cancer treatment, and severe viral pneumonia such as influenza pneumonia or coronavirus disease 2019 (COVID-19) pneumonia. Recently, COVID-19-associated aspergillosis (CAPA) and COVID-19-associated mucormycosis (CAM) have been described. These particular mold infections had high mortality. Treatment of CAPA and CAM are similar to those who had mold infections without COVID-19. However, the interaction between triazole and drugs used for the treatment of COVID-19 must be taken into consideration.

Timely and accurate diagnoses are crucial for the management of invasive fungal infections. Conventional fungal cultures from sterile clinical samples or blood are useful but they are time-consuming. Nevertheless, the diagnosis of invasive mold infections is challenging as the imaging is non-specific and the serological tests are not widely available in Asian countries. In some circumstances such as those with non-classical risk factors, serology revealed relatively low sensitivities. Molecular diagnostic tests are also the unmet needs among Asian countries for timely and accurate diagnosis of invasive fungal diseases. Several factors should be considered for the appropriate choice of antifungal agents, including antifungal coverage, adverse effects, underlying conditions, drug-drug interactions, and cost. Recently, novel antifungal agents such as novel triazoles or new classes of antifungal agents have been studied and may be a promising choice for the treatment of invasive fungal diseases.

S1.4d

Cryptococcus qPCR assays: the future for routine mycology labs and clinical trials dealing with cryptococcosis

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S1.4 Fungal infections in Asia, bringing it out of the dark, September 21, 2022, 11:00 AM - 12:30 PM

Background: Routine laboratory testing for cryptococcal meningitis currently consists of Cryptococcal antigen (CrAg) testing in blood and cerebrospinal fluid (CSF), CSF India ink, and CSF fungal culture. Quantitative cryptococcal culture (QCC) is labor intensive and not feasible in most settings.

Objectives: We evaluated quantitative (qPCR) and reverse transcriptase qPCR (RT-qPCR) assays to quantify cryptococcal load in CSF, plasma, and blood. We also investigated the dynamics of fungal DNA and RNA detection during antifungal treatment.

Methods: We developed a qPCR assay that can differentiate serotypes A, D, and B/C of *Cryptococcus neoformans* and *C. gattii* based on the amplification of a unique nuclear Quorum sensing protein 1 (QSP1) and a multicopy 28S rRNA gene and evaluated the assays on 205-patient samples from the AMBITTON-cm trial in Botswana and Malawi (2018-2021). CSF, plasma, and whole blood samples were stored per patient and were sampled at day 0 (baseline), day 7 and 14 for CSF and at day 1, 3 and 7 for plasma and whole blood post antifungal treatment initiation. A Roche LightCycler480 and Graph pad prism were used for data analysis.

Results: A total of 205/209 stored patient samples (85 from Botswana; 124 from Malawi), were used. For QSP1 qPCR tested in CSF at D0, 138 (81.7%) were serotype A, 28 (16.6%) were serotype B/C and 3 (1.8%) were a mixed infection of serotype A and B/C. There was no amplification with 36 (17.6%) samples. There was no difference in fungal loads at D0, D7, and D14 between serotype A and B/C with the QSP1 qPCR assay, and QCC. QCC showed a good correlation with qPCR quantification with QSP1 qPCR (slope = 0.797, R2 = 0.73) and with 28S rRNA qPCR (Slope = 0.771, R2 = 0.778) assays. The fungal load at D0 was significantly higher in patients who died at week 2 (w2) and at week 10 (w10) as compared with patients who survived post-week 10 ($P < .01$), with no significant difference in initial fungal load in both treatment regimens ($P > .05$). Detection of *Cryptococcus* DNA (28S rRNA qPCR) in plasma or whole blood within the first 24 h of treatment was significantly associated with early mortality at w2 and mortality at w10 ($P < .01$). QSP1 RT-qPCR showed that detection of DNA was due to viable fungal cells as the quantification of QSP1 whole nucleic acids was systematically higher (X2 to 5) than that of DNA.

Conclusion: Quantification of *C. neoformans* and *C. gattii* load in CSF and plasma at D0 is useful in identifying patients at risk of death and may be a promising tool for monitoring treatment response in the future.

