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SPECTRUM OF PULMONARY ASPERGILLOSIS – A CLINICAL REVIEW

Microbiology

Dr. Rohit Vadala MD,DNB (Pulmonary medicine), IDCCM, Senior Resident, Department of Pulmonary and Critical care medicine, Primus Super Speciality hospital, Chanakyapuri, New Delhi – 110021, India.

Dr. Isabella Princess* MD, DNB, Senior Registrar - Microbiology, Apollo Speciality Hospitals, Vanagaram, Chennai - 600095 *Corresponding Author

ABSTRACT

Fungi are ubiquitous in nature spreading across large geographic regions at any given timeframe. Non seasonal predisposition of fungi makes it convenient for them to cause rampant infections throughout the year. Moreover, wide range of incubating temperatures for their growth makes them resistant to adverse climatic conditions. Most commonly occurring environmental molds belong to the Aspergillus species which can cause variety of infections in humans ranging from simple colonization to more invasive pulmonary aspergillosis. Predisposition of Aspergillus in the respiratory tract is due to the common mode of transmission by inhalation of spores. Immune compromised and chronically infected patients are susceptible to invasive pulmonary aspergillosis which has significant morbidity and mortality. Knowledge on pathogenesis, distinct clinical and radiological features in various groups of individuals would be useful in early recognition and management of various spectrum of infection caused by aspergillosis.

KEYWORDS

Pulmonary aspergillosis, Immune compromised, Mortality.

INTRODUCTION

Aspergillosis is caused by the saprophytic mold aspergillus species. Among the hundreds of prevalent species, *Aspergillus fumigatus* is the commonest one causing human infection.[1]The burden of pulmonary aspergillosis has increased tremendously in the recent years due to evolving immune compromised conditions. Various forms of Aspergillosis have been documented based on clinical manifestation. Pulmonary aspergillosis most commonly manifesting as aspergilloma is associated with various underlying risk factors.The classical clinical presentation may be masked due to various coexisting infectious and noninfectious conditions. Therefore, this review intends to collate the entire clinical spectrum of pulmonary aspergillosis in a nut shell.

Epidemiological determinants of pulmonary aspergillosis

Epidemiological aspects of the disease play a vital role in understanding and anticipating infections based on factors which predispose to development of the same. Three aspects of the disease such as agent, host and environmental factors go hand in hand for disease pathogenicity. These epidemiological characteristics of pulmonary aspergillosis are explained.

I) Agent: Pulmonary aspergillosis is a systemic fungal infection caused by several species of Aspergillus species. They are ubiquitously found saprophytic fungi growing on dead and decaying organic matter and soil. Aspergillosis grouped as a mold since the vegetative hyphae germinate from spores/conidia. Aspergillus has branching hyphae which are septate and hyaline (colorless) in nature. Among 200 species of Aspergillus, commonly isolated ones from clinical samples are *A.fumigatus, A.flavus, A.niger, A.terreus, A.nidulans, A.oryzae, A.glaucus* etc.[2]Among these, *Aspergillus fumigatus* is the commonest cause of pulmonary aspergillosis compared to other species.About 80-90% cases of invasive pulmonary aspergillosis are caused by *A.fumigatus*.[3]The reasons for predisposition of *A.fumigatus* with pulmonary aspergillosis are : 1) Wide temperature (12-65°C) and pH (2.1-8.8) range for its growth[4,5], 2) Easy dispersion of spores in air due to small size, hydrophobicity and presence of melanin which makes spores resistant to ultraviolet irradiation[6,7], 3) Small size of conidia/spore (2-3 µm) compared to other Aspergillus species facilitates its passage up to distal airway[8], 4) Presence of cell wall melanin of *A.fumigatus* spores makes it resistant to Reactive Oxygen Species (ROS) and lysis by host cell, 5) Easy adaptability due to adherence (more sialic acid residues) and germination of *A.fumigatus* spores in the respiratory epithelium of immune compromised host.[9,10]Apart from *Aspergillus fumigatus*, *Aspergillus terreus* and *Aspergillus flavus* are also associated with pulmonary aspergillosis of various forms.[3]

