



Aspergillosis: an Update on Clinical Spectrum, Diagnostic Schemes, and Management

Rimjhim Kanaujia¹ · Shreya Singh² · Shivaprakash M. Rudramurthy³

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Abstract

Purpose of Review This review gives an overview of the diseases caused by *Aspergillus*, including a description of the species involved and the infected clinical systems. We provide insight into the various diagnostic methods available for diagnosing aspergillosis, particularly invasive aspergillosis (IA), including the role of radiology, bronchoscopy, culture, and non-culture-based microbiological methods. We also discuss the available diagnostic algorithms for the different disease conditions. This review also summarizes the main aspects of managing infections due to *Aspergillus* spp., such as antifungal resistance, choice of antifungals, therapeutic drug monitoring, and new antifungal alternatives.

Recent Findings The risk factors for this infection continue to evolve with the development of many biological agents that target the immune system and the increase of viral illnesses such as coronavirus disease. Due to the limitations of present mycological test methods, establishing a fast diagnosis is frequently difficult, and reports of developing antifungal resistance further complicate the management of aspergillosis. Many commercial assays, like AsperGenius®, MycAssay Aspergillus®, and MycoGENIE®, have the advantage of better species-level identification and concomitant resistance-associated mutations. Fosmanogepix, ibrexafungerp, rezafungin, and olorofim are newer antifungal agents in the pipeline exhibiting remarkable activity against *Aspergillus* spp.

Summary The fungus *Aspergillus* is found ubiquitously around the world and can cause various infections, from harmless saprophytic colonization to severe IA. Understanding the diagnostic criteria to be used in different patient groups and the local epidemiological data and antifungal susceptibility profile is critical for optimal patient management.

Keywords Aspergillosis · Invasive aspergillosis · Biomarkers · Molecular diagnosis · *Aspergillus fumigatus* · *Aspergillus flavus* · Management

✉ Shivaprakash M. Rudramurthy
mrshivprakash@yahoo.com; mrshivprakash@gmail.com

Rimjhim Kanaujia
rim.pgimer@gmail.com

Shreya Singh
shreya_thedoc@rediffmail.com

¹ Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research PGIMER, Chandigarh, India

² Department of Microbiology, Dr B R Ambedkar State Institute of Medical Sciences (AIMS), Mohali, Punjab, India

³ Mycology Division, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research PGIMER, Chandigarh, India

Introduction

The genus *Aspergillus* comprises several hundred species, many of which are known to be potentially pathogenic and implicated in serious infections. Aspergillosis includes various diseases caused by *Aspergillus* species dependent upon the host immunological responses, ranging from non-invasive allergic illness to chronic and invasive lung infection [1, 2••]. The conidia of *Aspergillus* are ubiquitous in the environment. Following inhalation or inoculation, depending on the host's immune state, an infection can either spread locally or disseminate to distant sites [3•]. The risk factors for this infection continue to evolve with the introduction of several biological agents targeting the immune system and the rise of viral infections such as coronavirus disease [3•, 4]. Despite significant advances in aspergillosis diagnosis and treatment, severe fungal diseases continue to occur and

are not easy to treat. Fatality rates remain high, particularly in immunocompromised people. Establishing a prompt diagnosis is often challenging due to the limitation of existing mycological test methods, and reports of rising antifungal resistance further hamper the management of aspergillosis. This review gives an overview of the risk factors and clinical spectrum of aspergillosis, followed by recent updates in the diagnostic armamentarium, management, and antifungal resistance, focusing on invasive pulmonary aspergillosis.

Agent

The most commonly implicated pathogens in aspergillosis are representative of the following species complexes: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger*. The most frequently reported agent accounting for 60 to 90% of all infections is *A. fumigatus* [1, 5]. Non-*fumigatus* species, on the other hand, are also now being identified in a wide range of cases led by *A. flavus*. This fungus is especially prevalent among cases of invasive aspergillosis (IA) from Asia, the Middle East, and Africa. It could be due to its potential to tolerate hot and arid environments. However, more research on the underlying biological characteristics of diverse *Aspergillus* species diving differences in prevalence is essential [6]. While *A. fumigatus* represents the majority of pulmonary aspergillosis, *A. flavus* causes around 10% of bronchopulmonary infections, with rhino-cerebral aspergillosis being the most prevalent presentation [6]. In hospital settings, *A. fumigatus* has been associated with pulmonary or sinus disease, whereas *A. flavus* outbreaks have been attributed to cutaneous, mucosal, and subcutaneous tissues [7].