S1.5a

Epidemiology of mycotic keratitis in developing countries

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S1.5 Mycotic keratitis, September 21, 2022, 11:00 AM - 12:30 PM

Mycotic keratitis (corneal infection due to a fungal etiology) is a well-recognized ophthalmological emergency warranting rapid initiation of specific antifungal therapy. However, the magnitude of the problem of mycotic keratitis in the community, especially in the Indian subcontinent and the developing world, is, perhaps, less apparent. A minimal annual incidence estimate of 1051, 787 cases (23.6/100 000 population [popln]) globally has recently been reported, with the highest rates being in Asia (33.9/100 000 popln, an absolute number of 939 895) and Africa (13.5/100 000; 75 196); if all culture-negative cases are assumed to be fungal, especially where the incidence of mycotic keratitis is known to be high, then the annual incidence would be about 1480 916 cases. A fungal etiology has been found to account for a very high proportion (> 45%) of microbial keratitis cases in countries in the Indian subcontinent. Countries where a fungal etiology accounts for >25% of microbial keratitis mostly tend to be the equator. Interestingly, the proportion of microbial keratitis patients with a proven fungal etiology shows a significant negative correlation with the gross domestic product per capita. Although it is clear that the most common fungal species are *Fusarium*, *Aspergillus*, and *Candida* species, marked regional variations in fungal etiology have been noted. It is important to realize that sensitivity of the culture of ocular fungal pathogens can vary, depending on the pathogen, as well as the competence of the testing laboratory. For some countries, multiple reports over time have been noted, with there being some evidence of an increasing trend in the proportion of all microbial keratitis cases being diagnosed as mycotic keratitis. Even in a single geographical location, cases of mycotic keratitis may be higher than the yearly average at certain times of the year, such as during the harvest or windy seasons, or when there is increased relative humidity. A disturbing statistic to note is that, in 8%-11% of patients with mycotic keratitis, the affected eye needs to be removed, representing an irreversible annual loss of 84 143-115 697 eyes. It is recognized that many people suffering from mycotic keratitis in rural distant communities never present to health care workers due to financial and other constraints. Hence, the actual number of people afflicted by mycotic keratitis, man-days lost due to the disease and during therapy, and reduced quality of life due to persistent disability (corneal scarring) in the Indian subcontinent and developing countries requires further study.

S1.5b

The burden of mycotic keratitis in West Africa

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S1.5 Mycotic keratitis, September 21, 2022, 11:00 AM - 12:30 PM

Background: Fungal infection of the cornea, known as mycotic keratitis, can cause permanent corneal scarring and perforation resulting in the loss of the eye. This paper reviews the prevalence and epidemiology of mycotic keratitis in different countries in West Africa to estimate its burden.

Methods: An exhaustive search of the literature was made on Google, PubMed, MEDfacts, Cochrane Library, and Web of knowledge using different sets of keywords, viz. mycotic keratitis, ocular fungal infection, West Africa, risk factor, prevention, etc.

Results: A study in Nigeria over a period of 4 years (1974-1977) dealt with 42 confirmed cases of mycotic keratitis with *Fusarium solani* as the predominant etiological agent (14 cases) followed by *Penicillium citrinum* (8 cases) and *Aspergillus fumigatus* (5 cases), *Candida* spp (3 cases). The remaining 12 cases were that of *Fusarium moniliforme*, *Aspergillus* spp, *Penicillium* sp, and *Cladosporium* spp. The predisposing factors identified were trauma from palm tree leaves, thorns, kernels, or other plant objects, mechanical tools, and frying oil. A 10-year review (2003-2012) of 152 cases of corneal ulcers at the University of Calabar Teaching Hospital, Calabar, Nigeria revealed only 2 (2.9%) cases due to *Aspergillus* sp, many patients in this study were farmers. Other studies from Nigeria only mentioned the prevalence of keratitis without any mention of fungal etiology. Of the two studies from Ghana, the one conducted in 1999 showed the predominant agents *Fusarium* spp. (52.3%) and *Aspergillus* spp. (15.3%), in the other one conducted in 1999-2001, these agents were represented by 42.2% and 17.4% respectively. In another prospective study of suppurative corneal ulcers in 290 cases in Ghana (June 1999-May 2001), the etiological agents identified in culturally proven 77 (85.5%) cases of mycotic keratitis were *Fusarium* spp-46, *A. flavus*-9, *A. fumigatus*-7, *A. niger*-1, *A. nidulans*-1, and *Aspergillus* sp-1 A Siera Leonian study of cases of suspected infectious ulcerative keratitis from January 2005 to January 2006 detected 35.6% of mycotic keratitis and 13.7% of mixed fungal and bacterial etiology. A study on the burden of serious fungal infections in Togo mentioned an annual incidence of 951 cases of mycotic keratitis but no details of fungal etiology were mentioned.

