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AN UNUSUAL CAUSE OF LUNG ABSCESS IN A PATIENT RECEIVING BIOLOGICS & IMMUNOSUPPRESSIVE DRUGS FOR ANKYLOSING SPONDYLITIS

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Introduction

The use of biological agents to treat inflammatory conditions and malignancies has remarkably increased over the past decade. However, biologic use can be associated with serious life threatening infectious complications. Duration of therapy on biologics determines the severity of immunosuppression. Generally it takes several months after starting biologics to produce lymphocytopenia and again several months for immune function to recover after stopping therapy. Mycobacterial infections, varicella infection and invasive fungal infections (IFI) are common infectious complications associated with TNF alpha inhibitors. This communication describes an invasive fungal infection due to an emerging fungal pathogen in a patient on biologics.

Case Report

A 40 years old male who had history of acute rheumatic fever in childhood, was diagnosed to have Ankylosing Spondylitis (AS) in 2011. Since then he was on regular follow up with a rheumatologist for AS. He was initially treated with NSAIDs and steroids. He required methyl prednisolone pulses intermittently due to disease flares. He had also received multiple biologics including infliximab (5 doses in 2014), etanercept (2 doses in 2014) and adalimumab (2 doses in February 2015). He was also receiving 7.5 mg Methotrexate once a week for last 2 years and recently was also prescribed prednisolone 10 mg once a day. He was diagnosed to have Diabetes in January 2015 for which he was started on metformin with HbA1C of 8.7. He was admitted to Sterling Hospital in February 2015 with bilateral multiple joint pains (small and large), abdominal discomfort, diarrhea alternating with constipation and dry cough, all for 10 days. Work up showed inflammatory bowel disease and was started on Mesalamine sachet 2 gm twice daily and was also advised 2 doses of Adalimumab 2 weeks apart.

He was readmitted at Sterling Hospital after two weeks with complaints of cough with yellow copious sputum, fever and breathlessness. His laboratory work up showed Hb- 8.8 gm%, WBC of 17000/ µl, DLC of 63/30/01/03/0, platelets of 397000/ µl and ESR 86 mm/hour. Biochemistry and electrolytes were within normal limits. CRP was 12 times upper level of normal. HIV/HBsAg were non reactive. 2D ECHO showed rheumatic heart disease, mild mitral stenosis, mild MR, PML mobility mildly restricted, normal EF, no PAH. USG abdomen showed oedematous terminal ileum/ ileocaecal junction/appendix, caecum and proximal ascending colon with max thickness of 4.2 mm with few regional mesenteric lymph nodes, largest 10X6 mm without necrosis or conglomeration.

CT thorax showed thick walled cavitatory lesion in middle and lower lobes of the right lung (Figure 1). Sputum was sent multiple times for direct microscopy. One of them showed 24-28 pus cells per L.P.F, few GPC in pairs and GNBs with occasional fungal hyphae with negative culture. BAL direct microscopy, cytology and GenXpert for MTB/Rif were negative. BAL culture was negative for TB, fungal and bacterial pathogens. Post bronchoscopy sputum showed fungal hyphae on direct microscopy and culture grew mycelial fungus, subsequently identified as Paecilomyces species (Figure 2). Transbronchial biopsy was reported as bronchiolitis obliterans organizing pneumonia (BOOP) with no evidence of fungal/mycobacterial infection in examined sample.

Patient was started on Voriconazole. He was readmitted after three weeks with increased cough and sputum, intermittent high-grade fever and gradually progressive breathlessness. Repeat HRCT thorax showed increase in the extent of thick walled cavitatory lesion involving even right upper lobe compared to previous HRCT thorax. He was started on Liposomal amphotericin B and voriconazole was continued. The patient was subsequently transferred to another hospital near his residence for further continuation of treatment. Thereafter he was lost to follow up.

Fig. 1: CT scan Thorax showing thick walled cavitatory lesion in Rt middle and lower lobe

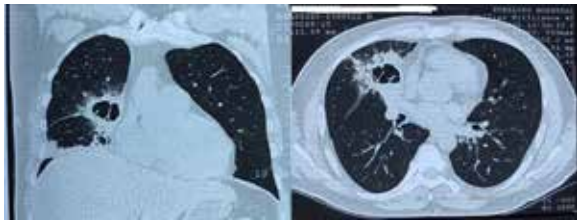

Fig. 2: LCB mount from isolate showing fungal hyphae

Dear Friends,

It gives me great pleasure to present to to you the 2nd newsletter of this year. In this newsletter we are discussing a very contemporary topic about the role of biomarkers in diagnosis of invasive fungal infections. These should be sent only in the setting of high pretest probability of an IFI, the sample should be collected carefully, tests sent to a standard lab and the results interpreted with caution. We also discuss the case of an immunocompromised host who develops a serious invasive fungal infection highlighting the immunosuppressive effects of biologics, emerging fungal pathogens and the limitations of currently available antifungal drugs against these pathogens.Finally we have a quiz that tests your "Fungal IQ"