ii) Host factors / Risk factors: Spores of Aspergillus are the infective form which gains entry into the human host through inhalation. The spores easily adapt to adverse environmental conditions outside the human body. The airway of immune compromised individuals is another such example which serves an adverse condition for adaptation of spores.[11]Aspergillus cause infection in immune competent individuals as well. Considering these two broad groups, immune competent host develop infection only due to repeated exposure to spores such as those exposed to mouldy hay, tree bark chippings,mushroom factory, near drowning etc.[12-14]This suggests that repeated exposure leading to colonization aids the fungi in establishing itself within the host thereby causing infection.

The major arm of host factor predisposing to pulmonary aspergillosis is the immune compromised state of individuals. Documented reports published in literaturehave shown strong correlation between immunosuppression and pulmonary aspergillosis. Immune suppressed individuals are broadly classified as neutropenic and non-neutropenic hosts. The major difference between these two groups is the higher incidence of disseminated aspergillosis occurring with neutropenic individuals.[15]Neutropenic individuals are those undergoing hematopoietic stem cell transplants, chemotherapy etc.[16] Non-neutropenic individuals are solid organ transplant recipients, long term corticosteroid therapy, autoimmune conditions such as chronic granulomatous disease, AIDS patients, critically ill, patients with Chronic Obstructive Pulmonary Disease (COPD), liver failure etc.[14,17]Risk factors for specific forms of pulmonary aspergillosis are elicited in table 1.

Table 1. Risk factors for clinical forms of pulmonary aspergillosis

Forms of pulmonary aspergillosis	Risk factors	Reference
Invasive Pulmonary Aspergillosis	Neutropenia(<500 cells/mm3), allogenichaematopoietic stem-cell (2.3 – 15%) and solidorgan transplantation, prolonged and high-dose corticosteroid therapy, haematological malignancy, cytotoxic therapy, advanced AIDS (CD4 count <100 cells/mm3, chronic granulomatous disease (CGD), severe Graft Versus Host Disease (Grade III-IV ; 78-112 days post transplantation)	Soubani AO et al, Segal BH et al, Zmeili OS et al.[18-20]

Allergic Bronchopulmonary Aspergillosis	Bronchial asthma, cystic fibrosis	Stevens DA et al, Knutsen AP et al.[21,22]
Chronic necrotizing aspergillosis	Underlying chronic lung diseases such as COPD, previous pulmonary tuberculosis, thoracic surgery, radiation therapy, pneumoconiosis, cystic fibrosis, lung infarction, sarcoidosis (less commonly), chronic lung disease, low dose corticosteroid therapy, alcoholism, liver disease, connective tissue diseases.	Zmeili OS et al, Denning DW.[20,23]
Aspergilloma	Pre-existing cavity in the lung caused by tuberculosis (most common),sarcoidosis, bronchiectasis, bronchial cysts and bulla, ankylosing spondylitis, neoplasm, and pulmonary infection	Kauffman CA, Zizzo G[24,25]

iii) Environmental factors: Aspergillus is ubiquitous in nature with worldwide distribution. They are prevalent from grasslands to mountainous terrain and have high temperature habitats. Aspergillus is an aerobic fungus and therefore found in most oxygenated environments. They are more common in cultivated and garden soil compared to uncultivated land, also found in compost, bird droppings, tobacco and stored foods such as potatoes.[26]Studies show supportive evidence for colonization of building materials by spores of Aspergillus, especially *Aspergillus fumigatus*.[27]There have also been studies showing positive correlation of Aspergillus spores dispersed in environments where constructions are taking place.Therefore, the diverse habitat and distribution of Aspergillus is proof enough to intrigue its suspicion in causing widespread pulmonary infections.

