Fungal Infections Study Forum

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A DUAL INVASIVE FUNGAL INFECTION FOLLOWING COVID-19

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An 81year old gentleman with chronic atrial fibrillation was admitted to our hospital with chief complaints of fever with chills fortwo days, urinary urgency, irrelevant talk and progressively increasing breathlessness. He was admitted elsewhere, one month before, with an acute right middle cerebral artery territory infarction, for which he was thrombolysed with alteplase. On admission for the stroke, his nasopharyngeal swab for SARS-CoV2 RT-PCR was found to be positive. He required supportive oxygen and received Remdesivir and IV steroids in the course of hospitalization. He was discharged on anticoagulants, antiplatelets, amiodarone and a prolonged tapering dose of oral steroids.Post-discharge he had a dry cough which had worsened over thepast week.

During the current admission, he was drowsy, tachypneic, hypoxemic and initially needed oxygen support of twoliters with nasal prongs. There were scattered crackles throughout his chest on auscultation. His chest X-ray revealed patchy reticular shadows. (Figure 1). Routine laboratory investigations, blood and urine cultures were sent and an urgent diagnostic bronchoscopy and lavage was performed. The patient was empirically started on meropenem, caspofungin and IV methylprednisolone by the primary team.High-resolution computer tomography (HRCT) of the chest showed confluent ground glass and consolidative opacities in both lungs and some nodules. There was evidence of subtle smooth septal thickening and ill-defined nodularity bilaterally. Relative sparing of subpleural parenchyma was noted.(Figure 2). Other investigations wereremarkable for anaemia (hemoglobin 9.6 g/dl), raised d-dimer levels (4.62 mg/l) and raised lactate dehydrogenase levels (745 U/L). C-reactive protein was raised (96 mg/L).Blood and urine cultures were negative.

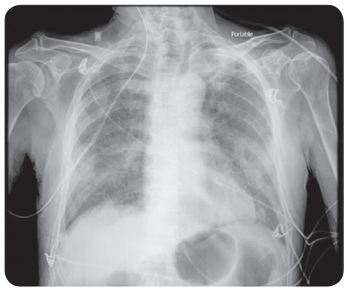

Figure 1

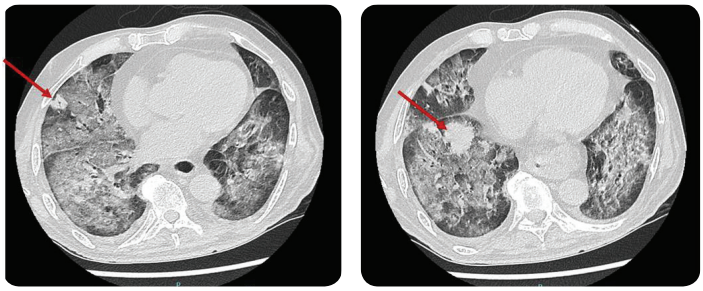

Figure 2a and 2b: Confluent ground glass shadows with nodules (Arrow)

Message from the Editor

Dear Friends

Presenting here the 2nd newsletter of the year a bit late though. All of us are aware of the increased risk of mucormycosis, invasive aspergillosis and candidiasis following COVID-19. However other fungi have also used this profound immunosuppressed state due to COVID-19 and use of immunomodulatory drugs to their advantage. In this newsletter we present four complex cases of serious invasive fungal infections following COVID-19 namely *Pneumocystis jiroveci, Histoplasma capsulatum, Fusarium solani* and *Cryptococcus neoformans*. Hope you find them interesting and are able to take home messages for your own practice. There is also a quiz in the end to test your diagnostic skills in mycology.

The FISF is actively engaged in research on COVID-19 related fungal infections. The second COVID associated mucormycosis study is ongoing, The FISF is also collaborating with Academy of Pulmonary Sciences for guideline development for COVID associated Pulmonary mucormycosis. Finally, FISF has set up an Fungal Registry for invasive fungal infections the details for which are mentioned below

It has been a tumultuous year and hopefully we will see an end to COVID-19. Looking forward to meeting some of you in person next year.

