Treatment of Invasive Aspergillosis by Non-*fumigatus* *Aspergillus* spp.

Subjects: Mycology

Contributor: Jannik Stemler, Christina Többen, Cornelia Lass-Flörl, Jörg Steinmann, Katharina Ackermann, Peter-Michael Rath, Michaela Simon, Oliver Andreas Cornely, Philipp Koehler

With increasing frequency, clinical and laboratory-based mycologists are consulted on invasive fungal diseases caused by rare fungal species. *A. flavus* is the second most common *Aspergillus* spp. isolated in patients with IA and the predominant species in subtropical regions. Treatment is complicated by its intrinsic resistance against amphotericin B (AmB) and high minimum inhibitory concentrations (MIC) for voriconazole. *A. nidulans* has been frequently isolated in patients with long-term immunosuppression, mostly in patients with primary immunodeficiencies such as chronic granulomatous disease. It has been reported to disseminate more often than other *Aspergillus* spp. Innate resistance against AmB has been suggested but not yet proven, while MICs seem to be elevated. *A. niger* is more frequently reported in less severe infections such as otomycosis. Triazoles exhibit varying MICs and are therefore not strictly recommended as first-line treatment for IA caused by *A. niger*, while patient outcome seems to be more favorable when compared to IA due to other *Aspergillus* species. *A. terreus*-related infections have been reported increasingly as the cause of acute and chronic aspergillosis. A recent prospective international multicenter surveillance study showed Spain, Austria, and Israel to be the countries with the highest density of *A. terreus* species complex isolates collected.

Keywords: invasive aspergillosis ; epidemiology ; invasive fungal disease

1. *A. flavus*

1.1. General Characteristics and Epidemiology

Aspergillus flavus is the second most frequently isolated *Aspergillus* spp. from clinical samples [1][2][3]. There seems to be a higher incidence of IA caused by *A. flavus* in warm and arid climate zones due to its ability to withstand higher temperatures [4][5]. Therefore, most reports of invasive infections are from sub-tropical countries like India, Pakistan, Saudi Arabia, Tunisia or Mexico [6].

IA due to *A. flavus* usually manifests as IPA but causes bronchopulmonary infections less frequently compared to *A. fumigatus*. Furthermore, it is the most frequently detected fungal pathogen of sinu-orbital and cerebral IFD as well as a frequent causative agent of invasive sinusitis and otitis externa in immunosuppressed patients. It also occurs in cutaneous IA and fungal keratitis, with the highest risk in patients after ocular surgery and diabetes [4][7]. Risk factors for the development of primary osteomyelitis due to *A. flavus* are trauma and diabetes [4][8]. In a review of bone and joint aspergillosis, *A. flavus* was the causative agent in 18% of patients, including the manifestations of mastoiditis, discitis, vertebral osteomyelitis and septic arthritis [9]. Arthritis and/or osteomyelitis usually develop as a secondary infection in disseminated aspergillosis after pulmonary aspergillosis or endocarditis with hematogenous spread [4]. Cardiac aspergillosis due to *A. flavus* has been described rarely and may present as endocarditis or after cardiac surgery and is mainly a healthcare-acquired infection [10][11].

1.2. Diagnosis and Microbiology

Aspergillus flavus grows well on Sabouraud dextrose agar or Czapek Dox and malt extract at 37 °C, and germination of conidia occurs at about 24 h [4].

The key microscopic finding for *A. flavus* is biseriate conidial heads with a yellow to green or brown appearance and dark sclerotia [3]. Besides the general diagnostic approach for IA and conventional microscopic approaches, immunohistochemistry with in situ hybridization with WF-AF-1 or EB-A1 antibodies specific for *A. flavus* (as well as *A. fumigatus* and *A. niger*) may be of use. However, this technique is time-consuming and not available in many centers [12][13].

For serologic testing, the sensitivity of GM was shown to be lower for *A. flavus* when compared to *A. fumigatus* [14]. A combination of an *A. flavus*-targeted PCR-ELISA and GM testing showed an improved rate of early IA diagnosis in patients with hematological malignancies [15]. This may specifically be applied to patients without antifungal prophylaxis.