A. terreus species complex is an emerging opportunistic fungus found in many environments, including soil and compost, with an increasing clinical prevalence in recent years [8]. Infection due to this agent is frequently seen in Innsbruck, Austria, Houston, the USA, and even India. Being amphotericin B resistant, *A. terreus* species assumes a very significant etiologic role constituting nearly 4% of all IA with an adverse outcome than IA caused by non-*A. terreus* species [9, 10].

Patients with chronic granulomatous disease (CGD) are commonly infected with *A. fumigatus*, followed by *A. nidulans* and other aspergilli, such as *A. tanneri* [11]. CGD patients are associated with a higher risk of *A. nidulans* infection than other immunocompromised patients. This agent is reportedly more virulent than the more commonly encountered *A. fumigatus*, with higher mortality rates and a greater propensity to spread [12].

A. niger is less commonly associated with IA than other species of *Aspergillus*. It is usually associated with otomycosis, and cutaneous infections, with few reports of pneumonia

[13]. A study in COPD patients with invasive pulmonary aspergillosis (IPA) revealed that 3.6% of cases were due to *A. niger* [14]. Interestingly, the pathologic demonstration of calcium oxalate crystals is a common indicator of infection due to this agent, as oxalic acid undergoes fermentation and precipitation to form these crystals [15].

A. versicolor is a ubiquitous fungus that has been widely described as an agent of onychomycosis, otomycosis, cutaneous infection, osteomyelitis, and ocular disease. It is regularly encountered as a respiratory tract colonizer with rare cases of IPA [16]. Many species of *Aspergillus* are also known to found to produce toxic metabolites (mycotoxins 3-nitro propionic acid, aflatoxins, and ochratoxin A), which impairs the phagocytosis [2••].

Clinical Spectrum

Aspergillosis can affect almost any organ of the human body. The lower respiratory tract and lungs are the most frequently infected, followed by the nasal sinuses and skin. Direct or hematogenous spread may affect the cardiovascular and central nervous system (CNS). While the disease spectrum depends on the immunological status of the infected host and the existence of pre-existing pulmonary illness, other environmental factors which raise the spore count in ambient air, such as reconstruction, refurbishment, and building demolition, particularly in the healthcare setting can even lead to outbreaks [17••].

Superficial and cutaneous aspergilloses affecting the outer layers of the skin, nails, cornea, or ear canal are infrequent and seldom infiltrate deeper tissue. Such infections are primarily acquired through direct traumatic injection, including fungal otomycosis, keratitis, onychomycosis, and cutaneous aspergillosis. While keratitis, otomycosis, and onychomycosis are common among immunocompetent patients, cutaneous aspergillosis is more frequent in immunocompromised patients [18]

Regarding pulmonary infection, *Aspergillus* spp. usually remain colonized in pre-existing pulmonary cavities resulting from tuberculosis, bronchiectasis, sarcoidosis, or cavitary neoplasia and cause conditions like chronic pulmonary aspergillosis (CPA) in otherwise immunocompetent individuals [19, 20]. Severe asthma with fungal sensitization (SAFS) and allergic rhinitis are common among hypersensitive individuals. In contrast, allergic bronchopulmonary aspergillosis (ABPA) is frequently seen among patients with underlying cystic fibrosis (CF) or asthma [21, 22]. Invasive pulmonary aspergillosis can be seen among immunocompromised, neutropenic individuals as a part of systemic involvement and also in non-neutropenic patients with prolonged intensive care unit (ICU) stay, mechanical ventilation,

chronic obstructive pulmonary disease (COPD), and more recently, coronavirus disease (COVID-19).

Among sinus diseases, non-invasive fungal rhinosinusitis can include saprophytic fungal infestation and fungal ball, which may be associated with previous mucosal injury or surgery, particularly dental treatment. Allergic fungal rhinosinusitis (AFRS) is a non-invasive form of the disease, typically seen in younger, immunocompetent, atopic individuals, with hypersensitivity reaction to fungal antigens, immune complex deposition, and inflammation. Invasive rhinosinusitis includes acute invasive fulminant rhinosinusitis (AIFRS), chronic invasive, and chronic granulomatous invasive fungal rhinosinusitis [22, 20]. AIFRS has a short course (<4 weeks) of presenting illness and is most commonly seen in immunocompetent individuals [23]. Though the prevalence of AIFRS in the developed and developing worlds is approximately equivalent, *A. fumigatus* is the most prevalent agent in developed countries, while *A. flavus* is becoming more common in underdeveloped countries [24].