Editor

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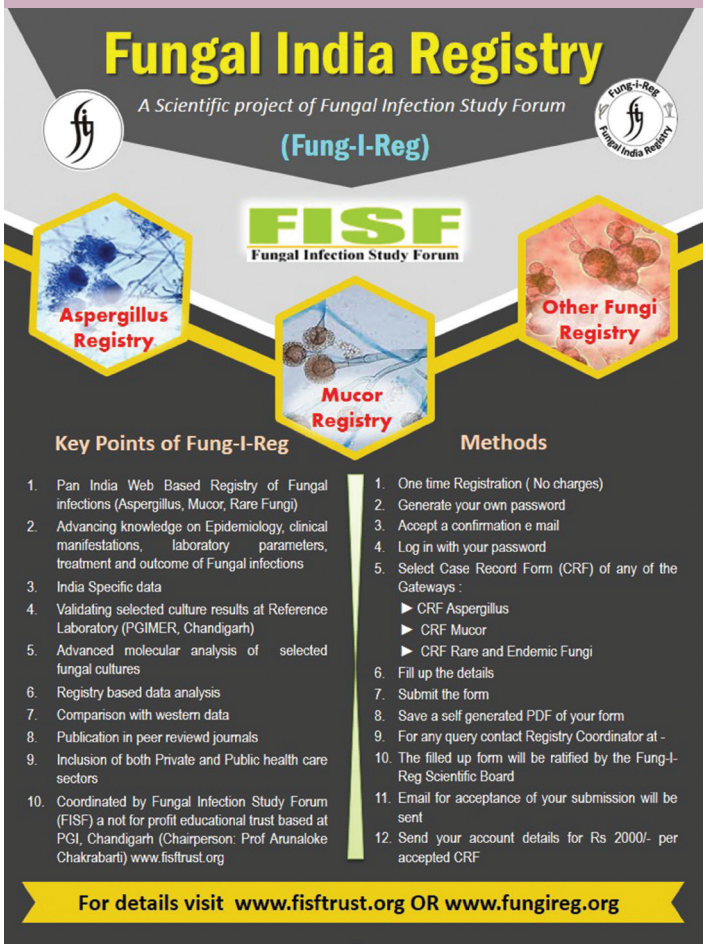

Serum Beta-D- glucan (BDG) levels were strongly positive (> 523 pg/ml).An infectious diseases reference was sought at this stage. Empiric TMP-SMX was added giventhe strong clinic-radiological suspicion of *Pneumocystis jirovecii* pneumonia (PJP). The BAL PCR for *Pneumocystisjirovecii* was also positive thus confirming the diagnosis of PJP. However, BAL galactomannan wasalso be positive with a value of 1.356. In view of the presence of nodules in the scan and a positive BAL galactomannan, the diagnosis of probable CAPA (COVID associated pulmonary aspergillosis) was made and injection caspofungin was substituted with voriconazole.Despite prompt initiation of treatment, the patient deteriorated rapidly with worsening respiratory failure and succumbed to his illness.

Discussion

COVID-19 has gained notoriety as a predisposing factor for various invasive fungal infections (IFIs) that pose diagnostic challenges and can be as fatal as the severe COVID-19 illness itself. The association of these IFIs withvarying severities of COVID-19 infection have led to the exploration of novel mechanisms and pathophysiology in this cohort. Patel et al reported uncontrolled diabetes, COVID-19 related hypoxemia and glucocorticoids asimportant risk factors in patients with COVID-19 associated mucormycosis[1]. The risk factors described in COVID-19 associated pulmonary aspergillosis (CAPA) patients include older age, lymphopenia, chronic respiratory diseases, corticosteroid therapy, antimicrobial therapy, mechanical ventilation or cytokine storm[2].Anecdotal reports of PJP have also been published over the past year in patients with severe/critical COVID-19 illness with associated risk factors of poorly controlled HIV with lymphocytopenia and multiple immunosuppressive therapies [3].