Conclusion: Investigators have estimated the annual global incidence of fungal keratitis at over 1 million cases. Reports of cases reported from some countries represent only a tip of the true burden of mycotic keratitis in West Africa. There is a need for comprehensive surveys (involving collaboration between ophthalmologists and microbiologists) of mycotic keratitis in representative communities in collaboration with primary health centers and hospitals in different countries. It should be possible to produce a combined antifungal antibacterial preparation for widespread and immediate prophylactic first-aid use after corneal trauma, especially in rural areas.

S1.5c

Proteomics in fungal keratitis research: a road map to personalized treatment

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S1.5 Mycotic Keratitis, September 21, 2022, 11:00 AM - 12:30 PM

Research becomes very significant and meaningful when it addresses a significant public health problem of a region. Fungal keratitis, ulceration of the cornea due to fungal infection, is one such serious problem. This infection that results in monocular blindness affects primarily the agrarian population and is considered to be a silent epidemic in India.

The current treatment for fungal keratitis is the topical application of antifungal drugs such as natamycin and voriconazole. Nearly 40% of the patients do not respond to this treatment and require corneal transplantation. Treatment with antifungal drugs is not a holistic approach as it addresses only killing the fungus. The exaggerated inflammatory response at the site of infection may be a crucial factor in the disease outcome. And, this is not taken into account during the treatment due to the lack of knowledge of the host response during the fungal infection. This was the starting point of our research in fungal keratitis—to understand the corneal immune response to fungal infection.

Using mass-spectrometry-based proteomics studies on a tear from keratitis patients, we identified that in response to the fungal infection, the complement and coagulation pathways were activated along with neutrophil-mediated defense responses, notably the neutrophil extracellular traps. These pathways and their cross-talk with each other were primarily responsible for the exaggerated immune response at the site of infection. We selected five tear proteins that were significantly altered and validated them to serve as indicators of the inflammatory status of the ulcer in keratitis patients. Further, we developed a predictive logistic regression model that incorporates tear biomarker levels and ulcer characteristics to identify the subset of patients who are unlikely to respond to the antifungal treatment. We are currently exploring the possibility of using tear-derived EVs or their cargo as adjuvant therapy to modulate the inflammatory response in these non-responder patients.

Through our efforts using proteomics approaches, we now have five tear proteins as indicators of the inflammatory status in keratitis patients. These proteins along with the clinical features can identify the subset of patients who are unlikely to respond to antifungal treatment. Additionally, we showed that keratitis patient tear-derived extracellular vesicles are enriched with proteasomes. As proteasomes have an established role in immune modulation, EVs with proteasomes are thus promising candidates for adjuvant therapy, which we are currently exploring. Thus, our journey of over a decade of research on fungal keratitis started with the basic research to understand the host response that in turn provided the leads for translational research, which is now advancing towards personalized treatment for these patients.

S1.5d

Spectrum of etiological agents of mycotic keratitis: A n 11- year review

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S1.5 Mycotic Keratitis, September 21, 2022, 11:00 AM - 12:30 PM

Background: To assess the spectrum of etiological agents of mycotic keratitis over an 11-year-period at a tertiary eye care center in southern India.

Methods: A retrospective review was made of microbiological data relating to corneal scrapings performed over a period of 11 years (January 2011-December 2021) on 1200 individuals who presented with suspected microbial keratitis. Each individual underwent corneal scraping and the scraped materials were subjected to meticulous microbiological analysis that included direct microscopy (Gram-stain and lactophenol cotton blue wet mount) and culture on multiple solid and liquid culture media.

Results: A total of 404 fungal isolates were recovered from the corneal scrapings of 1200 patients with suspected microbial keratitis. Of the 404 fungal isolates, *Fusarium* spp (133) were the predominant isolates, followed by *Aspergillus* (*Aspergillus* (*Aspergillus* spp (24), *Aspergillus fumigatus* (17), *Bipolaris* sp (7), *Alternaria* sp (2), *Colletotrichum* sp (2), *Cylindrocarpum lichenicola* (2), *Exserohilum* sp (1) and *Drechlera* sp (1); there were also 111 filamentous fungus isolates that defied identification in spite of various efforts made to induce sporulation. Of the 404 culture-proven cases of mycotic keratitis, 381 patients confirmed ocular trauma while engaged in agricultural activity.

Conclusion: *Fusarium* spp., followed by *Aspergillus* spp., were the most common organisms found in mycotic keratitis patients in this specific geographical area. Additional efforts are required to spread awareness among villagers about the dangers of not promptly treating mycotic and other forms of microbial keratitis so that blindness and visual disability caused by corneal scarring in rural areas can be reduced.