Acute Invasive Pulmonary Aspergillosis: Clinical Presentation and Treatment

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Abstract

Among all clinical manifestations of pulmonary aspergillosis, invasive pulmonary aspergillosis (IPA) is the most acute presentation. IPA is caused by *Aspergillus* hyphae invading the pulmonary tissue, causing either tracheobronchitis and/or bronchopneumonia. The degree of fungal invasion into the respiratory tissue can be seen as a spectrum, going from colonization to deep tissue penetration with angio-invasion, and largely depends on the host's immune status. Patients with prolonged, severe neutropenia and patients with graft-versus-host disease are at particularly high risk. However, IPA also occurs in other groups of immunocompromised and nonimmunocompromised patients, like solid organ transplant recipients or critically ill patients with severe viral disease. While a diagnosis of proven IPA is challenging and often warranted by safety and feasibility, physicians must rely on a combination of clinical, radiological, and mycological features to assess the likelihood for the presence of IPA. Triazoles are the first-choice regimen, and the choice of the drug should be made on an individual basis. Adjunctive therapy such as immunomodulatory treatment should also be taken into account. Despite an improving and evolving diagnostic and therapeutic armamentarium, the burden and mortality of IPA still remains high. This review aims to give a comprehensive and didactic overview of the current knowledge and best practices regarding the epidemiology, clinical presentation, diagnosis, and treatment of acute IPA.

Keywords
- invasive pulmonary aspergillosis
- EORTC/MSGERC
- intensive care unit
- clinical presentation
- diagnosis
- treatment

Invasive pulmonary aspergillosis (IPA) is a fungal disease caused by *Aspergillus* species. These filamentous fungi are ubiquitous and play important roles in biodegradation. Upon reproduction, airborne *Aspergillus* spores (conidia) efficiently disperse in the environment. Humans inhale 100 to 1,000 conidia daily, which are efficiently cleared from the respiratory tract by various components of the immune system. However, when elements of our antifungal immunity fail (inherited or acquired), *Aspergillus* conidia start germinating into hyphae which may invade the underlying tissue and blood vessels (angio-invasion), resulting in thrombosis, necrosis, and hemorrhage. In the absence of adequate

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interventions, this focal infection may lead to hematogenous dissemination.[1] Historically, IPA was recognized as an opportunistic infection in immunocompromised patients with presence of host factors as defined by the European Organization for Research and Treatment of Cancer and Mycosis Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions.[2] Over the past decades, specific risk factors have been identified. IPA is nowadays increasingly recognized in patients admitted to the intensive care unit (ICU) without classical predisposing immunodeficiency,[3–8] including patients with chronic obstructive pulmonary disease (COPD) exacerbations, severe liver disease, and severe viral pneumonia, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza A and B.[8–14] Other associations with invasive aspergillosis (IA) have been described in critically ill patients with human immunodeficiency virus infection/acquired immune deficiency syndrome, uncontrolled or new-onset diabetes mellitus, alcoholism, and sepsis.[15] In this review, we discuss the clinical presentation, diagnosis, and treatment of IPA in patients with and without EORTC/MSGERC host factors.

Epidemiology

The true disease burden of IPA is difficult to estimate, since comprehensive surveillance data are lacking. Fungal diseases are not obligatory reported, and globally, still many cases of invasive fungal diseases (IFDs) are undiagnosed.[16] The World Health Organization recently positioned *Aspergillus fumigatus* within the critical group of their first-ever fungal priority pathogens list, recognizing the major impact of aspergillosis on global morbidity and mortality.[17] Based on the limited available data, the global incidence of IPA is estimated to be at least 300,000 cases per year, but this is probably an underestimation of the real disease burden.[18]

Patients with Classical EORTC/MSGERC Host Risk Factors

The EORTC/MSGERC consensus criteria were established in 2002 to more homogenously define cases of IA in clinical research, and have been revised in 2008 and 2019, respectively.[2,19,20] Of note, these definitions were explicitly never intended to be used for clinical decision-making. Initially, the focus of these definitions was only on hematology (including hematopoietic cell transplantation [HCT] recipients) and cancer patients. Host factors predisposing to IPA were identified, with two groups at particular high risk: (1) patients undergoing allogeneic HCT and (2) patients with acute myeloid leukemia (AML) receiving intensive remission–induction or consolidation chemotherapy resulting in a period of prolonged and profound neutropenia.[21–23] A case–control study in patients with acute leukemia receiving intensive chemotherapy showed that the rate of IPA increased with 4% per day between the 24th and 36th days of neutropenia. A cumulative frequency of over 70% was observed beyond 34 days of neutropenia.[22] The annual incidence of IA (proven or probable) in allograft recipients was 7.3% (matched-related transplants) to 10.5% (HLA-mismatched or unrelated transplants), and the incidence is particularly increased after engraftment.[24] These data are consistent with data in other studies for allogeneic hematopoietic stem cell transplantation (HSCT) recipeints.[23,25,26] Mortality of IPA is high in patients with classical EORTC/MSGERC host risk factors and also depends on the patient's underlying condition. A multicenter prospective study showed 30% mortality in AML patients,[27] while a systematic review showed a case-fatality rate of 49.3% in patients with leukemia or lymphoma and 86.7% in bone marrow transplant recipients.[28]

Critically Ill Patients without EORTC/MSGERC Host Factors

Reported incidences of IPA in critically ill patients vary widely from less than 1 to 6.9% in some ICU populations.[5–7,29] This large variation is explained by important differences in clinical characteristics of included patients (cohorts with or without presence of classical host risk factors), differences in diagnostic definitions, and extent of fungal work-up. ICU physicians do not always consider the possibility of IPA in patients without classic host risk factors.[30] However, autopsy studies show that IPA remains one of the most common missed diagnoses among patients admitted to a medical ICU[31–34]: in one autopsy study on 893 ICU patients, only 40% of the IA cases diagnosed at autopsy had been recognized ante-mortem.[34] Although classical host risk factors might not always be present, most patients do have underlying comorbidities: in a prospective cohort study of 297 ICU patients with IA, only 5% had no underlying disease.[7] Hereafter, we focus on three medical conditions that increase the risk for IPA in ICU patients: COPD exacerbations, liver decompensation, and severe viral pneumonia. Treatment with systemic steroids is a common denominator in all these subgroups.