Given the importance of timely and appropriate antifungal regimens for these life-threatening infections,time is of the essence in making an accurate diagnosis.Our case highlights the significance of each step involved in the diagnostic process of IFIs, appreciating the various risk factors, radiological suspicion, the supportive role as well as the limitations of biomarkers like BDG in differentiation,dual infections and finally;the invaluable role of a promptly performed bronchoscopy in making a definite diagnosis.

The fatal fallouts of inappropriate prolonged steroid use in COVID 19, as seen in this case, cannot be over-emphasized.

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POST COVID-19 DISSEMINATED HISTOPLASMOSIS

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Case report

A 65 year old malehailing from rural area of Maharashtra presented to our hospital in January 2021 with a 2 month history of fever, marked weight loss, fatigue, swallowing difficulty and stomatitis. On evaluation after admission he was found to have pancytopenia, with severe abnormality in liver function testsand very high ALP and GGT. The HIV serology was negative. The primary unit suspected him to have malignancy & investigated further. The patient was also referred for an ID opinion.

On enquiry the patient had COVID-19 in September 2020 & hadreceived steroids for the same. He had papulo-nodular skin lesions some of which were umbilicated (Figure 1). Oropharyngeal swelling with ulceration was seen along with hepato-splenomegaly. The PET scan showed an adrenal lesion. (Figure 2)

In view of skin lesions, oral ulcers, pancytopenia, cholestatic hepatitis and

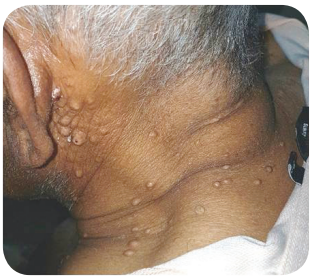

Figure 1

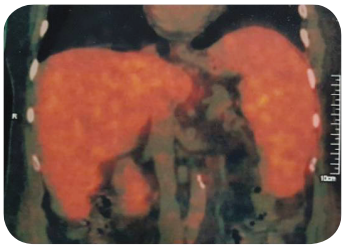

Figure 2

adrenal lesions a possibility of disseminated histoplasmosis was considered and the patient was subjected to a bone marrow and skin biopsy. Skin biopsy & bone marrow examination showed intra-cellular yeasts (Figure 3). The biopsies were culture positive and the fungus was identified as Histoplasma on the LPCB mount (Figure 4)

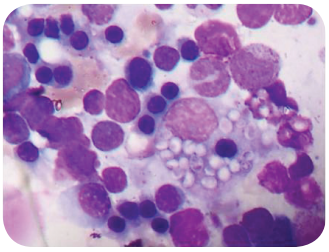

Figure 3

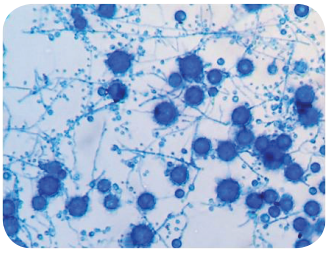

Figure 4

The patient was treated with 2 weeks of liposomal amphotericin B and later switched to posaconazole. Intravenous form was given for 2 days followed by the suspension and when he was able to take tablets orally, he was given oral posaconazole gastric resistant tablets. Therapeutic drug levels were achieved and the patient was discharged.

Discussion

Unlike mucormycosis, aspergillosis and invasive candidiasis, histoplasmosis is uncommonly described after COVID-19. Our index patient did not have any other risk factors for histoplasmosis apart from COVID-19 and steroids. Taylor et al have reported a 50 year old previously immunocompetent person from Ohio valley, USA who presented with disseminated histoplasmosis 2 weeks after severe COVID-19 pneumonia that was treated with remdesivir and 10 day therapy with dexamethasone (1).