Susceptibility testing should be performed on all clinically relevant *A. flavus* samples to focus on reported elevated MICs for AmB. *A. flavus* has shown broadly variable MICs with shares between 66.6% and 92% of isolates being higher than ≥2 mg/L for AmB. depending on study and region suggestive of intrinsic resistance [16][17]. In addition, azole resistance seems to play a role in India, with 2.5% to 5% of isolates tested being resistant to commonly used triazoles [18][19]. Resistance mechanisms superior to those described for IA, in general, have been detected for *A. flavus*, such as mutations in the *cyp51a*, *cyp51b* and *cyp51c* alleles as multiple mechanisms responsible for azole resistance [19][20].

1.3. Clinical Management and Treatment

The recommended first-line antifungal treatment agent is voriconazole. Posaconazole and isavuconazole are reasonable alternatives with better tolerability [17][21][22]. Posaconazole has been noted to inhibit the lanosterol 14α-demethylase to a larger extent than other azoles in vitro [4]. In cases with a MIC ≥ 2 mg/L for voriconazole, combination therapy with caspofungin has been suggested to improve overall survival in modeling studies [23], as all echinocandins seem to demonstrate good activity against *A. flavus* in vitro [17].

The proposed ECOFF for AmB by EUCAST ranges at 4 mg/L. Therefore, if available, this may be used to guide therapy in patients with IA due to *A. flavus*. A correlation between the MIC and the clinical outcome of *A. flavus* infections has been described. Thus, L-AmB should be avoided as first-line treatment [16][24].

In patients with fungal sinusitis, fungal endophthalmitis and fungal otitis, bone erosion and subsequent spread to the CNS may occur [4][25]. For these sites of infection, local treatment (e.g., intracameral and intravitreal application) with antifungals is recommended as an addition to systemic therapy [19][26]. In these cases, surgical debridement should be performed to improve outcomes [25].

2. *A. nidulans*

2.1. General Characteristics and Epidemiology

In patients with primary immunodeficiencies, *A. nidulans* is the second most common pathogen causing IA after *A. fumigatus* [27]. Among them, patients with CGD are to be mentioned as particularly susceptible to IFD due to disruption of the NADPH oxidase complex and subsequent lack of neutrophil extracellular trap formation [27][28].

Generally, *A. nidulans* is the third most common fungal pathogen causing osteomyelitis, and besides CGD, risk factors include hematological malignancies, SOT, diabetes, and chronic pulmonary diseases, among others [8]. Lungs are the primary site of infection, but less frequently, skin, lymph nodes and liver may be affected [29]. Furthermore, osteomyelitis due to *A. nidulans* occurs more often compared to *A. fumigatus* and more frequently involves small bones with substantially higher mortality [9][30]. *A. nidulans* infections seem to have a more aggressive course and cause disseminated disease more often [31].

IFDs are the most common cause of death in patients with CGD and are also caused by rare and resistant molds, which may partially be due to the broad and long-term use of antifungals [32][33]. For this population, therefore, systemic triazole-based prophylaxis is generally recommended [34][35]. The fact that *A. nidulans* is rarely found as a causative agent of IFD in other immunocompromised patients than CGD favors the hypothesis of a unique pathogen-host interaction and role of the NADPH-oxidase for innate host-defense, which still needs to be determined [36].

2.2. Diagnosis and Microbiology

Aspergillus nidulans (also referred to as *Emericella nidulans* in its teleomorph form) shows rapidly growing, (dark) green, cream-buff or honey-yellow/orange colonies and reverse dark purplish appearance in culture. Microscopically the conidial heads of *A. nidulans* are short, septated and columnar; the vesicles hemispherical, and the conidiogenous cells biseriate. MALDI-TOF MS analysis may be used to identify *A. nidulans* in clinical samples [37]. GM is detectable in serum in patients with IA due to *A. nidulans*, but is less sensitive [38].

To select antifungal treatment, susceptibility testing is mandatory. For AmB, the MIC was >1 mg/L and up to 4 mg/L in some cases, using the EUCAST method [39]. The lowest MICs were noted for anidulafungin, micafungin, and posaconazole (<1 mg/L). Isavuconazole seems to have good in vitro activity with rather low proposed ECOFFs for *A.*

nidulans as well [40]. In a study with patients with primary immunodeficiencies, *A. nidulans* isolates had low MICs for itraconazole, posaconazole and voriconazole but were elevated for AmB [27]. Thus, innate resistance of *A. nidulans* against AmB has been suggested [41].