COPD

COPD patients display chronically damaged and inflamed respiratory epithelium, mucociliary dysfunction, and impaired clearance of inhaled micro-organisms.[35] Incidence rates of IPA in COPD patients range from 1.13/1,000[36] to 3.6/1,000 COPD admissions.[37] However, in a recent systematic review, the global incidence of IA in COPD patients was substantially higher at 1.3 to 3.9% of hospital admissions.[38] Since there are many COPD patients hospitalized each year, some authors even suggest that they are the main driver of the global burden of IPA.[18,39,40] Almost all COPD patients with IPA are treated with steroids before and during hospitalization.[41] Even inhaled corticosteroids might predispose to IPA, but evidence remains limited to case reports.[42–44] Interestingly, also patients classified as having moderate disease severity (GOLD II stage) are prone to develop IPA when treated with steroids.[45] In patients admitted to the ICU with a COPD exacerbation and diagnosed with IPA, the mortality is estimated at 30 to 60%,[46,47] being twice as high compared to matched controls.[48]

Liver Disease

Both severe alcoholic hepatitis (SAH) and end-stage liver disease (ESLD) predispose patients to IPA. Patients with

severe liver impairment display a dynamic spectrum of immunological perturbations in both the innate and the adaptive immune systems[49,50] and patients with SAH are often treated with high doses of corticosteroids.[51] Most cases of invasive fungal infection in ESLD are caused by *Candida* spp. However, IA remains an important life-threatening fungal infection, occurring in 5 to 14% of critically ill patients with ESLD, mainly in Child C cirrhosis.[9,52] In SAH, reported incidences are even higher: 16 to 42%.[53,54] Despite antifungal treatment, the prognosis is bad: mortality rates reach 70 to 100% in most case series.[9,52,54,55]

Viral-Associated Pulmonary Aspergillosis

While bacterial co-infection in severe viral pneumonia is a well-known complication, fungal co-infection has gained more attention in recent years. Patients presenting with viral-induced acute respiratory distress syndrome caused by influenza A or B virus or SARS-Cov2 are at increased risk for IPA, leading to influenza-associated pulmonary aspergillosis (IAPA) and COVID-19-associated pulmonary aspergillosis (CAPA), respectively.

IAPA

In 2018, severe influenza was identified as an independent risk factor for IPA in critically ill patients.[11] IAPA was found as a superinfection in 19% of patients at a median of 3 days after ICU admission and was associated with an overall mortality of 53%, significantly higher than the 28% mortality in those without IPA.[11] Other studies have confirmed the high overall mortality in patients with IAPA, but incidences vary depending on geographical distribution and extent of fungal work-up.[56–59] In a recent Taiwanese cohort of ICU patients with severe influenza, IAPA incidence was 11% with adequate mycological work-up.[60] An incidence of 7% was reported in a recent Canadian retrospective cohort of ICU patients with severe influenza.[61] In contrast, some European multicentric retrospective studies report IAPA incidences as low as 1 to 2%,[62,63] but rates of galactomannan (GM) testing in these cohorts were very low, leading to underestimation of IAPA cases (see further). Typically, IAPA can be diagnosed early after ICU admission with up to 70% of IAPA cases within the first 48 hours.[58] While IAPA does occur in previously healthy individuals, most have one or more underlying diseases. Although it seems logical to assume that diagnosing and treating IAPA as early as possible will improve the outcome, the mortality remained 53% in a prospective study in which an aggressive diagnostic approach was followed.[58]

CAPA

The increased awareness on IAPA over the past decade has led to a rapid recognition of IPA as a complication in COVID-19 patients requiring mechanical ventilation.[12–14] CAPA incidences among studies vary greatly.[64] Summarizing data from 18 prospective studies on 2,953 critically ill COVID-19 patients, the pooled CAPA incidence (proven, probable, or putative) was 15.1% in a recent review.[65] However, while the absolute number of ICU COVID-19 admissions has gradually decreased, the proportion of severely immunocompromised patients among COVID-19 admissions is rising.[66–68] This shifted patient profile is associated with a dramatically high incidence of CAPA: in a recent monocentric study of COVID-19 patients admitted to a tertiary care medical ICU in Belgium (with high COVID-19 vaccination rates), CAPA was diagnosed in 59% of patients admitted since the vaccination era.[69] In contrast to IAPA, CAPA is diagnosed later during ICU stay: the median time from admission to diagnosis is 6 to 8 days.[12,14,64] Despite angio-invasion being far less frequent than in IAPA (20 vs. 60%), mortality rates are similar, with excess mortality of 16 to 25% compared to patients without aspergillosis co-infection.[70,71]

Clinical Presentation and Diagnosis

Pulmonary aspergillosis can cause diverse clinical manifestations, depending on the strength and type of the host antifungal immune response. We refer to other chapters in this issue concerning other subtypes like chronic pulmonary aspergillosis, aspergilloma, severe asthma with fungal sensitization, and allergic bronchopulmonary aspergillosis. IPA is an acute form of pulmonary aspergillosis, with a disease course of a few days to weeks.