Follow up therapy following the initial treatment with amphotericin B needs discussion. Itraconazolehas been the guideline- preferred -treatment. However a liquid preparation for optimal drug exposure is needed along with therapeutic drug monitoring (TDM). Fluconazole &Voriconazole both have lower genetic barrier to resistance due to Y136F mutation which is common with Histoplasma. While voriconazole hasbeen used in treatment of histoplasmosis, a retrospective cohort study showed increased mortality with voriconazole based therapy as compared to itraconazole (2). The long azoles itraconazole&posaconazolehave multiple binding sites to CYP51A & therefore have a higher barrier to resistance (3). PCZ has very low MIC but there is no outcome data to support its routine use.However it is considered as a reasonable alternative (4).

The case has several important messages to convey. First it is important to differentiate non-infectious & infectious possibilities in clinical practice. Secondly, COVID-19 is now so common that patients may not volunteer the history, which should be carefully elicited. Thirdly, the clinician needs to know

local epidemiology in order to suspect the diagnosis. Finally, the choice of drugs depends on guideline, available local preparations, TDM facilities & patient related factors.

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BREAKTHROUGH FUNGAL INFECTION IN AN IMMUNOCOMPROMISED PATIENT WITH SEVERE COVID-19

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Case report

This is a 17-year male with extramedullary granulocytic sarcoma who was started on induction chemotherapy with daunorubicin plus cytarabine (3+7) and antimicrobial prophylaxis with posaconazoleGR tablet and acyclovir. He developed febrile neutropenia with carbapenem-resistant *Klebsiella pneumoniae* bacteremiawhich was treated with ceftazidime-avibactam. However, a few days later, he developeddry cough and tachypnoea with bilateral infiltrates on chest X-ray. A nasopharyngeal swab for SARS-CoV-2 PCR was positive. He was shifted to the COVID ICU as he required high flow oxygen. Remdesivir and steroids were started. Multiple granulocyte and platelet transfusions were administered for prolonged cytopenia. Prophylactic posaconazole GR tablet was switched to liposomal amphotericin B as serial ECG's showed prolonged QTc intervals. His electrolytes were within range and no other QTc prolonging agents were used. Oddly thougha posaconazole level sent before discontinuation was found to be subtherapeutic.

A week later, while stillseverely neutropenic, he developed high-grade fever, multiple erythematous tender non-pruritic nodules on both extremities (Fig1), trunk and face along with bilateral leg pain. Important differentials includedcandidemia, disseminated mold infection, ecthyma gangrenosum and nocardiosis. A skin biopsy was inconclusive. Serum Beta-D-Glucan (BDG) was indeterminate (82) and Serum Galactomannan was negative at the time. Blood cultures grew CR *E.coli* (NDM) for which appropriate antibiotics were restarted. The skin nodules darkened with time.

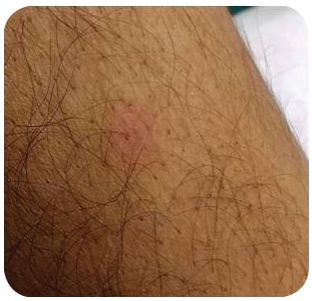

Fig 1 Skin nodule

With neutrophil recovery at D+35, the patient developed redness in the right eye followed by reduced vision, epiphora, and pain.CRP levels increased sharply, and a repeat S. BDGshowed an increase to 206. Anophthalmology opinion was sought, examination revealed a clear cornea with circumcorneal congestionand differentials considered included anterior uveitis, endophthalmitis and CMV retinitis. Plasma CMV viral load was undetectable, and the vitreous aspirate grew *Fusarium solanicomplex* (Fig 2,3,4).