IA is a life-threatening condition in immunocompromised people, with fatality rates ranging from 40 to 80% [25, 26]. The underlying risk factors are hematological malignancies, hematopoietic stem cell transplantation, solid-organ transplant and patients on prolonged chemotherapy/steroid, and previous viral cases of pneumonia such as influenza or COVID-19 [27, 28]. Tracheobronchitis is frequently seen among organ transplant recipients or people suffering from COVID-19. Rhino-cerebral aspergillosis is seen in neutropenic patients with hematological malignancies and disseminated infection affecting different organ systems. Genetic susceptibility to fungal infections, including aspergillosis, is seen among individuals with loss-of-function mutation in the signal transducer and activator of transcription 3 (STAT 3) gene, which results in a defective adaptive immune response against *Aspergillus* spp. [29].

Diagnosis

In the absence of a single “gold standard” test for the diagnosis of aspergillosis, the combination of microbiological and histopathology findings, host factors, and clinical and radiological evidence is needed to obtain rapid and accurate diagnosis. Several diagnostic criteria have been described to reach a diagnosis of IA in various patient groups and these are summarized in Table 1.

Radiological Findings

Radiological imaging provides a primary cue for diagnosing IPA; the presence of nodules, halo sign (ground glass attenuation surrounding a pulmonary nodule), wedge-shaped infiltrates, pleural effusion, etc. may be seen in invasive pulmonary disease. The EORTC guidelines describe the presence

of cavity or wedge-shaped, air crescent sign; dense, well-circumscribed lesions with or without a halo sign; and segmental/lobar consolidation as radiographic features suggestive of IA [38]. In neutropenic hosts, the “Halo sign,” which is ground glass opacity representing alveolar hemorrhage, is seen surrounding a pulmonary nodule or mass due to a focus on pulmonary infarction mass. A “Reverse halo” or “Atoll sign” consists of central ground-glass opacity surrounded by denser consolidation (usually > or = 2 mm, crescentic, i.e., forming > 3/4th circle or complete ring) but is less frequently seen in pulmonary aspergillosis and more frequently among patients with pulmonary mucormycosis (PM). Multiple nodules (more than 10) and pleural effusions also appear more regularly in PM than in IPA. Intracavitary mass separated from the wall by a crescent-shaped airspace is called the “Air crescent sign” and is usually seen late in the course of illness and is non-specific. Among non-neutropenic patients, multiple pulmonary nodules and non-specific findings, e.g., bronchopneumonia, consolidation, cavitation, pleural effusions, ground glass opacities, tree-in-bud opacities, and atelectasis may be noted with the absence of classical signs. In cases of clinical suspicion of IPA, it is recommended to perform a computed tomographic (CT) scan without chest contrast [38].

Bronchoscopy

It is recommended that all patients with suspected IPA be subjected to bronchoscopy examination for two main reasons: firstly, it allows the direct visualization of the affected area and allows localized sampling; secondly, bronchoalveolar lavage is a validated sample accepted in the most guideline for mycological testing. For *Aspergillus* tracheobronchitis, the pseudomembrane, tracheobronchial ulceration, plaque, nodule, or eschar detected by bronchoscopy is defined as a definitive sign [38]. However, since bronchoscopy is an aerosol-generating procedure, it is sometimes avoided, particularly in patients with COVID-19.

Culture-Based Methods

Demonstrating hyaline septate hyphae followed by isolation and identifying *Aspergillus* spp. from clinical specimens is the standard approach for establishing a proven case of IA. In cases of pulmonary aspergillosis, the burden of *Aspergillus* in the lung tissue is much higher than that obtained in BAL samples, due to which only culture from invasive samples such as lung tissue biopsy or other sterile sites in case of other systemic IA provides superior specimen for identifying invasive disease [39]. However, this is only sometimes possible in debilitated and critically ill patients making the diagnosis of proven IA difficult.