2.3. Clinical Management and Treatment

The 2018 ESCMID guideline on the management of *Aspergillus* infections marginally recommends therapy with voriconazole with a low level of evidence, mostly due to the lack of studies involving enough patients with IA due to *A. nidulans* [21]. No primary recommendation was made for AmB, especially for patients with CGD, due to high MICs and documented poor clinical outcomes [31].

In cases with fungal osteomyelitis, the treating team needs to strive for surgical resection alongside a broad systemic antifungal therapy to achieve a higher rate of positive outcomes [8][28]. In those cases, voriconazole should be used for treatment as it provides high oral bioavailability and good penetration into bone tissue [9]. Though rarely performed, allogeneic HSCT represents a therapeutic option for patients with CGD with recurrent and refractory IFD [28]. The novel fungicidal agent olorofim seems to exhibit reasonable activity against *A. nidulans* in a mouse model [42]. In summary, treatment for IA due to *A. nidulans* does not differ substantially from general treatment approaches with close response monitoring when AmB is selected as upfront monotherapy.

3. *A. niger*

3.1. General Characteristics and Epidemiology

The cumulative 1-year incidence of infections with *A. niger* complex in patients with SOT or HSCT was 0.048% in a multicenter cohort study [43]. It is a rarely detected species in IA of post-transplant patients [44][45]. There seems to be an increase in the incidence of IA due to *A. niger* spp. complex from a mean incidence of 0.023 per 10,000 patient days to 0.095 per 10,000 patient days between 2005 and 2011 [46]. Within the *A. niger* spp. complex, *A. tubingensis* was most frequently isolated [46]. A possible explanation for the selection of triazole-resistant *Aspergilli tubingensis* could be the use of fungicides in agriculture or azole-based prophylaxis in certain patient populations [47]. *A. niger* has been reported to be a spore contaminant in burn wards which elucidates the role of hygiene measures for the prevention of IA, including filtered ventilation and decontamination of surfaces and personal equipment [48].

Aspergilli of the section *Nigri* have been identified as colonizers of the nose and throat in immunocompetent patients with other predisposing conditions, such as chronic lung diseases [49]. In this population, *A. niger* was also the most frequently detected pathogen causing otomycosis [50]. In a series of eight proven/probable *A. niger* cases in patients with hematological malignancies, three of which were breakthrough IA during systemic antifungal prophylaxis, the infected sites were the lungs and paranasal sinuses. Patients with AML were particularly at risk, and most acquired the fungal infection during remission-induction chemotherapy. IA-related mortality was as high as 75% [51]. Furthermore, *A. niger* is the second most common *Aspergillus* spp. found in the setting of fungal peritonitis in peritoneal dialysis patients [52].

Overall, larger epidemiological studies have shown a higher survival rate in patients with IA due to *A. niger*—about 80% vs. 66% and 60.5% compared to *A. fumigatus* and *A. flavus*, respectively [53].

3.2. Diagnosis and Microbiology

Aspergilli of the section *Nigri* grow on all mycological media within one to two days, as they do not need special culture conditions. *A. niger* shows black colonies in culture. Microscopically the conidial heads radiate, the vesicles subspherical and the conidiogenous cells biseriate.

The species within the section are morphologically indistinguishable, but identification at the species level is usually not necessary [54]. If required, identification of *A. niger* can also be performed with the MALDI-TOF MS analysis [37]. Where *Aspergillus* isolates cannot be clearly identified by conventional phenotypic methods, DNA sequencing is often helpful [55].

There are no clinical data on the value of GM or (1-3)-ß-D-glucan in serum for diagnosis of invasive infection, but GM is detectable in serum in patients with IA due to *A. niger* [38]. Specific PCR-based assays to detect *A. niger* DNA in clinical specimens are commercially not available.

In general, *Aspergilli* of the section *Nigri* are susceptible to all antifungal drugs, and breakpoints for *A. niger* have been proposed by EUCAST [56]. However, data on antifungal susceptibility testing of *A. niger* isolates seem to be contradictory. CLSI methodology yielded epidemiological cutoff values of 2µg/mL, 1µg/mL, 4µg/mL and 1µg/mL for AmB, and

isavuconazole, itraconazole and voriconazole, respectively, and was the lowest for caspofungin and posaconazole (both 0.25 µg/mL) [57]. Breakthrough IFDs have also been observed under voriconazole therapy, which is why the use of voriconazole has been rather discouraged [46]. Among the *A. niger* spp. complex, *A. tubingensis* shows particularly high MICs to azoles, especially to itraconazole [46][58][59]. It was shown that *A. niger* isolates were 100% resistant to ketoconazole and 33% resistant to AmB and itraconazole but showed no resistance to caspofungin [60]. In summary, MIC values for *A. niger* were generally higher than those for *A. fumigatus*; whether this translates into a poorer clinical response is unknown [61].