Clinical forms and classification of pulmonary aspergillosis

The clinical spectrum of pulmonary aspergillosis is varied and broad. It can thus be classified as allergic and non-allergic clinical forms.[Table 2]Allergic forms are restricted almost always to immune competent individuals whereas non-allergic invasive and chronic forms are seen in immune compromised patients. Allergic forms of pulmonary aspergillosis are Allergic Bronchopulmonary Aspergillosis (ABPA), asthma, allergic alveolitis. Non-allergic forms include colonization (Aspergilloma, local infection) and invasive aspergillosis (Chronic necrotizing aspergillosis, disseminated aspergillosis).[2]This classification and categorization of patients is important in diagnosing them according to the clinical presentation.

Table 2. Classification of clinical forms of pulmonary aspergillosis

Pulmonary aspergillosis	
Allergic	**Non-allergic**
• Allergic Bronchopulmonary Aspergillosis (ABPA) • Aspergillus induced allergic asthma • Allergic alveolitis	**Colonization :** • Aspergilloma • Local pulmonary infection **Invasive disease :** • Invasive pulmonary aspergillosis • Chronic necrotizing aspergillosis • Disseminated aspergillosis

Allergic forms of Pulmonary Aspergillosis:

Allergic Bronchopulmonary Aspergillosis (ABPA) is the commonest manifestation of allergic aspergillosis worldwide. Individuals with cystic fibrosis or bronchial asthma are at higher risk of developing ABPA.[28]Following repeated exposure and inhalation of Aspergillus spores, the susceptible (asthma/COPD/cystic fibrosis) atopic individuals mount an IgE mediated Type I Hypersensitivity reaction to the allergen (spore).[28]More than repeated exposure and environmental factors, host susceptibility to ABPA depends on genetic factors. Approximately 8-11% asthmatics are definitely prone to develop ABPA, probably prone asthmatics are about 22%.[29]Supportive evidences to clinically diagnosed ABPA in asthmatics are a higher serum IgE level, elevated total leukocyte count and absolute eosinophil count (eosinophilia), pulmonary infiltrates, fleeting shadows, sputum culture growth of *Aspergillus fumigatus*. A disclaimer to this high serum IgE level is for patients on systemic corticosteroids. The allergic immune response gets blunted due to corticosteroids thereby masking IgE levels and eosinophil count in ABPA patients who are on corticosteroid therapy.

Diagnosis of ABPA is made based on diagnostic criteria proposed by Rosenberg et al in 1977.[30] He and his colleagues have divided the ABPA diagnostic criteria into major and minor criteria. [Table 3]. Apart from this, three other ABPA diagnostic criteria exist such as "Minimal essential criteria", "Truly minimal criteria", "ISHAM working group criteria", "Diagnostic criteria for ABPA in cystic fibrosis".

Table 3. ABPA diagnostic criteria (Rosenberg – Patterson criteria)

Major criteria	Minor criteria
1. Asthma 2. Presence of transient pulmonary infiltrates (Fleeting shadows) 3. Immediate cutaneous reactivity to A.fumigatus antigen 4. Elevated total serum IgE 5. Precipitating antibodies against A.fumigatus 6. Peripheral blood eosinophilia 7. Elevated serum IgE and IgG to A.fumigatus 8. Central/proximal bronchiectasis with normal tapering of distal bronchi	1. Expectoration of golden brown sputum plugs 2. Positive sputum culture for Aspergillus species 3. Late type III hypersensitivity (Arthus type) skin reactivity to A.fumigatus

It is noteworthy that not all 8 major criteria be present in an individual with ABPA. Some individuals may manifest features mentioned above only during acute stage and some only during chronic stage. As a general rule, patients on corticosteroids may not manifest all major criteria of ABPA.Being an immunologically mediated disease, ABPA has a protracted clinical course. The clinical manifestations may range from subtle symptoms to very severe respiratory distress and failure with intermittent remission and episodes of exacerbation.[29,31]Age at presentation / diagnosis of ABPA is usually in 20s, 30s. It has however been reported from small children and infants as well.[32]Commonest clinical manifestations in over 90% individuals are cough, expectoration, breathlessness, wheezing. Less common manifestations (less than 50% individuals) arehemoptysis, expectoration of mucus plugs, upper respiratory tract symptoms.[33]Physical signs such as rhonchi, bronchial breathing and crepitations are varied based on extent of lung involvement and severity of ABPA. Extensive fibrosis in chronic cases may produce persistent crackles, cyanosis, clubbing, cor pulmonale.[29,34]