Table 1 Diagnostic schemes and criteria used to define various spectrum of diseases caused by *Aspergillus* spp

Criteria used	Details	Comment	Reference
Consensus definitions of the Infectious Diseases Group of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC-MSG): invasive aspergillosis in immunocompetent or immunocompromised patient	<p>Proven IA: histopathologic, cytopathologic, or direct microscopic demonstration or culture from sterile aspiration or biopsy specimen with associated tissue damage. Positive PCR with DNA sequencing from FFPE tissue</p> <p>Probable IA (immunocompromised patients): presence of one each of:</p> <ol style="list-style-type: none"> 1. Host factor like neutropenia, transplantation, and immunosuppressant use 2. Clinical feature or pulmonary, sino-nasal, or CNS infection 3. Mycological evidence, e.g., galactomannan antigen, <i>Aspergillus</i> PCR 	<ul style="list-style-type: none"> • There is a paucity of data to support the clinical application of nonculture-based fungal biomarkers such as the GM test • The ISHAM-working group FPCR1 has made progress on developing an <i>Aspergillus</i> PCR standard • The GM test for IA is rendered ineffective when exposed to mold-active antifungals 	[30]
Bulpa Criteria: in COPD patient with GOLD stage III or IV, with recent exacerbation of dyspnea	<ol style="list-style-type: none"> 1. Positive microscopy/or culture for <i>Aspergillus</i> from lower respiratory tract 2. Positive serum antibody test (including precipitins) for <i>A. fumigatus</i> 3. Two consecutive positive serum galactomannan tests 		[31]
Modified Asp/ICU criteria: in ICU patients	<p>One positive blood biomarker (PCR and/or GM) and > 1 criterion among the following:</p> <ol style="list-style-type: none"> 1. Endotracheal aspirate: repeated culture/PCR positive 2. Compatible clinical signs 3. Chest radiography — abnormal 4. 4a. Underlying host risk factors or 4b. Direct microscopy positive + BAL: semiquantitative culture/PCR positive for <i>Aspergillus</i> 		[32]
Influenza-associated pulmonary aspergillosis (IAPA)	<p>Airway plaque, pseudomembrane, or ulcer and at least one of the following:</p> <ol style="list-style-type: none"> 1. Serum GM index > 0.5 2. BAL GM index \geq 1.0 3. Positive BAL culture 4. Endo-tracheal aspirate culture: positive 5. Sputum culture: positive 6. Hyphae consistent with <i>Aspergillus</i> 	<p>May include probable <i>Aspergillus</i> tracheobronchitis or IAPA without documented <i>Aspergillus</i> tracheobronchitis</p>	[33]
Patient with COVID-19 needing intensive care and a temporal relationship — COVID-associated pulmonary aspergillosis (CAPA)	<p>Pulmonary infiltrate, not attributed to another cause (probable pulmonary IA) or tracheobronchial ulceration, pseudomembrane, nodule, eschar, or plaque (Probable tracheobronchitis)</p> <p>And at least one:</p> <ol style="list-style-type: none"> 1. BAL: microscopic demonstration of fungal elements 2. BAL culture: positive 3. Serum GMI > 0.5 4. Serum LFA index > 0.5 5. BAL GMI \geq 1.0 6. BAL LFA index \geq 1.0 7. Plasma, serum, whole blood, or BAL: positive <i>Aspergillus</i> PCR 		[34•••]

Table 1 (continued)

Criteria used	Details	Comment	Reference
Chronic pulmonary aspergillosis (CPA)	<p>CPA is defined as illness for > 3 months and all of the following:</p> <ol style="list-style-type: none"> 1. Persistent cough, hemoptysis, and/or weight loss 2. Chest radiography: progressive cavitary infiltrates and/or pericavitary fibrosis or infiltrates or pleural thickening and/or fungal ball 3. Positive <i>Aspergillus</i> IgG assay or other evidence of <i>Aspergillus</i> infection 		[35]
Allergic bronchopulmonary aspergillosis in patients with asthma: modified ISHAM-ABPA working group criteria	<p>Predisposing condition: presence of asthma or cystic fibrosis</p> <p>Presence of both:</p> <ol style="list-style-type: none"> 1. <i>A. fumigatus</i> specific IgE > 0.35 kU/mL 2. Serum total IgE > 500 IU/mL <p>And two of the following</p> <ol style="list-style-type: none"> 1. <i>A. fumigatus</i> specific IgG > 27 mg/mL 2. Peripheral blood eosinophil count > 500/μL 3. CT thorax: high attenuation mucus (ABPA-HAM); bronchiectasis (ABPA-B) or normal findings (ABPA-S) 	Earlier, the Rosenberg-Patterson criteria were the most commonly used for ABPA diagnosis but there was no agreement on the number of criteria needed and the cut-off value immunological tests were not specified	[36]
Bent and Kuhn criteria — allergic fungal rhinosinusitis	<p>Major criteria:</p> <ol style="list-style-type: none"> 1. History of type I hypersensitivity 2. Nasal polyposis 3. Characteristic CT findings 4. Eosinophilic mucin without invasion 5. Positive fungal stain of sinus contents <p>Minor criteria: history of asthma, unilateral predominance of disease, radiological imaging showing evidence of bone erosion, positive fungal cultures, serum eosinophilia and presence of Charcot-Leyden crystals in surgical specimens</p>	Patients must meet all the major criteria for diagnosis	[37]