3.3. Clinical Management and Treatment

Compared to *A. fumigatus*, infections due to *A. niger* appear to be less virulent and are less frequently associated with invasiveness, as they are up to 10 times more frequently reported in superficial mycoses [45][62]. Nonetheless, invasive *A. niger* infections remain difficult to treat due to varying susceptibility patterns. Guidelines recommend avoiding azole monotherapy due to higher MICs and moderately recommend against the use of itraconazole and isavuconazole for the same reason, however, with a low level of clinical evidence [21][63]. First-line AmB treatment in hematological patients showed a response in only 25% of cases. However, with a very low number (n = 8) of patients treated for IA due to *A. niger* [51]. In cases of peritoneal IA in peritoneal dialysis patients, removal of the indwelling catheter and intra-peritoneal antifungals improves outcome [52]. With the lung being the most frequently affected site in invasive *A. niger* infections, surgical approaches may hardly be feasible. Overall mortality seems to be higher in patients with hematological malignancies and in the ICU compared to those with SOT and chronic pulmonary diseases [46].

4. *A. terreus*

4.1. General Characteristics and Epidemiology

Within the genus *Aspergillus*, *A. terreus* species complex takes a special position, as most representatives are AmB resistant, and invasive infections are frequently associated with the dissemination and poor outcome [64].

A. terreus accounts for four to 12% of clinically relevant *Aspergillus* isolates [65][66]. It is described as the most important species within the section *Terrei* and is found commonly in soil, dust, compost and other environmental sources [67]. It appears that *A. terreus* is a fungal pathogen of increasing importance in patients with IA. In an international multicenter study, the prevalence of *A. terreus* was 5.2% among all reported aspergillosis cases [66].

Generally, patients with hematological malignancies, especially acute leukemia and long-term neutropenia, are at risk of developing *A. terreus* IA. Reporting or observer bias may play a role in the description of the epidemiology of such rare fungal species depending on the local research efforts and the variable contribution to international studies in the field.

4.2. Diagnosis and Microbiology

Molecular and phylogenetic studies divide the *Aspergillus* section *Terrei* into a total of 17 accepted species distributed over three series (*Ambigui*, *Nivei*, *Terrei*) [64]. Only micromorphology or targeted molecular-based analyses support discrimination of this pathogen from other *Aspergillus* species, such as from the sections *Fumigati, Flavi* and *Nigri* [68].

On Czapek or Sabouraud dextrose agar, *A. terreus* colonies grow from beige to buff to cinnamon and are able to become floccose, the reverse is yellow, and yellow soluble pigments are frequently present. Conidial heads are compact, columnar (reaching up to 500 µm long by 30 to 50 µm in diameter), and biseriate; conidiophores are hyaline and smooth-walled; conidia are globose-shaped, smooth-walled, 1.5 to 2.5 µm in diameter, and vary in color from brown to light yellow [64]. Only *A. allahabadii* and *A. neoindicus* produce white conidia. The production of accessory conidia seems to be specific to *A. terreus* species complex, as described for *A. terreus* sensu strictu, *A. citrinoterreus*, *A. hortai*, *A. alabamensis* and *A. neoafricanicus* [64]. The formation of these non-pigmented aleurioconidia, which are settled directly from vegetative hyphae—reveal under both in vitro and in vivo conditions. Additionally, accessory conidia have been described for species of the *Aspergillus* section *Flavipedes*, which seem to form an evolutionary sister branch of the section *Terrei* [64].

4.3. Clinical Management and Treatment

The most relevant clinical feature of *A. terreus* is the intrinsic resistance to AmB. Therefore, identification of *A. terreus* at the species level is crucial to exclude AmB treatment. MIC testing should be performed while clinical breakpoints are available for isavuconazole, itraconazole and posaconazole.

First-line treatment should be based on triazoles which have shown better response rates and greater survival in hematology patients [69].

A. terreus infections seem to be associated with a poorer outcome than compared to *A. fumigatus* infections [69][70]. An almost twice as high rate of disseminated infections has been described in one single-center study, and higher mortality compared to IA caused by other species [69].

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