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Discussion

TNF is also important for immune responses and host defense. It is also useful for granuloma formation and clearance of intracellular pathogens. Risk of infectious complications in patients receiving TNF-α factor inhibitor decrease if patient has not developed infection in the first two years of treatment.

Paecilomyces species are saprophytic fungi that commonly inhabit the air, soil, decaying plants, and food products. They are usually uncommon pathogens but sometimes can produce serious infections in immunocompromised patients.[1] *Paecilomyces lilacinus* and *Paecilomyces variotii* are the two species most frequently associated with human disease. Despite its apparently moderate virulence, *P. lilacinus* is able to infect both immunocompromised and immunocompetent hosts. The portal of entry of the fungus usually involves breakdown of the skin barrier, indwelling catheters or inhalation. *Paecilomyces variotii* is associated with many types of human infections, such as fungemia, endocarditis, peritonitis, and osteomyelitis.[1] Pneumonia & Lung Abscess due to *Paecilomyces variotii* has been rarely reported in medical literature. Except for one patient, all patients including ours have been immunocompromised due to diabetes mellitus, hematological malignancies, or the use of chronic corticosteroids. Our patient has multiple risk factors for invasive pulmonary fungal infection. He had received steroids, methotrexate and multiple dosages of biologics (TNF α inhibitors) since last one year and also developed diabetes.

The clinical presentations of the reported patients included fever, pleuritic chest pain, productive cough, and dyspnea. Chest imaging abnormalities included hilar lymphadenopathy and nodules, in our case it was thick walled lung cavity.

In-vitro drug susceptibility data showed variable results in different studies but voriconazole and posaconazole have lowest MICs, ranging from 0.12 to 0.5mg/L. Newer triazoles including albaconazole, ravuconazole also shows good in-vitro activity against *Paceliomyces lilacinus*. Amphotericin B has poor in-vitro activity against *P. lilacinus*. MIC values of this drug were always > 2 mg/ l, and usually > 8 mg/ l. The response of our patient to voriconazole was dismal since he was readmitted with progressive disease. Though the final outcome is not known it is likely to be bleak. Surgical resection of the cavity may have been associated with better results.

This case also illustrates the fact that pulmonary infections in immunocompromised patients requires invasive diagnostic work up and sometimes repeated microbiological assessment are needed to reach final diagnosis. Merely direct microscopy may not be enough, fungal cultures are needed for accurate identification and appropriate therapy. Paceliomyces is an emerging human pulmonary pathogen.

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MCQ'S IN FUNGAL INFECTIONS

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1. A 1 kg preterm newborn is in the NICU for the past 3 weeks. He has had previous problems of hyaline membrane disease and necrotizing enterocolitis. He develops features of sepsis and blood cultures show yeast 24 hours later. He has received IV fluconazole 6 mg/kg IV twice weekly for prophylaxis. Which is incorrect?
   a. Amphotericin B deoxycholate is the drug of choice
   b. CSF examination is a must
   c. He should not have received fluconazole for prophylaxis
   d. Echinocandins are not the drugs of first choice for neonatal candidiasis

2. A child with acute myeloid leukemia who has undergone an allogenic stem cell transplant develops this lesion on his leg on day 10 transplant when his neutrophil count is 20. He also has nasal stuffiness and a necrotic lesion in the nose. Which of the following fungus are you most likely to isolate from the blood?

   a. Aspergillus
   b. Mucor
   c. Cryptococcus
   d. Fusarium

3. A patient with chronic liver disease develops fever, headache and vomiting. CSF shows lymphocytic meningitis and is positive for cryptococcal antigen. Which of the following is true about treatment
   a. The duration of induction therapy with amphotericin B and flucytosine is 4-6 weeks
   b. Induction therapy can be with amphotericin B alone since the patient is relatively less immunocompromised
   c. The prognosis is definitely better than a patient who has HIV and cryptococcal meningitis
   d. There is no recommendation about using liposomal amphotericin B for treatment in this setting due to lack of data