Abbreviations: BAL: Broncho-alveolar lavage; CT: Computed tomography; CNS: central nervous system; COPD: Chronic Obstructive Pulmonary Disease; DNA: deoxyribonucleic acid; FFPE: Formalin-Fixed Paraffin-Embedded; FPCR: Fungal PCR initiative; GM: Galactomannan; GMI: Galactomannan Index; GOLD: Global Initiative for Chronic Obstructive Lung Disease; IA: Invasive aspergillosis; IV: Intravenous; LFA: Lateral flow assay; ISHAM: International Society for Human & Animal Mycology; PCR: Polymerase chain reaction

Non-culture-Based Biomarkers

The difficulty in collecting samples for culture or histological examination to diagnose invasive or local fungal infection has stimulated interest in non-invasive diagnostic tests [40]. The fungal cell wall component, galactomannan (GM), has been used to identify patients with IA. At the same time, another popular biomarker, 1,3- β -d-glucan, although suggestive, is not typically specific for mold infection and is not used in Aspergillosis diagnosis [41, 42]. The GM enzyme-linked immunosorbent assay (ELISA) by Platelia and lateral flow assay by IMMY diagnostics are both accepted for the diagnosis of IA [43]. The revised (EORTC/MSGERC) recommends different GM cut-off for various specimens, viz 0.7 for combined single serum/plasma, 0.8 for BAL, and 1.0 for serum/plasma/BAL/CSF for defining IA among neutropenic patients [38]. In non-neutropenic patients, since the immune response is not affected, there is a lower GM in the blood. The disease is usually more locally invasive, due to which a lower GM index cut-off value of 0.5 is used to define positive test among non-neutropenic cases. The detection of GM is simple nevertheless, the presence of false-positive results is a significant disadvantage if the patients are on beta-lactam therapies (piperacillin-tazobactam), and gastrointestinal chronic graft-versus-host disease [44]. Consecutive samples, as well as clinical and radiographic evidence, are thus required to provide a definitive diagnosis [44, 45].

Nucleic Acid Amplification-Based Methods

A polymerase chain reaction (PCR) assay is now accessible, albeit mostly at reference laboratories. The revised EORTC guidelines have also now included *Aspergillus*-positive PCR tests in plasma, serum, or whole blood, as well as BAL fluid for diagnosing IA [38]. Many in-house PCR tests and even some commercially available assays are used for the molecular detection of *Aspergillus* spp. The main utility of PCR has been seen in immunocompromised patients, those with underlying malignancies, and those not taking antifungal prophylaxis. The main concern with using PCR is that the standardization of blood PCR remains tricky. Most tests are validated using *A. fumigatus*, due to which inferior detection of non-*A. fumigatus* species is common. There are efforts made to standardize the various aspects of *Aspergillus* PCR, including the type and volume of specimen used, the DNA extraction procedures, PCR targets and primer, and detection chemistries; however, there is no general recommendation on which PCR tests are preferable. Despite the costs, commercial assays, AsperGenius®, MycAssay Aspergillus®, and MycoGENIE® have the advantage of better species-level identification and simultaneous resistance detection compared to in-house platforms. The PathoNostics AsperGenius assay is a commercially available multiplex real-time PCR that can identify *Aspergillus* and detect four resistance-associated mutations (RAMs), i.e.,

TR34, L98H, T289A, and Y121F in the cytochrome Cyp51A gene. The AsperGenius assay was reported to have a sensitivity and specificity of 84% and 80%, respectively, for diagnosis of IA in suspected patients with underlying hematological malignancies. This assay had a good diagnostic performance on BAL. In a recent study conducted to evaluate the analytical and clinical performance of AsperGenius species in serum samples, the assay's sensitivity and specificity were 78.6% and 100%, respectively, and RAMs were linked to the failure of azole treatment [46].

Next-generation sequencing (NGS) is another emerging technology involving the sequencing of several short DNA fragments followed by computational alignment against a reference genome for the rapid detection, characterization, and genotyping of fungi in clinical diseases with unknown etiology [47]. It also identifies RAMs and virulence-associated genes and studies genetic relatedness during epidemics [48]. Unfortunately, the need for more specialized reference databases makes evaluating sequencing data challenging. While cost is also an impediment, it has decreased over time due to the emergence of cheaper/smaller devices.