Acute Invasive Pulmonary Aspergillosis: Spectrum of Invasiveness

Pulmonary aspergillosis can be seen as part of a continuum starting from respiratory tract colonization on the one end, to hyphal invasion of bronchi and bronchioles and angio-invasion with subsequent dissemination to other organs at the other end of the disease spectrum (►**Fig. 1**).[67] Colonization can be defined as the repeated isolation of *Aspergillus* from respiratory samples in patients without evidence of associated disease.[72] However, colonization increases the risk of subsequent invasive disease at the time of immunosuppression and/or broncho-epithelial damage, as also described in lung transplant recipients.[73–75] Angio-invasive pulmonary aspergillosis is typically seen in patients with severe and prolonged neutropenia, as neutrophils are primordial in the killing of *Aspergillus* hyphae.[1] Nonneutropenic IPA patients tend to have more tissue invasion than angio-invasion with less outspoken symptoms and slower disease progression.[3] Less frequently, and more typical in a critically ill patient with viral-associated pulmonary aspergillosis (VAPA)[76,77] or after lung transplantation,[78] it can present as invasive *Aspergillus* tracheobronchitis (IATB). IATB is a very aggressive form of IPA with mortality rates in some series exceeding 80%.[76] IATB is the result of a disrupted respiratory epithelium caused by viral lytic effects or following surgery. Since radiological signs of IATB may be absent or very subtle, diagnosis is typically made during bronchoscopy, where it presents as ulcerations, nodules, pseudo-membranes, or plaques in the large airways (►**Fig. 2**). It may be present in up to 25 to 50% of patients with IAPA and in 10 to 20% of patients with CAPA.[10,76,77,79] IATB has also been sporadically reported in patients with hematological malignancy, neutropenia, and COPD.[80] In the next paragraphs, we discuss the clinical signs and symptoms followed by the radiological and mycological

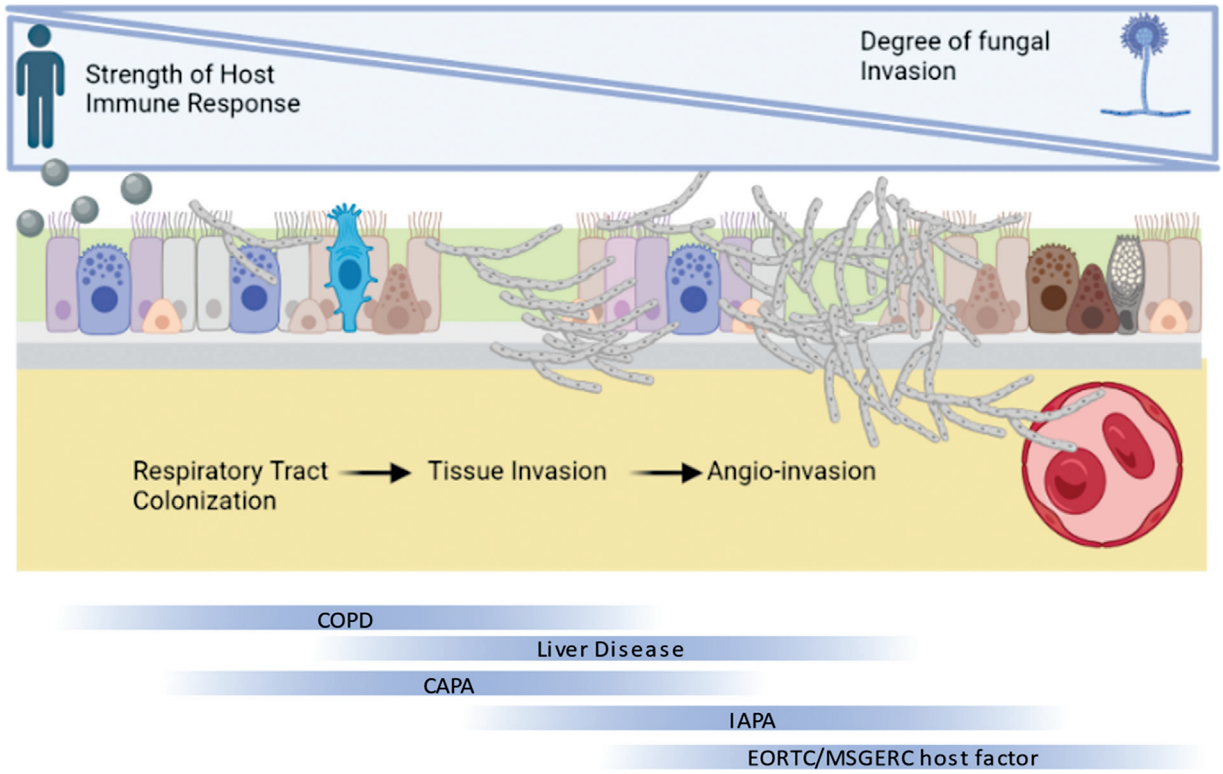

Fig. 1 Acute invasive pulmonary aspergillosis: spectrum of invasiveness. *Acute pulmonary aspergillosis can be seen as a continuum starting from respiratory tract colonization to hyphal invasion of bronchi and bronchioles and finally angio-invasion (with subsequent dissemination to other organs). COPD patients typically display lower grades of angio-invasion, while immunocompromised patients with EORTC/MSGERC host factors are at highest risk of angio-invasion.* COPD, chronic obstructive pulmonary disease; EORTC/MSGERC, European Organization for Research and Treatment of Cancer and Mycosis Study Group Education and Research Consortium. Created with support of BioRender.com.

features of acute IPA. We refer to other chapters in this issue for more details on the complete diagnostic armamentarium.

Clinical Features

Patients with IPA can present a broad range of rather nonspecific symptoms: (prolonged) fever, nonproductive cough, pleuritic chest pain, and hemoptysis (as sign of angio-invasion).[81] In mechanically ventilated patients without typical host risk factors, worsening respiratory insufficiency or unexplained fever in spite of appropriate antibiotic therapy and ventilatory support could also be a sign of IPA presence.[73]

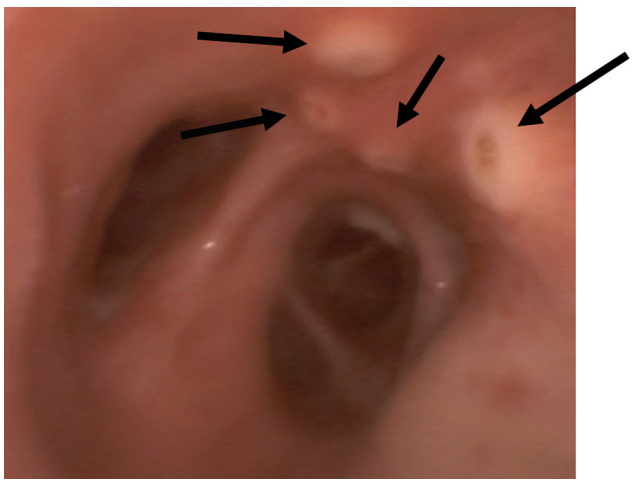

Fig. 2 Invasive Aspergillus tracheobronchitis. Case of invasive *Aspergillus* tracheobronchitis in a critically ill patient with COVID-19. Bronchoscopy shows presence of white nodules with central ulceration in the large airways (arrows).

