Results: We observed that H. capsulatum has the capability to adhere and internalize within these HSCs; nonetheless, this process did not affect the survival of the fungus. The interaction of H. capsulatum with HSCs induced a significantly increased expression of TLR2 and Dectin-1 but not TLR4. In addition, this fungal interaction significantly induced an augmented expression of IL-6, IL-1β, IL-10, IL-17, TNF-α, TGF-β, as well as the immune mediators Arg-1 and iNOS. Interestingly, blockade of these receptors significantly decreased the phagocytosis process as well the expression of all inflammatory mediators evaluated, especially when blocking TLR4 and Dectin-1. Of note, H. capsulatum induced apoptosis but did not inhibit the proliferation of these stem cells.

Conclusions: These results indicate that HSCs are capable of phagocytosing *H. capsulatum* but do not affect its survival; moreover, this fungal pathogen could induce changes in the expression of pattern-recognition receptors (PRRs), especially TLR2 and Dectin-1, and could subsequently activate the HSCs leading to the expression of inflammatory mediators as well as affecting the viability of these stem cells. Altogether, these findings indicate that *H. capsulatum* could affect the hematopoiesis process as reflected in an increase or decrease in leukocytes, erythrocytes, and platelets as observed in patients with severe and disseminated disease, especially in those with dissemination to bone marrow.

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Cytokine gene Polymorphism in superficial *Malassezia* associated skin diseases

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives:

ü To isolate and characterize *Malassezia* species from patients of Pityriasis Versicolor (PV), Atopic Dermatitis (AD), Seborrhoeic Dermatitis (SD), and healthy controls.

ü To study single nucleotide polymorphism in IL-10 and IFN- γ genes of the host and its relation with susceptibility to *Malassezia* infection.

Methods: It was a prospective observational study done in University College of Medical Sciences and GTB Hospital, Delhi. Sample size comprised of 38 cases each of AD. Skin scrapings were used for fungal culture on Sabouraud Dextrose Agar (SDA) and Modified Dixon Agar (MDA) and isolates were identified as per conventional phenotypic methods. Genomic DNA was extracted from blood samples and Cytokine genotyping was carried out by Amplification Refractory Mutations System-Polymerase Chain Reaction (ARMS-PCR) with sequence-specific primers. Three SNPs (IL10-1082A/G; IL10-819/592C/T; IFN-g +874A/T) in two cytokine genes were assessed in all the patients and healthy controls. Chi-squared Test or Fisher's-Exact Test and Bonferroni's correction were used for statistical analysis

Results: *Malassezia* yeast was isolated in 94.7%, 63.1% each and 52.6% in PV, AD, SD, and healthy controls respectively. *Malassezia globosa* was the most commonly isolated species from both patient and healthy control. *Malassezia sympodialis* was the second most common followed by *M. furfur* and *M. restricta*. Association between specific cytokine gene polymorphism and clinical outcome was found to be significant in PV, AD, and SD group. IFN-γ +874 T allele and IFN-γ +874 A allele were significantly associated with PV and AD respectively. IFN-γ +874 AA genotype frequency was found to be higher in PV and AD patients than in controls. This finding suggests that PV and AD patients may produce a lower IFN-γ. IL10-819/592 C/T alleles were also significantly associated with PV. IL10-819/592 CT genotype frequency was found to be lower, and CC genotype frequency was found to be higher in PV patients as compared to healthy controls, suggesting that IL10 production may be higher in PV patients. AD patients were more likely to carry the IL10–1082 G allele and, it was significantly associated with this disease. Moreover, IL10-1082 AG genotype was significantly associated with AD and SD, which corresponds to high production of IL10.

Conclusion: The identification of *Malassezia* yeast to a species level is of a great importance to determine which species are implicated in certain skin diseases.

The use of phenotypic methods for identification of *Malassezia* species is a reliable, easily executed method, that is also inexpensive. Molecular methods are necessary to decrease the turnaround time, especially for slow-growing *Malassezia* species.

Cytokine gene polymorphism studies in IL10 and IFN γ genes demonstrated susceptibility of host to *Malassezia* infections. Comparison with the serum cytokine levels will help in understanding the evolution of *Malassezia* infections in susceptible host.

Population genetics studies require inclusion of a larger number of subjects to evaluate the probability or the frequency of occurrence of the genotype.

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Chronic pulmonary aspergillosis in a Tuberculosis c enter of Assam—a 2-year study

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Objectives: Chronic pulmonary aspergillosis is a slow and progressive lung disease that occurs in apparently immune-competent patients. Pulmonary tuberculosis is considered to be a major predisposing factor. Early diagnosis of the condition can improve morbidity and mortality. As the number of studies to detect this disease in India is sparse, this study was undertaken in District Tuberculosis center attached to Assam Medical College and Hospital, Dibrugarh to find out the occurrence of chronic pulmonary aspergillosis in treated pulmonary TB patients.

Methods: This prospective hospital-based cross-sectional study was done in Assam Medical College and Hospital, Dibrugarh from December 2015-November 2017. Every consecutive pulmonary TB case after completion of intensive therapy attending the outpatient department with respiratory symptoms was included in the study. Cases were identified with clinical, serological/mycological, and radiological criteria.