Volatile Organic Compounds Detection

The detection of volatile organic molecules (VOCs) is another developing diagnostic test. Exhaled breath contains thousands of VOCs produced by different metabolic pathways [49]. A promising biomarker for IA is 2-pentyl furan which has been detected in neutropenic patients with IA by analysis of exhaled breath using the eNose technology [50]. The sensitivity of assays based on VOCs depends on the host's immunological state, the site of infection, previous antifungal use, and the specimen used.

Combining diagnostic tests may benefit in circumventing the constraints of any one test. Hence, various guidelines have been proposed by the different committees, including the clinical, radiological, and mycological evidence. These guidelines help decide whether or not to start the patient on antifungal agents.

Antifungal Resistance

Antifungal resistance in *Aspergillus* spp. may be intrinsic or develop due to exposure to antifungal agents. Intrinsic resistance to amphotericin B is seen in *A. terreus*, *A. alliaceus* (*A. flavus* complex), *A. tanneri*, and *A. nidulans* with poor response to treatment due to which it is avoided for managing aspergillosis due to these species. Although the molecular mechanism underlying this intrinsic resistance is poorly understood [51], typically, resistance to amphotericin B is mediated by the upregulation of ergosterol biosynthesis genes (ERG5, ERG6, and ERG25) [52]. Azoles should be avoided in IA due to the intrinsic resistance of *A. calidoustus*, *A. tubingensis* (*A. niger* complex), and *A. lentulus* (*A. fumigatus* complex).

The usage of agricultural antifungal drugs that share structural similarities with triazoles, which are mold-active, has been seen to result in a rise in azole resistance, particularly in *A. fumigatus* [53, 54]. The prevalence of azole resistance in *A. fumigatus* isolates is quite high in Europe, such as the UK (6.6–27.8%), the Netherlands (3.1–4.6%), and Germany (3.2%) while relatively lower resistance rates are seen in India (1.75%) [55–58]. This may be attributable to Asia's restricted use of azole fungicides or the lack of sufficient surveillance [54, 55].

In *A. fumigatus*, point mutations in the 14'-lanosterol demethylase (LAD) genes (Cyp51A) [59] and overexpression of Cyp51A [60, 61] have been reported from azole-resistant *A. fumigatus*. The duplication of a few elements in the Cyp51A promoter region results in specific mutations, i.e., Y121F/T289A and L98H in *A. fumigatus* isolates, originating from azole use in agriculture [61, 62]. These mutations cause structural and functional alterations that interfere with the enzyme's binding affinity [63, 64].

Generally, antifungal susceptibility testing is required in areas where the reported resistance rates are above 10%;

otherwise, we can use species-level identification (to rule out intrinsic resistance), national epidemiology, and local susceptibility data to guide the choice of treatment.

Management of Aspergillosis

The most crucial predictor of a favorable outcome remains early and targeted systemic antifungal treatment, particularly in immunocompromised persons. The various antifungal agents used for managing aspergillosis and their pharmacokinetic properties are summarized in Table 2. In general, the duration of treatment for IPA therapies should be 6–12 weeks, considering the extent and length of immunosuppression, location of disease, and signs of improvement. Cavitary and chronic necrotizing pulmonary aspergillosis may require long-term medical therapy for > 6 months.

Table 2 Clinical use and pharmacokinetics of broad-spectrum triazoles, liposomal amphotericin B, and echinocandins in aspergillosis [44, 65]

	Voriconazole	Isavuconazole	Posaconazole	Itraconazole	Liposomal amphotericin B	Caspofungin
Approved indication	Primary therapy: IA Second line: CPA, ABPA	CPA: in case of intolerance/toxicity, resistance, or clinical failure to first and second line (limited data)	Prolonged neutropenia prophylaxis for IA CPA: in case of intolerance/toxicity, resistance, or clinical failure to first and second line (limited data)	Salvage IA therapy ABPA, CPA: primary therapy	IA primary Therapy, empiric febrile neutropenia therapy	IA Salvage or empiric febrile neutropenia therapy
Dosage and formulation	IV: 6 mg/kg Q 12×24 h, 4 mg/kg Q12 starting day 2 PO: 200 mg Q12 h	IV: 200 mg Q8 h×48 h, 200 mg daily starting day 3 PO: 200 mg Q8 h for 48 h, 200 mg daily starting day 3	IV: 300 mg Q12×24 h, 300 mg daily starting day 2 Delayed-release: 300 mg Q12×24 h, 300 mg daily starting day 2 Oral suspension: 200 mg TID	200 mg IV/PO BID-TID×3–4d then 200 mg IV/PO QD-BID	IV: 3 mg/kg/day	IV-70 mg daily on day 1, 50 mg daily starting day 2
Half-life (h)	6	110–115	27–35		7–10	9–11
CNS penetration	High	High (animal model)	Low		High (animal model)	Low (animal model)
Metabolism	CYP2C19, CYP2C9, CYP3A4	CYP3A4/5	UGT		Unknown	