Radiological Features

The ESCMID-ECMM-ERS (2017) and Infectious Diseases Society of America (IDSA) guidelines (2016) recommend to perform a high-resolution computed tomography (HRCT) in patients at risk for IPA and with fever of unknown origin or with a clinical picture of a lower respiratory tract infection not responding to antibiotics.[82,83] According to the EORTC/MSGERC consensus criteria for IPA, radiographic signs indicative of IPA are presence of nodule(s) with or without a halo sign, air-crescent sign(s), or presence of a cavity within an area of consolidation.[20] In nonneutropenic patients (such as patients on the ICU), however, any radiological image can be seen, ranging from uni- or bilateral consolidations, ground-glass opacifications, cavitations, tree-in-bud features, and pleural effusion.[84] Computed tomography (CT) patterns can vary in time, and it is advised to perform the CT at an early time point, because more specific radiological signs, such as the halo sign, are transient and visible early, while air-crescent signs are seen later in the disease course, reflecting neutrophil recovery and fibrosis of necrotic areas.[85–93] The exact place of positron emission tomography/CT—which shows poor specificity—in the evaluation of fever in a neutropenic patient without response to antibiotic therapy remains to be defined in clinical studies.[94,95]

Mycological Features

Microscopy and Culture

Microscopic visualization of fungal hyphae in inflamed or necrotic tissue or growth of *Aspergillus* species from a normally sterile body site remains the gold standard for diagnosis of proven IFD.[2] This is currently the only technique able to differentiate between colonization and invasive disease; nonetheless the sensitivity of microscopy is only 50%.[82] However, invasive tissue sampling, such as a lung biopsy, is often not possible or too risky. Early bronchoscopy with bronchoalveolar lavage (BAL) sampling, which can be used for culture, microscopy, antigen, or DNA detection techniques, is the most sensitive diagnostic approach. Moreover, bronchoscopy with BAL in critically ill patients is well tolerated with low risk of complications.[96] Culture has the advantage that it can lead to identification to the (sub) species level and phenotypic as well as genotypic antifungal resistance testing.[97,98] While a positive fungal BAL fluid (BALF) culture is compelling evidence in favor of an IPA in the appropriate host, its sensitivity is low (around 30%) and cannot be used to rule out IPA.[11] Blood cultures are almost never positive, even in disseminated IA.[99] A positive fungal culture of sputum or a tracheal aspirate should be interpreted with caution as it may reflect colonization or infection.[100–102] Thus, without a proven diagnosis, diagnosing IPA is subject to estimation of the likelihood, using host factors combined with clinical, radiological, and mycological criteria.[103] Given the limitations of culture and microscopy, non-culture-based diagnostics for IA is an area in full evolution, both on invasive samples (such as biopsy, BALF) and less invasive samples (whole blood, serum, plasma, urine, but also exhaled air).

Galactomannan

GMs are fungal cell wall polysaccharides released by *Aspergillus* (and a few other pathogens) during fungal growth.[104] Detection of Aspergillus GM in blood or BALF is mostly done via an enzyme immunoassay reporting an optical density index (ODI). The double-sandwich ELISA (Bio-Rad, Platelia, Marnes-la-Coquette, France) is the most commonly used. Diagnostic performance of GM detection in BALF is good with an acceptable sensitivity and specificity when using a cutoff ODI of 1.0, even in critically ill nonneutropenic patients.[104–112] Sensitivity of GM on BALF is substantially higher than culture.[106–109] A positive serum GM at a ODI cutoff of 1.0 in a compatible host is highly indicative for angio-invasion but its sensitivity is low early during the disease course, in particular in nonneutropenic patients.[113–118] Concomitant use of mold-active antifungal agents or pretreatment of the BALF with a mucolytic agent to enable GM testing can cause false-negative GM results.[117,119] A positive GM test can precede clinical or radiological features by several days and thus contribute to earlier diagnosis. Several more user-friendly methods (e.g., lateral-flow assays [LFAs] and lateral flow devices [LFD]) have been developed to detect antigens of the cell wall on single patient samples. These assays require less costly instruments and are economically more appealing for centers with lower volumes of immunocompromised patients. Some have been shown to have a diagnostic accuracy comparable to the Platelia test described above.[120,121]

*B*eta(1-3)-D-glucan

Beta(1-3)-D-glucan (BDG) is part of the cell wall of many fungi. Its poor specificity precludes its use to diagnose IA and it is currently not retained in the EORTC/MSGERC diagnostic criteria.[122] Specificity is improved in combination with GM or polymerase chain reaction (PCR).[82] Moreover, false positivity is described in certain contexts, e.g., when antimicrobials such as carbapenems, ampicillin-sulbactam, and some cephalosporins are administered.[123]