The importance of repeated radiographic imaging in ABPA suggested by Hinson et al highlights the difference in radiological signs one may encounter in these patients over the course of illness. The term "fleeting shadows" represents the transient changes in lung radiographs appearing in different parts of lungs during acute exacerbations of ABPA. The various transient changes documented are perihilar infiltrates, massive consolidation, 'tooth paste' shadows, 'gloved finger' shadows, 'tramline' shadows, lobar or segmental collapse. Permanent changes seen in chronic cases may appear as central bronchiectasis, parallel line shadows, ring shadows, pulmonary fibrosis, other late changes such as cavitation, upper lobe contraction, localized emphysema.[35,36]The hallmark of permanent change characteristic in ABPA is central bronchiectasis with normal peripheral bronchi. The five stages of ABPA are acute, remission, exacerbation, corticosteroid dependent asthma, fibrotic lung disease. Each patient should ideally be evaluatedand stage of ABPA be recorded at each clinical visit to assess treatment prognosis or clinical deterioration.

Aspergilloma

Aspergilloma, commonly known as fungal ball is nothing but the late manifestation of chronic pulmonary aspergillosis. They are rounded aggregates of fungal hyphae, mucus and cellular debris, mostly solitary although may be bilateral. They arise in already existing pulmonary cavities and therefore localized with no tissue invasion. In contrast with ABPA, this condition is common in middle aged men probably because of its attribution to repeated colonization occurring over time. Risk factors for developing aspergilloma are pre-existing cavities (>2cm diameter) in the lung caused by tuberculosis (most common), sarcoidosis, bronchiectasis, bronchial cysts and bulla,

ankylosing spondylitis, neoplasm, and pulmonary infection.[24,25]Other predisposing conditions to aspergilloma include bronchogenic cyst, pulmonary sequestration, And pneumatoceles secondary to *Pneumocystis jirovecii* pneumonia in patients with Acquired Immunodeficiency Syndrome (AIDS).[37] Although all symptoms of lung infection may be present chronically in these patients, hemoptysis is the commonest complicating life threatening condition.[38] The typical radiological sign elicited in aspergilloma is 'air crescent sign'due to presence of a mass with soft tissue opacity within a lung cavity separated from the wall of the cavity by an air space. Pleural thickening is another common sign which occurs earlier than the air crescent sign.[39]The major distinguishing characteristic of air crescent sign in aspergilloma with other conditions is the absence of preexisting cavity in the latter.[40]

Invasive Pulmonary Aspergillosis (IPA)

Any invasive form of disease has been attributed to immune compromised condition of individuals. Invasive aspergillosis is no exception occurring mostly in neutropenic individuals, however non-neutropenic individuals cannot be excluded from this clinical condition.[41]The commonest cause of fungal pneumonia in any intensive care unit is Aspergillus species. Major risk factors for the development of IPA are previous treatment with corticosteroids and COPD. Apart from these common risk factors, there appear to be many such as hematological malignancy, hematopoietic stem cell transplant, solid organ transplant patients, severe burns, multi-organ dysfunction syndrome, prolonged ICU stay, prolonged administration of antibiotics etc.[42]Since this condition is invasive, fever is the most common presentation, other features being hemoptysis, pleuritic chest pain, dyspnea etc. Patients with IPA are predisposed to developing secondary bacterial pneumonia especially when they are neutropenic. This may be the reason for failure of isolating Aspergillus in these patients. However in non-neutropenic individuals, IPA cannot be diagnosed and treated unless Aspergillus species is isolated from respiratory specimens.[3]

There are two clinical forms of IPA : the airway invasive form and the angio-invasive form. Airway invasive form suggests invasion of bronchial basement membrane by the Aspergillus mimicking bronchiolitis. The angio-invasive form is more aggressive involving small and medium sized pulmonary arteries causing occlusion by fungal hyphae. Haemorrhagic infarcts are also seen within the alveoli.[37,43]Due to the difference in lung involvement, clinical presentation in patients with IPA ranges from asymptomatic macronodules to overt respiratory failure.

