therapy. Biofilm was detected in 3 isolates one of which was from a patient with re-current cryptococcal meningitis but the association is not statistically significant.

Discussion: Non-adherence to HAART among HIV patients is known to increase the rate of hospitalization and mortality. Recurrence of cryptococcal meningitis has been thought to be due to drug resistance. But Illnait-zaragozi MT et al. based on STR typing technique, concluded that recurrence was due to co-infection with different strains or strains genetically modified during the long maintenance therapy. Biofilms produced by bacteria as well as fungus have been associated with more stubborn, recurrent, and persistent infections, especially among the immunocompromised population. Although biofilm production was detected in only one out of 3 isolates from recurrent cryptococcal meningitis, it may be a contributing factor along with non-adherence to treatment.

Conclusion: Non-adherence to ART and/or antifungal therapy is an important cause of relapse of cryptococcal meningitis. Biofilm production may be responsible for recurrence, especially among non-adherent patients. Further studies with a larger sample size may shed more light on the association between biofilm formation and recurrence.

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Diagnostic performance of MucorGenius® real-time polymerase chain reaction assay on tissue samples for the diagnosis of mucormycosis

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Objectives: To assess the diagnostic utility of MucorGenius® real-time PCR in tissue samples for the diagnosis of mucormycosis in patients suspected of having invasive mucormycosis (IM) during the second wave of the COVID-19 pandemic.

Methods: A total of 193 clinically suspected cases of IM presenting at our tertiary care center from May to July 2021 were included and defined as proven, probable, possible, or negative for invasive fungal disease (IFD) according to EORTC/MSGERC guidelines. One sample from each patient (nasal/sinus biopsy, nasal crust, or orbital tissue) was subjected to conventional methods for diagnosis of IM and MucorGenius® real-time PCR (hereafter called 'the assay').

Results: A total of 5 (1.92%), 124 (47.6%), and 44 (16.9%) cases respectively were classified as having proven, probable, and possible IM. The remaining 20 (7.69%) were classified as not having invasive fungal infections and were used as controls to calculate the specificity of the test. The majority of cases were classified as 'probable' because specimens received included biopsy from the nasal or sinus cavity.

According to radiological findings, sino-nasal involvement was seen in 26/173 (15.02%), sino-orbital involvement in 122/173 (70.5%), and additional intracranial extension in 25/173 (14.4%) of the 173 cases of IM.

Among 129 proven and probable cases, direct microscopy of samples showed only aseptate hyphae in 70 cases, and both aseptate and septate hyphae in 36 cases; the assay was positive in 53 and 13 of these cases respectively. In the remaining 23 cases, direct microscopy of samples showed only septate hyphae and the assay was negative.

Additionally, the assay was able to detect the presence of Mucorales among 44 possible cases of IM in which direct microscopy of samples showed no fungal elements, but the patients displayed clinical and radiological features of IM and improved with antifungal therapy.

The overall sensitivity and specificity of the assay were 63.21% and 90.48% respectively.

The sensitivity of the assay in proven and probable cases of IM was 60% and 66.7% respectively, while specificity was 90% for both, using the presence of aseptate hyphae in direct microscopy as a gold standard. Sensitivity and specificity in possible cases were 27.27% and 90% respectively, using the presence of clinical and radiological features of IM and response to antifungal treatment as a reference.

When sensitivity and specificity were determined independently in cases of mucormycosis and mixed infection (mucormycosis + aspergillosis), they increased to 75.71% and 90.48% respectively in the former, and both decreased to 38% in the latter.

Conclusion: The MucorGenius® real-time PCR performs well in detecting IM as a single infection, especially in cases of possible IM which are not detected by conventional methods. However, it is inefficient in detecting co-infections of invasive mucormycosis and aspergillosis, possibly because *Aspergillus* can suppress the growth of Mucorales. With further studies using the results to guide clinical intervention and measuring the impact on the outcome, it can be a useful tool to make an early diagnosis of mucormycosis in patients with a high index of clinical suspicion.

P406

Rise in HIV negative Cryptococcal infection in liver disease patient: epidemiology, risk factor, antifungal susceptibility profile from tertiary care hepatobiliary center

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: *Cryptococcus neoformans* is an encapsulated yeast found ubiquitously in the environment, considered important in immunocompromised individuals with HIV. Non-HIV susceptible groups include malignancies, long-term use of corticosteroids, solid organ transplantation, sarcoidosis, immunosuppressive therapy, and cirrhosis. Cryptococcal infections are rising in HIV-negative cirrhotic patients, accounting for 6-21% of the systemic infections. Chronic liver disease (CLD) is a neglected risk factor for fungal infections. To analyze epidemiology, risk factors, and antifungal susceptibility profile of HIV-negative liver disease patients.

Material Methods: The retrospective study was conducted in tertiary care hepatobiliary center in New Delhi, India from January 2017 to March 2022 after ethical approval. Demographic data, laboratory data, and clinical history of the patients admitted with liver disease and suspected cryptococcal infections were obtained from Hospital records for epidemiology and risk factor analysis. Blood, CSF, serum, urine, and body fluid (Ascitic fluid, peritoneal fluid, pleural fluid, MiniBAL) samples were processed for fungal culture on SDA and SDA with cyclohexamide. Blood and body fluid were also inoculated in an automated blood culture system (BacT/ALERT 3D bioMérieux). Samples were processed for gram stain, India ink, and Cryptococcal antigen. Identification was done by an automated identification system (VITEK 2 compact bioMérieux). The micro-broth dilution method (Sensititre YeastOne colorimetric plate, Thermo Fisher Scientific, MA, United States) was used to determine the susceptibility of all *Cryptococcus* strains to the six antifungal drugs, fluconazole, 5-fluorocytosine, amphotericin B, itraconazole, posaconazole, and voriconazole. The results were reported as wild-type (WT) or non-wild-type (non-WT) in accordance with the epidemiological cutoff value (ECV) set for *Cryptococcus* spp. (Espinel-Ingroff *et al.,* 2012a, b; CLSI, 2018).