Fig 2 White powdery Fusarium colonies

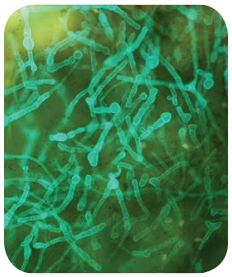

Fig 3 Calcoflour stain -Septate fungal filaments

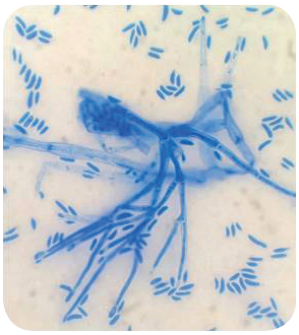

Fig 4-LPCB mount-Banana shaped macro-conidia

Systemic as well as localintravitrealvoriconazole therapywere started. Voriconazole trough levels were monitored. LAmB@ 5 mg/kg/day was continued as part of combination antifungal therapy in this profoundly neutropenic patient with disseminated fusariosis. He required a vitrectomy and lensectomy. His clinical course was complicated by frequent episodes of dyselectrolytemia, and one episode of *torsades de pointes*. However,an attempt to reduce the dose of L-AmB from 5 m/kg to 3mg/kg at D21 of therapy was met with new vitreal infiltrates. Despite repeated intravitreal antifungal instillations, aggressive attempts to continue an optimal antifungal regimen with close electrolyte monitoring and correction, he lost vision in the affected eye and developed phthisis bulbi. A relentless 49 day stay in the COVID isolation unit away from his family resulted in severe depression.

After six weeks of combination treatment, he was continued on Tab. voriconazole alone. A repeat PET scan at three months showed recurrence of the sarcoma after which the patient was transferred to another centerfor further treatment and was lost to follow up.

Discussion

The case discussed here highlights the important entity of breakthrough Invasive Fungal Infections(BIFI) in this vulnerable subset of patients with malignancy and COVID-19.

BIFI is defined as "any IFI occurringduring exposure to an antifungal drug, including fungi outside the spectrum of activity of an antifungal" (1). Breakthrough fungal infections usually occur due to inadequate antifungal levels, or due to organisms that are not covered by the prescribed antifungals,either as intrinsic or acquired resistance(2). Our patient may have developed fusariosis at the time when the posaconazole levels were sub-therapeutic. It is likely that the infection was suppressed but not eradicated byLiposomal Amphotericin Band that he developed a classical paradoxical exacerbation with neutrophil recovery.

Both LAmB as well as voriconazole have been used successfully as monotherapy for invasive fusariosis. Recent data reveals a trend towards better clinical outcomes with voriconazole. Variability in species-dependant in vitro susceptibility profiles, compounded by the poor prognosis of fusariosis especially inimmunocompromised hosts with disseminated diseasehave led to the recommendation of combination therapy with voriconazole plus terbinafine or LAmB as used in our patient.As with other rare opportunistic infections, randomised trials for evidence-based treatment protocols are not feasible and data on treatment outcome is anecdotal or at best restricted to retrospective case series. In an analysis of 233 cases of invasive fusariosis, a benefit of combination therapy over monotherapy was not observed (3). In a literature review of 97 patients done in Massachusetts General Hospital, the mortality rates of patients receiving combination therapy were higher than those of patients receiving monotherapy(Either L-AmB or Voriconazole). But the baseline characteristics were not matched, and it was an observational non-randomised study. Also, there could have been bias in choosing dual therapy for much sicker patients, which translated to high mortality4. Malignancy in remission, adequate neutrophil counts, and lack of significant (grade II or greater) graft-versus-host disease are considered as good prognostic factors (4,5).

Despite adhering to the composite checklist of a broad mould active prophylaxis, screening for drug toxicities, therapeutic drug monitoring, switching to an alternate agent when warranted, our patient went on to develop an unrelenting disseminated mould infection and lost vision in his right eye.Since the patient was immunocompromised, he couldn't be de isolated as per standard guidelines.

SARS-CoV-2 infection in severely immunosuppressed individuals poses myriad challenges for both patients and health care workers. Prolonged viremia requiring isolation, progression to severe COVID 19, immune exhaustion or iatrogenic immune suppression and the development of secondary opportunistic infections are a cascade of unending obstacles. Providing timely multi-disciplinary care and performing diagnostic or therapeutic interventions poses significant logistical difficulties as well. Most importantly, dedicated efforts to preserve the morale of these patients with the constant help of relatives and counsellors is essential to overcome the anxiety and despair that develops with prolonged isolation.