Results: A total of 26 cases of CPA were confirmed out of 211 enrolled cases. Positivity rate of CPA in the enrolled patients was 12.3% (95% CI 8.19-17.51%). The hospital-based occurrence rate was found to be 6/1000 (95% CI 3.8-8.5) in the outdoor attendants in DTC, Dibrugarh. Most of the cases (88.46%) were late cases presenting with aspergilloma. Majority (51.72%) of the aspergilloma cases were detected within 5 years of diagnosis of PTB. Very few (15.38%, $n = 4$) early cases were detected. The sensitivity of the ELISA test (DRG, Germany) was found to be 72.72% as compared with reference Immunocap method used in this study. A section (37.90%) of the patients with fungal ball was seronegative by *Aspergillus fumigatus* serological test. Two of them were positive for *Aspergillus flavus* in sputum culture.

Conclusion: A serological test to detect *Aspergillus flavus* IgG may complement the *Aspergillus fumigatus* IgG test in detecting CPA. CPA is a neglected disease with a high morbidity and mortality. Routine follow-up protocol for the cavitary patients in tuberculosis center by a sensitive serological test for a minimum period of 5 years may be necessary for early detection of cases in PTB high prevalence countries.

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Optimization of MTT assay protocol in *Pythium insidiosum* zoospores incubated together with neutrophils

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Objectives: The tetrazolium-based MTT assay is widely used to assess cell viability. This colorimetric assay measures cell viability through the enzymatic reduction of MTT, a yellow water-soluble tetrazolium dye, to purple-colored formazan crystals. The zoospores of *Pythium insidiosum* are the infective form causing pythiosis. There remains a lack of standardized protocol for *P. insidiosum* zoospore viability assay. Here, we optimized the MTT assay to assess the activity of neutrophils on zoospores.

Methods: Neutrophils and zoospores were incubated for 2 h, 4 h, and overnight at 37°C. Neutrophils were lysed in water (pH = 11), MTT was added to each well and incubated at 37°C for 2 h. Then, SDS-HCl solution was added to each well, and absorbance at 570 nm was measured using a microplate reader. The percentage of viable zoospores was then determined.

Results: The viable zoospores metabolized MTT to insoluble purple formazan product. The zoospores germinated into hyphae when incubated for >2 h. Because of the rapid germination of zoospores into hyphae, reliable results would be obtained with an optimum incubation time of 2 h for the zoospores.

Conclusion: The simplicity and low cost of the assay make it a valuable tool for zoospore viability studies, with a focus on immunology and immunotherapy.

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Post-COVID-19 recurrent fungal urinary tract infections: A case series

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Background: COVID-19 has opened a pandora's box of opportunistic infections and immune alteration. We hereby present a series of patients with recurrent fungal urinary tract infections triggered by an episode of COVID-19.

Description: Case 1: A 66-year-old male, diabetic, known chronic kidney disease, had moderate COVID-19 in September 2020 needing remdesivir and steroids; 2 months later, he was treated for a fungal UTI elsewhere. In May 2021, he presented with right-sided pyelonephritis, hydroureter, and pyonephrosis. DJ stenting was done and cultures grew *C. tropicalis*. Record of anti-fungal susceptibility from the fluconazole resistance and was hence treated with 21 days of micafungin with significant improvement. Despite 3 weeks of directed anti-fungal treatment, he had recurrent episodes of candiduria with azotemia. Cystoscopy and selective sampling yielded *C. tropicalis* from the right pelvi-calyceal system, but as he was unwilling for nephrostomy and local antibiotic instillation he was started on anidulafungin and long-term 5-flucytosine suppression.

Case 2: A 61-year-old male, diabetic, with uncontrolled sugars, had severe COVID-19 in September 2020 needing oxygen, remdesivir, and steroids; 1 month later, presented with right-sided flank pain and four episodes of painless passage of fleshy tissue per urethra in the last 1 year (Fig. 2). He had multiple episodes of bacterial UTIs which were treated, with complete resolution. The fleshy mass showed septate fungal elements and culture grew *Aspergillus flavus* sensitive to voriconazole, itraconazole, and posaconazole. He received multiple prolonged courses of voriconazole and caspofungin prior to presenting to us. CT revealed right-sided pyelonephritis with dilated pelvi-calyceal system. The patient was started on 2 weeks of micafungin followed by a prolonged course of voriconazole and 5-flucytosine with close clinical and therapeutic drug monitoring.

Case 3: A 68-year-old female, diabetic, had moderate COVID-19 in April 2021, and was given remdesivir and steroids. She was admitted 1 month later with UTI (Pyelonephritis with early forming renal abscess) with urine culture growing CR Klebsiella p. (OXA-48) and *C. glabrata* for which she was given ceftazidime-A, vibactam, and voriconazole and underwent bilateral DJ stenting followed by stent removal after 1 month along with switching to micafungin in view of repeated candiduria on treatment. She has developed multiple UTIs in the subsequent months with *C. auris* being isolated twice and bacterial UTIs, treated with antibiotics and micafungin. Subsequent CT revealed retroperitoneal fibrosis encasing the ureters causing obstruction, a biopsy was inconclusive but was empirically started on methotrexate for IgG4 disease.

Discussion: The proposed immune alteration mechanism of COVID-19 of decreased phagocytic function, uncontrolled sugars, and steroid-related neutrophil dysfunction predisposes to several opportunistic infections including fungal infections. It is intriguing that these patients with refractory funguria never underwent any instrumentation The challenges associated with the management of these cases included deranged renal functions precluding the use of intravenous contrast for imaging and several anti-fungal drugs; inadequate urinary penetration of antifungals especially with deranged renal functions and hesitancy with local instillation of antibiotics, and the need for repeated surgical intervention including insertion of stents in an infected urinary tract.