Radiological signs pathognomonic of IPA are worsening of existing signs of aspergillosis such as infiltrates, cavity, nodule, pleural effusion, consolidation. Various descriptive terminologies for radiological appearance seen in IPA are “tree-in-bud” appearance, “halo sign” denoting bronchiolitis with patchy centrilobular nodules and large nodules surrounded by groundglass attenuation respectively.[43,44]Air crescent sign, consolidation, cavitary lesion, infarct, macronodules etc. are other radiological signs reported by authors in previous published reports. Studies have shown that chest Computed Tomographic (CT) imaging is more sensitive than plain chest radiography for IPA.

Management of pulmonary aspergillosis

Direct as well as supportive evidence of pulmonary aspergillosis using diagnostic modalities make the clinical diagnosis exhilarating. A profound knowledge on laboratory diagnosis will aid physicians in diagnosing arduous clinical patterns of pulmonary aspergillosis which is not uncommon.Various conventional and newer methods for diagnosis of pulmonary aspergillosis have evolved over the years. Unavailability of these tests in various countries is the challenging reality. Direct as well as supportive evidence of Aspergillus infection is of major diagnostic value.

Laboratory diagnosis of pulmonary aspergillosis can be divided into microscopy, culture, molecular diagnosis and serological diagnosis.Samples used for performing diagnostic tests are sputum, tracheal aspirates, bronchoalveolar lavage, lung biopsy tissue etc. The specification of each staining method is described in table 4.

Table 4. Microscopic diagnosis of Pulmonary Aspergillosis [45]

S.No	Fungal stains and their implications
1.	KOH (Potassium hydroxide) mount: KOH acts as a keratinolytic agent thereby clearing keratin in tissue/sample and makes the fungal hyphae visible. Characteristics such as colour of hyphae, septations, branching pattern are seen.
2.	Gram stain: Gram stain can stain fungal hyphae and sometimes the finding may be incidental. Entangled fungal hyphae and spores can be seen in aspergilloma.
3.	Calcofluor white: Fluorescent stain used directly on clinical specimen for easy visualization of the fungal hyphae. Calcoflour white binds to polysaccharides with beta glucan linkages which are predominant in fungal cell wall.
4.	Lactophenol Cotton Blue mount: This stain is used to prepare tease mount / scotch tape method to stain fungi after culture.
5.	Hematoxylin& Eosin (H&E) stain: Tissue response as well as fungal morphology is detected from tissue biopsy using H&E stain. Entangled fungal hyphae and spores can be seen.
6.	Periodic acid Schiffstain: This stain is used for identification of most fungi. However addition of diastase enzyme to this makes the visualization of fungi from tissue much clear by leaving the glycogen unstained.
7.	Gomori`s Methanamine silver (GMS) stain: This is an excellent stain for fungal detection as it gives a good contrast of fungi with its background. GMS stains non-viable fungi as well.

Fungal culture is still considered the gold standard in diagnosing fungal pulmonary infections. The only critical issue is distinguishing true infection with colonization or contamination. This issue is more so with pulmonary aspergillosis since Aspergillus is ubiquitous and a common environmental contaminant in the laboratory. Commonest culture media used for isolation of fungi is the Sabouraud`s Dextrose Agar (SDA). Since various species of Aspergillus have different temperature of optimal growth, two SDA plates should be inoculated and incubated at 25°C and 37°C each. In case of suspected superadded bacterial infection, clinical samples should be inoculated SDA supplemented with antibiotics. Following primary culture on SDA, sporulation is necessary for species level identification of Aspergillus. Other culture media such as corn meal agar, potato dextrose agar and banana peel technique can be used for sporulation of Aspergillus. Slide culture technique is another way of demonstrating undisturbed intact morphology of Aspergillus hyphae and spores. Antifungal susceptibility of Aspergillus is done by microbroth dilution method and interpreted using the Clinical Laboratory Standards Institute (CLSI) guidelines.