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TRIPLE TROUBLE: A MYCOBACTERIA, A VIRUS AND A FUNGUS

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Case report

This 54 year old male with preexisting diabetes and hypertension presented in October 2020 with history of fever since 1-2 monthswith imaging findings of multiple necrotic nodes in neck, mediastinum and abdomen. Empiric treatment with first line ATT was initiated with no response. He was then admitted to our hospital where a CT guided neck node biopsy was smear positive for acid fast bacilli. Xpert MTB/Rif ULTRA was positive for MTB with rifampicin resistance. Line probe assay showed resistance to rifampicin, INH resistance with Kat g mutation and no fluoroquinolone resistance. HIV serology was negative. He was initiated on all oral regime with linezolid,moxifloxacin, cycloserine, clofazimine and bedaquiline. Three weeks later he presented with sudden onset right hemiparesis and aphasia. Contrast MRI showed leptomeningeal enhancement and granulomas and infarct. The CSF was abnormal but Xpert was negative. The phenotypic susceptibility tests of the previous LN biopsy showed sensitivity to all 2nd line drugs as well as pyrazinamide and ethambutol. Anticoagulation, low dose aspirin,steroids(dexamethasone 8 mg thrice daily) and cotrimoxazole prophylaxis for PJP was started. He was discharged after a 3 week stay on tapering doses of steroids. ATT was continued with moxifloxacin replaced by levofloxacin due to the occurrence of QT prolongation.

Two weeks after discharge, the patient developed fever, cough and breathlessness. He presented to the ER with hypoxia (saturation of 86% on room air). Nasopharyngeal RT-PCR was positive for SARS-CoV-2. His absolute lymphocyte count was only 150 and CRP was 126 mg/l. Treatment with oxygen by NRBM at 15 liters/ minute, remdesivir was initiated and the ongoing oral dexamethasone was converted to IV methylprednisolone 30 mg twice daily. He progressively worsened and needed intubation, mechanical ventilation after 48 hours of admission. He also developed refractory thrombocytopenia because of which linezolid was stopped. Bedaquiline was also withheld. The patient was gradually weaned and extubated 2 weeks later. He was also given polymyxin B, meropenem and echinocandins for suspected secondary infections.Bedaquiline was restarted and the rehabilitation for the stroke reinitiated. Over the next 3 weeks the patient got better, steroids were tapered and stopped and discharged was planned.

However 6 weeks into the current admission, the patient again became drowsy. Relapse of TBM in view the ATT interruption and COVID related immunosuppression was suspected. A CSF examination was done which showed only 2 WBC but a full field of encapsulated yeast suggestive of Cryptococcus (Figure 1). The cryptococcal antigen test was positive by lateral flow assay in a titer of 1:640. Xpert MTB/ Rif was negative. Treatment with IV liposomal amphotericin B in a dose of 3 mg/kg/day and flucytosine in a dose of 100 mg/kg/day was initiated. CSF pressure was checked which was below 20 cm of H2O. The CSF culture grew *Crytpococcusneoformans* with fluconazole MIC of 2 and amphotericin B MIC of 1. Repeat CSF after 2 weeks of treatment continued to show full field of yeast on India Inksmear and was still culture positive. Cultures after 3 weeks of initiation of therapy were negative. However the India ink smear continued to remain positive. Flucytosine was stopped after 32 days as the patient developed neuropathy. Liposomal amphotericin B was stopped after 6 weeks and treatment switched to fluconazole @ 800 mg/day. However the patient continued to remain drowsy. He also had multiple other problems including refractory *C. difficile* colitis (which needed a fecal transplant), *Candida aurisandcarbapenem* resistant *Klebsiella pneumoniae* blood stream infection and finally a ventilator associated pneumonia with septic shock to which he succumbed. His total duration of hospital stay was 4 months.