Results: We analyzed 30 patients of suspected cryptococcal infection and obtained 40 *C. neoformans* isolates from different samples from these patients. Out of 40 samples, C *neoformans* was isolated from blood (25,62.5%), urine (6,15%), ascitic fluid (4,10%), mini-BAL (2,5%), bone marrow (1,2.5%), CSF (1,2.5%), pleural fluid (1,2.5%). All samples were positive for Cryptococcal antigen. India ink positivity was observed in 56%. Alcoholic liver disease was the most common risk predictive factor seen in 30% of patients. Hepatitis B and C-associated with CLD was seen in 20%. Other risk predictive factors were AKI (70%), diabetes mellitus (20%), TB (10%), autoimmune hepatitis (6.6%), autoimmune disease (autoimmune hemolytic anemia, Sjogren syndrome) (6.6%), Sarcoidosis (3.3%), Hepatocellular carcinoma (3.3%), HIV (3.3%). Hepatic encephalopathy was seen in 70% of patients which mimics the symptoms of *C.* meningitis.7.5% (3/40), 5% (2/40), 2.5% (1/40), 7.5% (3/40), and 2.5% (1/40) of *C. neoformans* strains were non-WT to fluconazole, 5-fluorocytosine, amphotericin B, posaconazole, and itraconazole, respectively, but all strains were WT to voriconazole. Overall mortality was 66.6%.

Conclusion: Above study shows that liver diseases are an important risk factor for cryptococcal infection owing to immunosuppressed state, use of steroids, and associated comorbidities like DM, TB, AKI, autoimmune disease, Sarcoidosis, hepatocellular carcinoma. Therefore, it is necessary to investigate and be vigilant about the isolation of *Cryptococcus*. The use of appropriate antifungals can improve clinical outcomes. As there is increasing resistance to amphotericin B, 5-fluorocytosine, azoles; antifungal susceptibility testing should be done. Cryptococcal antigen detection can be useful in non-CSF samples as well.

P407

Standardization of PCR for the diagnosis of invasive aspergillosis and invasive mucormycosis from blood samples

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: (1) Standardization of PCR-based diagnosis of invasive aspergillosis and invasive mucormycosis in spiked blood samples. (2) Standardization of PCR-based diagnosis of invasive aspergillosis and invasive mucormycosis in proven cases. (3) Validation of the above-standardized protocol in patients with proven/probable invasive aspergillosis or mucormycosis.

Materials and Methods: Blood samples were collected from healthy volunteers and patients with proven mucormycosis, proven invasive aspergillosis, and suspected invasive fungal infection. Standard strains of Mucorales and *Aspergillus* spp. were collected from the National Culture Collection of Pathogenic Fungi, PGIMER and DNA was extracted using a modified protocol of the fungal DNA extraction method by Lee and Taylor (1990). Mucor-specific primer (ZM13), described by Bialek R et al. (2005) was used for mucormycosis samples. *Aspergillus* specific primer pair was designed from mitochondrial DNA of A. *fumigatus* using Primer3 software. Genomic DNA was used to determine the optimum annealing temperature and concentration for ZM13 assay. DNA extracted from whole blood samples of healthy volunteers after spiking with a known quantity of A. *fumigatus* DNA and only genomic DNA was used separately to optimize annealing temperature and concentration of *Aspergillus*-specific primers. Similarly, whole blood, plasma, and serum samples from healthy volunteers were spiked with various concentrations of *Rhizopus arrhizus* and *A. fumigatus* DNA. DNA extracted from spiked samples was used to determine the analytical sensitivity of PCR assays. DNA extracted from blood samples of ten proven mucormycosis and five invasive aspergillosis patients were used to determine the optimum sample dilution factor and template volume for the assay. The final standardized protocols were validated using 28 proven/probable cases of invasive aspergillosis and invasive mucormycosis and thirty cases of suspected invasive fungal infection.

Results: The optimum annealing temperature and primer concentrations for the assay were 54.3°C and 0.4μM respectively for ZM13 assay and 53°C and 0.45μM respectively for *Aspergillus* assay. The limit of detection was 0.06fg/μl for ZM13 assay and 0.08fg/μL for *Aspergillus* specific assay. For mucormycosis assay, the slope of the standard curve was −3.2687 and the percentage efficiency of the assay was 104% with 0.9936 as the coefficient of determination (R2). Likewise, the slope of the standard curve was −3.2695, the percentage efficiency of the assay 102%, and the coefficient of determination (R2) 0.9858 for aspergillosis assay. The best combination of template volume and sample dilution factor was 2.5 μl and 1000x in whole blood samples and, 2.5 μl and 100x in serum samples for mucormycosis assay. It was 1.0 μl and 10x in serum samples for *Aspergillus* specific assay. The sensitivity, specificity, positive predictive value, and negative predictive value of mucormycosis qPCR using serum samples were 79.3%, 86.4%, 65.7%, and 92.7% respectively. The assay had a positive likelihood ratio of 5.92 and a negative likelihood ratio of 0.24.

Conclusion: The real-time PCR protocols standardized for the diagnosis of mucormycosis and invasive aspergillosis from blood samples showed promising results. However, the assay needs further validation in a larger study population.