Molecular Testing: Aspergillus PCR

As PCR has become a well-established diagnostic method in most microbiologic labs, the use of PCR testing to detect DNA of aspergillus on BAL, blood, and tissue biopsies is increasing. An additional advantage is that certain PCR-based tests can simultaneously detect the presence of mutations in *A. fumigatus* that are associated with azole resistance.[124] However, similar to a positive culture from a nonsterile body site, a positive PCR cannot distinguish between colonization and invasive disease.[125] A positive PCR on BALF seems to have a moderate sensitivity and good specificity.[126–128] However, a recent study suggests that an isolated positive PCR test on BALF (i.e., while the GM test and culture are negative) has limited clinical impact.[129] Sensitivity and specificity on blood tend to be lower,[130–132] but the presence of two consecutive positive PCRs increases specificity, suggesting PCR is a better tool for confirmation of the diagnosis rather than screening.[131,132] A combination of GM and PCR (in hematology patients) also improves diagnostic accuracy for both confirming and excluding IA.[118,133,134] Anti-mold prophylaxis decreases PCR specificity without affecting sensitivity.[135] In these patients, a combination of diagnostic tests, such as GM, LFA, LFD (on serum and BALF), conventional methods (microscopy and culture), and molecular assays can help to diagnose breakthrough infections.[136]

From Definitions of IPA to Diagnostic Algorithms

The abovementioned clinical, radiological, and mycological features culminate into definitions for IPA. While the widely used EORTC/MSGERC definitions have been developed for immunocompromised patients, more recent algorithms focus on the other risk populations. One should however realize that all these algorithms were developed to classify patients in the context of epidemiological and interventional trials. While they can guide clinicians in assessing the likelihood of IPA, they should not be seen as criteria to withhold or start antifungal treatment.

EORTC/MSGERC Definitions for Invasive Fungal Disease

Three disease categories are described, based on degree of certainty of invasive disease: proven, probable, and possible disease. These definitions are summarized in ►**Tables 1** and **2**. Proven disease is defined as a positive biopsy (or

Table 1 EORTC/MSGERC definitions—proven invasive mold disease

Microscopic analysis: sterile material	Culture: sterile material	Blood	Tissue nucleic acid diagnosis
Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage.	Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine.	Blood culture that yields a mold.	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue.

Abbreviations: BAL, bronchoalveolar lavage; EORTC/MSGERC, European Organization for Research and Treatment of Cancer and Mycosis Study Group Education and Research Consortium; PCR, polymerase chain reaction.
Source: Adapted from Donnelly et al 2019[2].

Table 2 EORTC/MSGERC definitions—probable invasive mold disease applied for IPA

Host factors
Recent history of neutropenia ($<0.5 \times 10$ 9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of invasive fungal disease
Hematological malignancy (active malignancy, in receipt of treatment for this malignancy and those in remission in the recent past)
Receipt of allogeneic stem cell transplant
Receipt of solid organ transplant
Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of $\geq$0.3 mg/kg corticosteroids for $\geq$3 weeks in the past 60 days.
Treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days
Treatment with recognized B-cell immunosuppressants, such as Bruton's tyrosine kinase inhibitors, e.g., ibrutinib
Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency)
Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids
Clinical features
The presence of one of the following four patterns on CT:
Dense, well-circumscribed lesions(s) with or without a halo sign
Air crescent sign
Cavity
Wedge-shaped and segmental or lobar consolidation
Mycological evidence
Microscopy and culture
Aspergillus species recovered by culture from sputum, BAL, bronchial brush, or aspirate
Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold
Galactomannan antigen, any one of the following:
Single serum or plasma: $\geq$1.0
BAL fluid: $\geq$1.0
Single serum or plasma: $\geq$0.7 and BAL fluid: $\geq$0.8.
CSF: $\geq$1.0
Aspergillus PCR, any one of the following:

Table 2 (*Continued*)

Plasma, serum, or whole blood two or more consecutive PCR tests positive
BAL fluid two or more duplicate PCR tests positive
At least one PCR test positive in plasma, serum, or whole blood and one PCR test positive in BAL fluid

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; CT, computed tomography; IPA, invasive pulmonary aspergillosis; PCR, polymerase chain reaction.
Source: Adapted and reprocessed from Donnelly et al 2019[2].

positive culture from a primary sterile body site), confirming the fungal infection, regardless of host risk factors and radiological features, and irrespective of the patient's immune status. Regarding GM as a mycological criterium in probable and possible disease, the ODI cut-offs are higher than those proposed by the manufacturer to improve specificity (with little effect on sensitivity).[104]

For nonneutropenic critically ill patients at risk for IPA, using the EORTC/MSGERC consensus criteria for diagnosing IPA is problematic since these criteria require the host to be immunocompromised for possible and probable disease. For these patients, various alternative IPA case definitions for the ICU have been proposed.[8,11,70,100,137–140]

AspICU

The AspICU algorithm was the first diagnostic approach focusing on critically ill patients.[137] The algorithm distinguishes three disease categories reflecting the disease spectrum: proven IPA, putative IPA, and *Aspergillus* colonization and uses a positive *Aspergillus* culture on a respiratory tract sample as entry criterion.[11] The criteria are less strict regarding presence of a host factor and radiological signs. If a BALF culture is positive for *Aspergillus* spp. in combination with microscopic evidence for branching hyphae, the algorithm does not require the patient to have a host factor for diagnosis of putative IPA. Furthermore, the algorithm recognizes that radiological findings are nonspecific. Compared to the histopathological gold standard diagnosis, the AspICU definition of putative IPA reached a sensitivity of 92% and a specificity of 61% in discriminating between *Aspergillus* colonization and invasive disease. However, the AspICU algorithm also has its limitations. First, it can only be used in the presence of a positive *Aspergillus* culture as it did not include a positive serum or BALF GM as diagnostic mycological criteria, despite solid evidence on its diagnostic performance in critically ill patients.[105] Second, with only a specificity of 61%, false-positive rates are relatively high.[141]

IAPA Consensus Case Definition

In 2020, a multidisciplinary expert panel published the expert IAPA consensus case definitions, classifying patients into proven IAPA or probable IAPA.[138] Entry criterion for the algorithm is ICU admission for respiratory distress with a positive influenza PCR or antigen test temporally related to ICU admission. Although most IAPA patients display at least one underlying condition or steroid use, further host factors are not required. The algorithm emphasizes the need for early bronchoscopy with BAL for airway visualization, *Aspergillus* GM testing, culture and microscopy, besides serum GM[138] (see ►**Table 3**).