Abbreviations: *ABPA*, allergic broncho-pulmonary aspergillosis; *BID*, bis in die; *CPA*, chronic pulmonary aspergillosis; *CNS*, central nervous system; *IA*, invasive aspergillosis; *IV*, intravenous; *PO*, per oral; *TID*, ter in die.

Adapted from Jenks, J. D., & Hoenigl, M. (2018). Treatment of Aspergillosis. *Journal of Fungi*, 4(3). <https://doi.org/10.3390/jof4030098> [66]

Azoles

Extended-spectrum triazoles are usually the most preferred antifungals used in aspergillosis and include itraconazole, fluconazole, voriconazole, posaconazole, and isavuconazole [67]. Voriconazole is the primary agent of choice for treating all forms of IA including CNS aspergillosis, *Aspergillus* fungal sinusitis, and *Aspergillus* endocarditis [44]. For the treatment of *Aspergillus* endophthalmitis, systemic (oral/i.v), along with intravitreal voriconazole or AmB deoxycholate, is used [44]. Where possible, surgical intervention is indicated for treating *Aspergillus* osteomyelitis and arthritis, as well as sino-nasal aspergillosis in combination with voriconazole [44]. For chronic pulmonary aspergillosis and ABPA, itraconazole therapy, along with glucocorticoids, is the primary therapy [20, 21].

Posaconazole has been approved for prophylaxis in immunocompromised patients with IA, refractory or intolerant to conventional therapy [68]. In a prospective phase 3 study, posaconazole was compared with voriconazole and was found to be well tolerated and non-inferior to voriconazole in patients with IA [69•].

Voriconazole and posaconazole are CYP3A4 inhibitors, and drug interactions are possible when coupled with drugs metabolized by this same pathway. Therapeutic drug monitoring (TDM) is recommended for individuals who receive a long course of azoles, as it helps to maximize therapeutic efficacy and drug toxicity and detect suboptimal drug levels [70]. TDM is recommended for itraconazole, voriconazole, and posaconazole. A trough concentration of > 1 mg/L or a trough:MIC ratio of 2 to 5 is a minimal lower target concentration for voriconazole therapy of underlying illness. Voriconazole trough concentrations of 4–6 mg/L are advised to prevent drug-related toxicity [67, 71]. In patients receiving posaconazole prophylaxis and with established infection, a trough concentration of > 0.7 mg/L and > 1.0 mg/L is a lower target concentration [71]. Itraconazole therapy aims at a trough concentration of 0.5–1 mg/L in preventing and treating IFI.

Isavuconazole is an FDA-approved triazole for treating IA or as an alternative treatment for aspergillosis [72]. It has lower drug-drug interactions, and TDM is not necessary in most cases. Isavuconazole causes QT interval shortening, albeit the clinical importance is undetermined. In the SECURE trial, it exhibited non-inferiority to voriconazole, resulting in FDA approval for IA treatment [73]. Unfortunately, mutations in the *cyp51A* gene reduce isavuconazole efficacy against *Aspergillus* species, rendering it inappropriate for treating IA caused by voriconazole-resistant *Aspergillus* [74].

Echinocandins

Caspofungin, micafungin, and anidulafungin are important fungistatic drugs [75, 76]. Echinocandins are not preferred as monotherapy/primary therapy and are used primarily as salvage therapy in refractory cases or if azoles and amphotericin B are contraindicated. In IPA patients, IDSA recommends combining echinocandin with voriconazole [44]. Furthermore, echinocandins have low blood–brain barrier penetration and cannot be used to treat infections involving the central nervous system [76].

In some cases, combining voriconazole with an echinocandin may be preferable to monotherapy. A systematic evaluation of animal and human studies found improved overall survival when a combination therapy of echinocandin and azole was given. However, to investigate the efficacy of the combination therapy, well-designed RCTs and better clinical trials are required [77].