4. Which of the following is incorrect about invasive pulmonary aspergillosis (IPA) in a patient with hematologic malignancy?
   a. Isolation of aspergillus from a BAL specimen is "proven" IPA
   b. The sensitivity of serum galactomannan for diagnosis is close to 80%
   c. Voriconazole is the drug of choice
   d. Radiologic features are indicative but not very specific/ sensitive

5. What is true about mucormycosis?
   a. Higher doses (5-10 mg/kg) of liposomal amphotericin B are more efficacious than lower doses (3-5 mg/ kg)
   b. Combination of liposomal amphotericin B with posaconazole is synergistic
   c. There is no proven role of adjuvant therapy with deferasirox/ statins
   d. Caspofungin has no role to play in mucormycosis since mucor are inherently resistant to echinocandins

Answers

1. There is a level 1A recommendation about administering fluconazole prophylaxis to all infants weighing below 1 kg to prevent neonatal candidiasis. AMB- D is the drug of choice for neonatal candidiasis due to excellent renal penetration. Due to high degree of CNS translocation in neonatal candidiasis, CNS studies are a must. Data on echinocandin use in neonates is scarce and their poor CNS and renal penetration is a clear impediment to their use. Hence the correct answer is (c)

2. Fusarium is the most common mould with a tendency to invade the blood stream due to its yeast like properties. Hence the correct answer is (d)

3. Cryptococcal meningitis in the non HIV setting is a more difficult disease to treat probably due to delayed diagnosis. It needs longer induction therapy, flucytosine is a must and prognosis is poorer. However liposomal amphotericin B can be used for therapy if the deoxycholate preparation cannot be tolerated. Hence (a) is the right answer

4. For a "proven" diagnosis of IPA, the fungus should be demonstrated in a biopsy specimen. Isolation in BAL specimen indicates "probable" disease since it may also reflect colonization. The sensitivity of serum galactomannan is good and that of BAL is even better. For most cases of IPA, monotherapy with voriconazole is sufficient. While the presence of "halo"sign and "crescent"sign are indicative they are not specific enough since they may be present in other infections including tuberculosis and may often be absent in late disease. Hence (a) is the right answer

5. In mucormycosis unlike aspergillosis, higher doses of amphotericin B are better than standard doses. Synergism is possible between L-AmB and echinocandins but combination of L-AmB and posaconazole is generally to allow switch over to oral therapy when adequate posaconazole levels build up. There is some data supporting the use of defeasirox and statins as adjuvant therapy for mucor. Hence the correct answer is (a).

BIOMARKERS IN INVASIVE FUNGAL INFECTIONS (IFI)

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Background

The principle fungal pathogens involved in IFI are *Candida* and *Aspergillus*. Unfortunately blood or other body fluid cultures are not often positive, and invasive procedures to make a tissue diagnosis are not possible due to many factors like thrombocytopenia, neutropenia etc in patients at risk for infection with these pathogens. To overcome this problem, non-culture-based methods like fungal biomarkers can be useful clinical tools.

Biomarkers in the diagnosis of invasive candidiasis

The incidence of ICU-acquired candidemia in India is 6.51 cases/1,000 ICU admissions which is 20–30 times higher compared to western world. Blood culture is the gold standard for the diagnosis of candidemia, but it takes more than 48 hours to become positive and rate of culture positivity in India is only 21%. It has been shown that a delay of each day in initiating antifungal therapy after the onset of candidemia increases the risk of mortality. For this reason, non culture-based methods can be the key to early diagnosis. (Table 1)

Table 1: Biomarkers for diagnosis of invasive candida infections

Biomarker for candida	Usefulness	Limitations
Antigen		
1. β-D-glucan		
It is a cell wall component of major fungi except Mucor and Cryptococcus	- Pan fungal marker - Positive result may occur days to weeks prior to positive blood culture	False positive result can be seen in gram-positive and gram-negative bacteremia, IV amoxicillin-clavulanate, hemodialysis, fungal colonization, IV albumin or IVIG, use of surgical gauze or other material containing glucan and mucositis - Uncertainties about the best cutoff value for a positive result - Usefulness in children? - Testing on sample other than serum
2. Manan Ag		
Mannan is a component of *Candida* cell wall (7% of total dry cell weight) released in blood circulation during candidemia. It is short-lived due to rapid clearance followed by appearance of anti-mannan antibody	- Sensitivity being highest for *C. albicans* - Positive test has been recorded several days before radiological detection of hepatosplenic candidiasis	- Rapidly cleared from blood and frequent testing is required in high risk patients
Antibody		
Anti-mannan Ab	- Good performance for albicans, tropicalis and for glabrata where blood culture is typically negative - When the test is combined with simultaneous mannan detection, the sensitivity and specificity values improved to 83% and 86%, respectively	- Antibody detection is unreliable in immunocompromised patients
PCR	High sensitivity (95%) & specificity (92%)	Lack of standardized methodologies

Biomarkers in the diagnosis of Aspergillosis

Patients with neutropenia, hematologic malignancy and allogenic bone marrow transplantation recipients are at highest risk of developing IPA. Cirrhosis of liver, COPD and prolonged ICU stay are emerging risk factors.