Molecular diagnostic methods such as Polymerase Chain Reaction (PCR) are limited for use only in laboratories with good resources and research facilities.Routine diagnosis need not depend on these methods as they are expensive and limited for use in small laboratories. In developing countries where fungal culture by itself is a challenge, there is no question to these PCR based diagnosis. Further advanced gene sequencing of Aspegillus is done by detecting the internal transcribed spacer region, calmodulin and β-tubulin of various species.[46]

Supportive serological evidence for Aspergillus infection is detection of Galactomannan antigen from serum and other respiratory specimens such as sputum, endotracheal aspirate, bronchoalveolar lavage etc. The common methodologies used for Galactomannan detection are latex agglutination, Enzyme Linked Immunosorbent Assay (ELISA). This diagnostic test plays a much important role in diagnosing invasive aspergillosis from colonization by Aspergillus. The reason behind this differentiation is due to Galactomannan release occuring only during hyphal growth of Aspergillus rather than from the conidia.[47]Table 5 portrays importance of diagnostic tests in each type of pulmonary aspergillosis. Once a colonization and invasive infection is distinguished, appropriate antifungal therapy should be initiated.

Table 5. Diagnostic tests done for types of Pulmonary Aspergillosis

Type of pulmonary aspergillosis	Microscopy	Fungal culture	Skin test	Supportive tests (Serology)
Invasive Pulmonary Aspergillosis	Yes	Yes	No role	Yes

Allergic Bronchopulmonary Aspergillosis	Yes	Yes	Yes	No role
Chronic necrotizing aspergillosis	Yes	Yes	No role	Yes
Aspergilloma	Yes	Yes	No role	Yes

Treatment of pulmonary aspergillosis depends on the type of disease. Therefore a thorough clinical and diagnostic evaluation is mandatory before initiation of therapy. Type of antifungals used in different types of pulmonary aspergillosis is varied. Therefore, the indications, action and clinical implication for use of antifungals is collated and elicited in table 6.

Table 6. Treatment of Pulmonary Aspergillosis [40]

Type of pulmonary aspergillosis	Treatment and indication
Invasive Pulmonary Aspergillosis (IPA)	First line drugs : Amphotericin B, liposomal Amphotericin B, Voriconazole. Salvage therapy : Posaconazole, Echinocandins (Caspofungin, Micafungin, Anidulafungin) Due to high mortality rate in IPA, therapy must be initiated as soon as IPA is suspected even before diagnostic workup is complete. Combination therapy of Voriconazole with Caspofungin is shown to have better outcome.
Allergic Bronchopulmonary Aspergillosis (ABPA)	Oral corticosteroids (Prednisolone) are the mainstay of therapy. Inhaled corticosteroids have shown less evidence of clinical improvement. Itraconazole has shown to augment the effect of corticosteroids. Voriconazole has demonstrated significant clinical and serological improvements. anti-IgE monoclonal antibody (omalizumab) may be beneficial.
Chronic necrotizing aspergillosis	Amphotericin B, Itraconazole (better alternative). Voriconazole – recently recognized for partial or complete recovery when used as initial and salvage therapy for a duration of 4-24 weeks. Surgical resection has a minor role.
Aspergilloma	Antifungal: Itraconazole. Surgical resection is done in patients with recurrent symptoms and good pulmonary function; however mortality after surgery is 7-23%. Itraconazole is preferred due to its high tissue penetration into an existing cavity. Intravenous and CT guided instillation of antifungals are of less value and clinical success.

CONCLUSION

Risk of acquiring pulmonary aspergillosis has many epidemiological determinants which most of the times are not modifiable. In the current era, the rise in fungal pulmonary infections is attributed to increase in host factor related risk factors. A major rise in communicable and non-communicable diseases in the recent past is the key to this increase. Since non-modifiable riskfactors cannot be reversed, the only option is to keep an open mind while diagnosing patients with signs of lung infections. Suspicion and early diagnosis of pulmonary aspergillosis play a key role in appropriate management and recovery of these patients.

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