Polyenes

When voriconazole is contraindicated, amphotericin B deoxycholate and its lipid derivatives are used as primary and salvage therapy for *Aspergillus* infections [44]. Amphotericin B is not recommended in patients with infection due to intrinsically resistant species [8].

Other Considerations

The treatment of chronic or saprophytic aspergillosis differs depending on the condition. Except in symptomatic or immunocompromised patients, where bronchoscopy removal of mucoid impaction is possible, tracheobronchitis does not require antifungal treatment. Single pulmonary aspergillomas, osteomyelitis, endocarditis, or focal CNS disease, and fungal ball of the paranasal sinus require surgical resection [44]. In fungal sinusitis, sinus ostomy enlargement may be required to increase drainage and avoid recurrence, although surgery and antifungal therapy can be performed.

Immunosuppressive medications should be reduced or eliminated as part of anti-*Aspergillus* therapy. The use of recombinant interferon as prophylaxis in patients with CGD or colony-stimulating factors and granulocyte infusions in neutropenic patients may also be administered [44].

The treatment of allergic aspergillosis entails a combination of medical and anti-inflammatory therapy [44]. Systemic glucocorticoids remain to be the most effective medicines in people with ABPA. However, the ideal dose regimen for prednisolone is not currently established due to a scarcity of clinical trials. The most frequent treatment plan is an initial

dose of 0.5 mg/kg daily for 14 days, followed by 0.5 mg/kg every other day, then progressively decreased and finally terminated at 3 months. Omalizumab is a humanized monoclonal antibody that inhibits IgE production. Previous research has revealed that omalizumab could be utilized to treat ABPA, particularly in asthmatic patients [78].

Other antifungals in the pipeline:

1. **Fosmanogepix:** Fosmanogepix is the precursor of manogepix which inhibits glycosylphosphatidylinositol (GPI) synthesis by inhibiting Gwt1. These GPI molecules play a crucial role in cell wall formation and homeostasis maintenance via a highly conserved route. Multiple *Aspergillus* species have shown susceptible MICs in different species. This drug is effective in treating pulmonary aspergillosis in pre-clinical investigations using animal models of the disease [79, 80].
2. **Ibrexafungerp:** This is a titerpinoid oral glucan synthase inhibitor with a slightly different binding site than the echinocandins. It exhibits remarkable in vitro efficacy against both azole-resistant and wild-type *Aspergillus* strains. A phase 2 combination research comparing voriconazole monotherapy with ibrexafungerp + voriconazole in treating IPA is ongoing (SCYNERGIA Study).
3. **Rezafungin:** It is a unique echinocandin that was discovered as a result of a continuous search for new echinocandins that provided alternate dosing regimens to medicines that had already received approval [81]. Rezafungin showed MIC₅₀ and MIC₉₀ values for *A. fumigatus* and *A. flavus* that were within one dilution of the already marketed echinocandins at 0.008–0.015 and 0.015–0.03, respectively [82]. Using the CLSI and EUCAST reference techniques, numerous groups have established echinocandin equivalence. The stability characteristics provide it an advantage by allowing for weekly rather than daily dosing. Studies using neutropenic mice have demonstrated effectiveness against *Aspergillus* and the capacity to delay the onset of sickness and increase survival in prophylactic models.
4. **Olorofim:** This substance is a member of a newly discovered class of antifungals called orotomides, which work by inhibiting the dihydroorotate dehydrogenase enzyme necessary for pyrimidine production. Nearly all *Aspergillus* species have low MICs for olorofim. A mouse model of pulmonary and sinus infection showed a comparable response to posaconazole.

Conclusion

Aspergillosis is a complex of diseases caused by fungi belonging to the genus *Aspergillus*. Clinical manifestations can range from mere saprophytic colonization to allergic illness, superficial infections, and locally invasive

or disseminated invasive aspergillosis. While patients with classical host factors such as neutropenia, immunosuppression, transplantation, and hematological malignancies are at a higher risk of IA, new risk factors, including past influenza, COVID, and critical illness in ICU, are also increasing. However, *A. fumigatus* is the most commonly implicated species in infection; *A. flavus*, *A. terreus*, and *A. versicolor* are also being increasingly reported. An understanding of the diagnostic criteria to be used in different patient groups helps make a diagnosis due to the lack of a single, convenient reference test. Local epidemiological data regarding implicated agents and their antifungal susceptibility profile is also essential to guide management.

Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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●● Of major importance

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