Galactomannan

Galactomannan (GM) is a heat stable hetero-polysaccharide which is released during hyphal growth. Being an early indicator of disease, it can be detected in blood even before clinical or radiologic features of disease appear.

The value of GM (serum and BAL) in aiding the diagnosis of IPA has been studied extensively, especially in the neutropenic, and hematologic malignancy populations, and has been included in the EORTC/MSG criteria.

But there are some false positive and false negative results of GM.

False positives – Other fungi like Penicillium, Histoplasma capsulatum, Fusarium, can give rise to a false positive GM. Plasmalyte used in BAL, generic piperacillin-tazobactam and GI mucositis (due to translocation of food-borne GM or bacteria with cross-reactive epitopes including Bifidobacterium esp in neonates) are other reasons for a false positive test.

False negatives- Aspergillus tracheobronchitis, non-neutropenic patients with low fungal burden, presence of anti-aspergillus antibodies and prior mould-active prophylaxis are the reasons for false negativity.

Sensitivity and Specificity of Galactomannan in Neutropenic population

	Sensitivity	Specificity
Serum GM	70 %	92 %
BAL GM	100 %	80.4 %

Sensitivity and Specificity of Galactomannan in Non-Neutropenic population

	Sensitivity	Specificity
Serum GM	36.8%	76.1%
BAL GM	94.7%	86.2%

The cutoff for BAL GM is still debated but, an optical density (OD) of <0.5 virtually rules out the diagnosis of IPA, while a value of >3 has near 100% specificity

Rational Interpretation of Galactomannan assay

Value	Sensitivity	Specificity	Significance
>0.5	High	Low	Rules out IPA if negative
>3	Low	100% specificity	Rules in regardless of pre-test probability
0.8	86.4%	90.7%	PPV 81%, NPV 93.6%
0.5 – 3			Pretest probability is crucial for interpretation

GM is also useful in the follow-up for assessment of therapeutic response.

PCR

A meta-analysis of PCR methods applied to blood, serum and plasma to detect IPA was published in 2009. Analysis using a single positive PCR gave a sensitivity of 88% and specificity of 75%, whereas, the requirement of two positive samples, made the sensitivity 75% and specificity 87%. Majority of studies involved patients with hematologic malignancies, however, some studies also looked at solid organ transplant (SOT) recipients.

Limitations

Publication of multiple assays with differences in DNA extraction, PCR, and product detection with little or no standardization that allowed easy comparison of studies. Hence it has not been incorporated into the EORTC/MSG criteria

Breath Tests

It has been found that in patients with suspected IPA, aspergillus secondary metabolite signatures in breath (alpha-trans-bergamotene, beta-trans-bergamotene, beta-vatirenene-like sesquiterpene) identified IPA patients with a sensitivity 94% and specificity of 93%. These results provided proof-of-concept that direct detection of fungal metabolites in breath can be used as a novel, non-invasive, pathogen-specific approach to identify patients with IPA.

Lateral Flow Device (LFD)

A novel and simple lateral flow device (LFD) using monoclonal antibody JF5 that targets an extracellular glycoprotein has been developed. The performance of this LFD was compared to real-time PCR (targeting 28s rRNA gene) and galactomannan detection when testing serum from an EORTC/MSG defined haematological population.

In proven/probable IPA versus no IPA population the LFD performance was comparable to both PCR and galactomannan EIA. Specificity (98.0%) was similar to PCR (96.6%) and slightly superior to GM (91.5%). Sensitivity (81.8%) was inferior to PCR (95.5%) but better than GM (77.3%). In combination with PCR it provided both 100% sensitivity and specificity

Conclusions

Invasive fungal infections are an important challenge in critically ill patients. Since early diagnosis of definite infection is difficult and treatment delay is to be avoided, new means of making early diagnosis is essential. Since the sepsis syndrome could be due to other causes, empirical antifungal therapy may lead to overuse of antifungal agents. Hence, the use of biomarker-assisted diagnosis can achieve the twin goals of maximizing outcomes for the individual patient and minimizing the collateral damage to the fungal ecology.

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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.