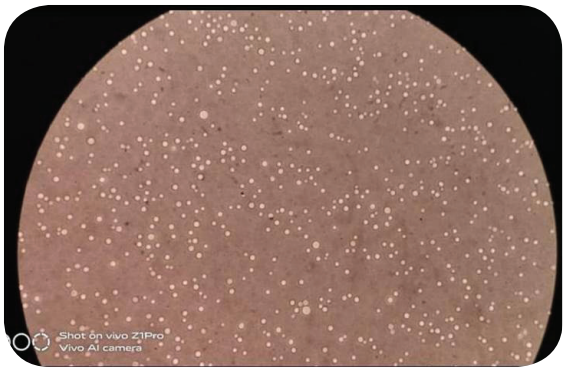

Figure 1: India Ink preparation showing the yeast with surrounding capsule

Discussion

This case illustrates the unfortunate journey of a middle aged man who fell from the frying pan into the fire not once but multiple times. The evolution of the MDR TB into TBM and subsequent dense hemiplegia interfered with mobilization later and contributed to significant morbidity. The steroids for TBM caused severe lymphopenia which was a risk factor for the serious COVID-19 disease. He was however fortunate to recover from COVID-19 (patient's who are ventilated for COVID-19 at our center have a mortality rate around 90%). And finally just as the situation was brightening up for him, he developed cryptococcal meningitis. The diagnosis of CM was clearly delayed since by the time the CSF was done the fungal burden was very high. Though the patient microbiologically recovered from the CM, he did not do so clinically and it was possibly the attributable cause for his mortality.

Unlike other fungal infections like aspergillosis and mucormycosis, cryptococcosis has been rarely reported with COVID-19. Till date there are only 3 case reports of cryptococcal disease in previously immunocompetent patients with COVID-19 (1-3). All patients had severe COVID-19 disease, two required mechanical ventilation, all three received steroids and two were also given tocilizumab. The outcomes were poor with one patient dying and the other two discharged to skilled nursing care facilities with tracheostomy and gastrostomy. Severe T cell dysfunction resulting from steroid therapy and COVID-19 itself were postulated as underlying factors.

Finally, it is important to consider the possibility of CM in a patient presenting with meningoencephalitis after COVID-19.

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QUIZ IN DIAGNOSTIC MYCOLOGY

Tanu Singhal, Rajeev Soman

1. Which of the following is true about diagnosis of invasive candidiasis (IC)
   a. A negative BDG rules out IC with 100% certainty
   b. A positive BDG confirms the diagnosis of IC
   c. A negative BDG is useful in decision making about stopping anti fungals
   d. Serial monitoring of patient with positive BDG can help in stopping antifungals early
2. Which of the following is true about invasive aspergillosis (IA)?
   a. The sensitivity of serum galactomannan (GM) in non-neutropenic patients for IA is around 20-30%
   b. The cut off of non-BAL galactomannan in CAPA (COVID associated pulmonary aspergillosis) is 4.5
   c. A cut off of BAL GM of 3 is highly specific for diagnosis of IPA
   d. All the above
3. Which is the most reliable method for diagnosis of mucormycosis?
   a. Histopathology and culture
   b. Calcoflor white stain
   c. PCR on tissue
   d. Fungal cultures
4. What is true about PJP?
   a. Serum BDG > 523 pg/ ml is almost diagnostic of PJP
   b. The sensitivity of PJP PCR is better than the immunofluorescent stain
   c. The actual Ct value of the PJP PCR is not helpful
   d. All of the above
5. Which of the following is the most sensitive method for diagnosis of cryptococcal meningitis?
   a. CSF India Ink stain
   b. CSF Cryptococcus PCR
   c. CSF Cryptococcal antigen detection
   d. CSF Fungal culture

Answers: 1c, 2d, 3a, 4b, 5c

About FISF

The purpose of the Fungal Infections Study Forum is to conduct educational activities, undertake epidemiological and clinical studies and to promote research activities on invasive fungal infections. The results of such research would benefit the clinicians, mycologists and the general public. The trust was formed in view of emergence of Invasive fungal infections (IFIs) in India which is posing a serious challenge to the haematologists, critical care providers, ID specialists, pulmonologists, neurologists, medical mycologists and many other clinicians handling serious and immunocompromised patients.The trust is the independent working consisting of clinicians and mycologists and instituted on 3rd March 2012 at Mumbai, India. To know more about us visit www.fisftrust.org.