CAPA: ECMM/ISHAM CAPA Consensus Criteria Followed by the Taskforce Report

In December 2020, the European Confederation for Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM) published the ECMM/ISHAM CAPA expert consensus criteria for diagnosis and management of CAPA, classifying patients as proven, probable, or possible CAPA.[100] It is the first ICU-specific definition to include an *Aspergillus* PCR on BAL fluid or serum as mycological criteria. Given the initial reluctance to perform bronchoscopy in COVID-19 patients, it also includes the possibility to diagnose possible CAPA using nonbronchoscopic lavage fluid (NBLF). The minimum requirement for possible and probable CAPA diagnosis is the combination of one clinical characteristic (e.g., refractory fever or chest pain), one imaging characteristic, and one mycological criterion. The first two requirements are poorly specific and frequently present in critically ill COVID-19 patients. As a consequence, CAPA diagnosis relies heavily on mycological criteria,[100] possibly overdiagnosing CAPA in colonized patients.[142] A later taskforce report removed the possibility of using NBLF and positioned bronchoscopy with BALF as a cornerstone of CAPA diagnosis.[102] This strategy erases the "possible CAPA" label and should increase specificity. Still, the diagnostic accuracy of the individual mycological tests (e.g., fungal culture, PCR, GM) on BALF of patients with COVID-19 is not well-established because this would require a study with many biopsy- or autopsy-proven cases as the gold standard diagnosis. However, given the unfavorable prognosis with increasing positive mycological markers,[143] compromising on specificity might be an acceptable strategy in the clinic.[144]

Future Developments in Diagnostics

The ICU population at risk for IPA is highly heterogenous and different diagnostic criteria exist with limited applicability outside their target population. Therefore, a multidisciplinary expert panel (FUNDICU) is currently developing a standard set of definitions for IFDs in nonneutropenic ICU patients without classical host factors, to harmonize research and clinical management.[145]

Table 3 IPA definitions in the ICU

	Host factors	Clinical signs and symptoms	Radiology	Mycological criteria
AspICU: putative IPA[137]	Neutropenia Underlying hematological or oncological malignancy treated with cytotoxic agents Glucocorticoid treatment (prednisone equivalent >20 mg/day) Congenital or acquired immunodeficiency (Host factors not required if mycological criterion B is present)	Fever refractory to at least 3 days of appropriate antibiotic therapy Recrudescent fever after a period of defervescence >48 hours Pleuritic chest pain or rub Dyspnea Hemoptysis Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support	Abnormal medical imaging by portable chest X-ray or pulmonary CT scan	*A. Aspergillus*-positive lower respiratory tract specimen culture *B.* Semiquantitative Aspergillus-positive culture of BAL fluid without bacterial growth together with a positive cytological smear showing branching hyphae
IAPA expert consensus: probable IAPA[138]	Positive influenza PCR or antigen test temporally related to ICU admission	ICU admission for respiratory distress Airway plaque, nodule, eschar, pseudo-membrane or ulcer on bronchoscopy for diagnosis of IATB	Any pulmonary infiltrate + mycological criterion A Cavitating infiltrate (not attributed to another cause) and mycological criterion B	A. Serum GM index > 0.5 or BALF GM index ≥ 1.0 or positive BALF culture B. Positive sputum culture or positive tracheal aspirate culture
ECMM/ISHAM CAPA: probable CAPA[100]	Positive COVID-19 PCR temporally related to ICU admission	ICU admission for respiratory distress Airway plaque, nodule, eschar, pseudo-membrane or ulcer on bronchoscopy for diagnosis of IATB	Pulmonary infiltrate preferably documented by chest CT Cavitating Infiltrate not attributable to another cause	Microscopic detection of fungal elements in BALF positive BALF culture Serum GM >0.5 or serum LFA >0.5 BALF GM ≥1.0 or BALF LFA ≥1.0 ≥2 positive aspergillus PCR tests in blood 1 positive A*spergillus* PCR in BALF (<36 cycles) 1 positive *Aspergillus* PCR in blood and a single positive PCR in BALF

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CT, computed tomography; ICU, intensive care unit; IATB, invasive *Aspergillus* tracheobronchitis; IPA, invasive pulmonary aspergillosis; PCR, polymerase chain reaction.
Source: Adapted from Blot et al,[137] Verweij et al,[138] and Koehler et al[100].

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Diagnostic Flowcharts

Based on the aforementioned criteria and definitions, we propose two flowcharts for diagnosis of IPA in patients hospitalized outside or inside the ICU, respectively (►**Fig. 3A and B**). By combining clinical features, radiological features (especially HRCT) and mycological features in appropriate hosts and according to the diagnostic definitions, a diagnosis of proven, probable, putative, or possible IPA can be made. If safe and feasible, bronchoscopy (or lung biopsy) should be performed given the better diagnostic performance of culture, microscopy, biomarkers, and molecular testing on BALF.

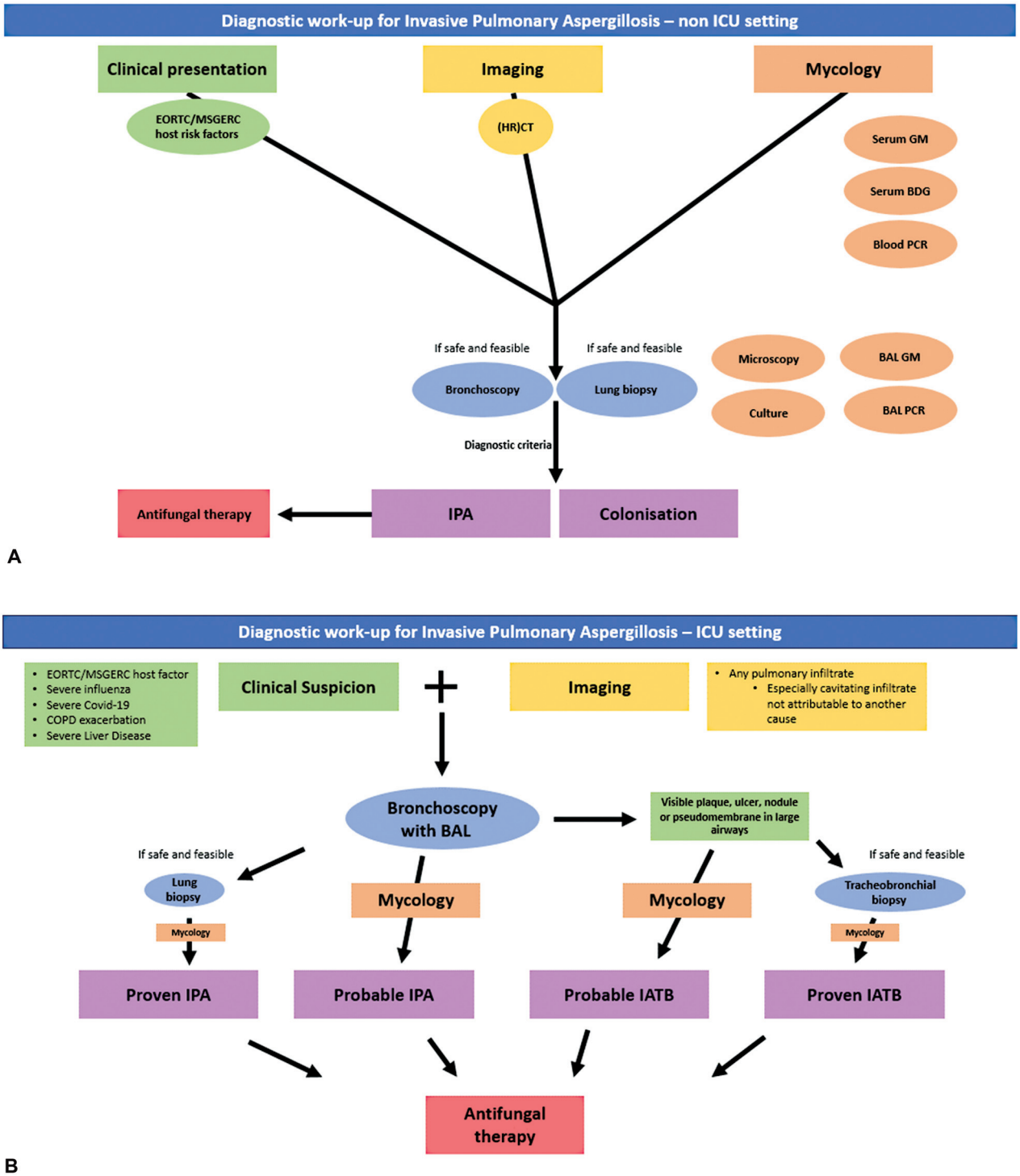

Fig. 3 (**A**) Diagnostic flowchart for IPA in the non-ICU patient. **(B)** Diagnostic flowchart for IPA in the ICU patient. *Cornerstone of IPA diagnosis in the ICU is bronchoscopy with bronchoalveolar lavage (BAL). In most cases, bronchoscopy can be performed bed-side and allows to both macroscopically screen the large airways for Aspergillus tracheobronchitis and take samples for mycological testing.* ICU, intensive care unit; IPA, invasive pulmonary aspergillosis.

Especially in ICU patients, bronchoscopy with BALF is the key to IPA diagnosis. First, bronchoscopy allows to macroscopically visualize the larger airways to pick-up IATB and it is the way to obtain a representative BAL sample for mycological analysis. Only low BALF volumes are required (maximum 40 mL) for mycological analysis and bronchoscopy is generally well tolerated in (mechanically ventilated) critically ill patients, with low risk of complications (even in patients in prone position or on extracorporeal membrane oxygenation).[96] Second, performing HRCT in mechanically ventilated patients is often complex or even impossible due to safety and/or logistical reasons. Those diagnostic flowcharts, in combination with the aforementioned IPA definitions, can be used to make a bed-side diagnosis of IPA, as a trigger antifungal therapy,

Treatment

Early and reliable diagnosis of IA and subsequent rapid initiation of appropriate antifungal therapy is of highly importance for survival.[146]

First-Line Antifungal Treatment

The IDSA, ESCMID-ECMM-ERS, ECIL-6 guidelines and recently updated Australian guidelines recommend primary treatment with a mold-active azole (voriconazole, isavuconazole, or posaconazole).[82,83,97,147] To date, there are no specific guidelines concerning treatment of critically ill patients with IPA in the ICU. Isavuconazole and posaconazole proved to be noninferior to voriconazole with fewer drug-related adverse events.[58,148] The azole of choice depends on drug availability, patient characteristics, location of infection, and concomitant drug administration. For patients in whom primary therapy with azoles is contraindicated, liposomal amphotericin B (AmB) is the alternative drug of choice.[149] The role of combination therapy remains unproven.[81]

A few aspects of triazole therapy are important; see the following sections.

Drug–Drug Interactions and Therapeutic Drug Monitoring

Triazole antifungals show important drug–drug interactions (mainly via the CYP3A4 and CYP2C19 pathways) and may exhibit important pharmacokinetic variability.[150] Polymorphism of CYP2C19 is important for voriconazole.[151] Therapeutic drug monitoring (TDM) is recommended for itraconazole, voriconazole, and posaconazole[82,83] but not routinely for isavuconazole.[150] In addition, TDM is generally recommended in patients with variable or changing pharmacokinetics, such as impaired gastrointestinal function (e.g., severe graft-versus-host disease [GVHD], mucositis), hepatic failure, renal failure, critical ill patients and upon intravenous-to-oral switch. The IDSA guidelines also recommend TDM in patients with major drug–drug interactions and severe disease (central nervous system [CNS] infection, disseminated infection) and it should be performed in the diagnostical work-up of refractory, relapsed disease, or breakthrough infection.[83]

Azole Resistance

Azole resistance is important and therefore testing for drug susceptibility by conventional and/or molecular methods should be considered in patients suspected to have an azole-resistant isolate or who are unresponsive to antifungal agents.[82,83]

Toxicity

All azole antifungal agents show hepatoxicity and toxicity of the peripheral nerves, the latter due to long-term exposure. Itraconazole, voriconazole, and posaconazole increase heart-rate corrected QT interval (QTc). On the contrary, isavuconazole shortens QTc. Intriguingly, posaconazole is associated with pseudo-hyperaldosteronism. Important to mention is the transient central nervous system adverse events of voriconazole, such as visual disturbances and hallucinations. Long-term side effects of voriconazole include skin cancer and periostitis.[150,152]

Salvage Therapy

Treatment of refractory or progressive Aspergillosis is considered as salvage therapy.*[153] Treatment failure implies that the patient's compliance is checked, adequate drug exposure is verified by TDM, immune reconstitution syndrome is excluded as well as alternative diagnoses. Indeed, co-infection of IA with mucormycosis is not uncommon and has important therapeutic implications and impact on survival.[154,155] Retrospective data show that mortality in patients with proven/probable azole-resistant IPA is much higher when they are treated with an azole first and switched to second-line therapy at a later point in time.[156] In patients showing radiological progression after 10 to 14 days of therapy despite adequate azole drug levels, we therefore typically try to repeat the bronchoscopy and do (or repeat) molecular testing for the presence of Mucorales species DNA and mutations associated with azole resistance. Testing for the presence of Mucorales species DNA on plasma may also be of value in this context.[157]

*Persistent IFD is any IFD that has not changed/is stabile since treatment initiation with ongoing need for antifungal therapy. Refractory IFD is defined as progressive disease under treatment. Relapsed IFD occurs after a completed treatment with the same pathogen at the same infection site.

To date there is no high-quality evidence for salvage therapy partly because of the heterogeneity of the studies, small sample size, use of several definitions, and confounding factors such as progressive underlying disease and co-infections.[158,159] Generally, it is advised to use a drug from a different antifungal class.[83] Patients who are progressing under triazole therapy despite adequate serum drug levels can therefore be treated with liposomal AmB, or an echinocandin.[160–162] When a co-infection with Mucorales species cannot be excluded with PCR, liposomal AmB is preferred. The possibility of a surgical intervention should be evaluated as well.[83]

Surgery

Surgery, adjunctive to antifungal therapy, can be considered in localized disease that is easily accessible, such as

infections of the respiratory sinuses and skin infections with IA. Moreover, neurosurgical removal of isolated large CNS lesions and surgical removal of growing solitary pulmonary lesions close to large blood vessels should be considered. In case of uncontrolled bleeding, surgical intervention or embolization of the afferent vessel should also be considered.[83]

Immune Reconstitution

Colony-stimulating factors (CSFs) in prophylaxis reduce the incidence of neutropenic fever and infection-related mortality, in the absence of serious adverse events.[163] Also, in vitro and murine studies suggest a potential role for CSF in patients diagnosed or suspected with IA, although clinical data are lacking.[164,165] According to a prospective study, there could be a potential role for high-dose granulocyte transfusions in neutropenic patients with bacterial and fungal infections.[166] The IDSA guidelines consider the role of granulocyte infusions in neutropenic patients with severe infections, such as IA, who are unlikely to respond to standard treatment or adjunctive to salvage antifungal therapy.[83] Adverse reactions include acute lung injury (especially when simultaneously administered with AmB)[167] and graft failure in allogeneic HSCT recipients due to alloimmunization.[168]

It is hypothesized, on the basis of in vitro and in vivo data, that immune checkpoint inhibitors could have a role in the treatment of IFD, such as IA, in patients with AML. Immune checkpoint inhibitors could attenuate the progression of IA and synergize with antifungal therapy, although evidence from clinical studies is lacking.[169]

There is limited evidence in favor of immunomodulation with recombinant interferon-gamma (r-IFN-γ) as adjunctive therapy for IFDs in chronic granulomatous disease[170] and leukemia.[171] r-IFN-γ could be considered in patients with severe or refractory IA according to the IDSA guidelines, taking into account that high-quality clinical evidence is lacking.[83]

Treatment Strategies

On the one hand, there is the preventive approach in which prophylactic antifungal therapy is started in high-risk patients to prevent IFDs. To date, evidence is mainly available in hematological patients. On the other hand, there is the empiric and pre-emptive approach in which treatment is initiated in high-risk patients based on clinical, radiological, and microbiological features.

Primary Prophylaxis

In primary prophylaxis, an antifungal agent is initiated based on a risk assessment, before the onset of any clinical manifestations of IFD. Typical at-risk patients include those with prolonged and severe neutropenia,[82,83,172] lung transplant patients,[173] or patients at the ICU with severe viral pneumonia.[58] For instance, posaconazole was shown to be more effective than fluconazole or itraconazole in preventing IFD in patients undergoing chemotherapy for AML or myelodysplastic syndrome or in allogeneic HCT recipients with grade 2 or higher GVHD.[174] Itraconazole, voriconazole, echinocandins, and aerosolized liposomal AmB (in combination with fluconazole) are less-well studied alternatives.[82,83,172,175,176] Data on isavuconazole[176] in primary prophylaxis and rezafungin[177] in primary prophylaxis are limited and studies are still ongoing.

The Empiric Approach

In the empiric approach, antifungal therapy is initiated in high-risk patients with persistent neutropenic fever despite broad-spectrum antibiotics. This strategy reduces morbidity and mortality when a diagnostic-driven approach is out of reach.[178–182]

The Diagnostic-Driven (Pre-emptive) Approach

In the pre-emptive approach, antifungal treatment is initiated based upon biomarker positivity or diagnostic imaging in high-risk neutropenic patients not receiving mold-active prophylaxis, as this implies the diagnosis of probable IFD which should be covered by treatment. Studies have shown that this strategy is effective while reducing the consumption of antifungals.[180,181,183] A Cochrane review confirmed that the pre-emptive approach did not increase all-cause mortality nor IFD-related mortality and that it may reduce the duration and use of antifungal agents, compared to the empirical approach.[184] A recent randomized prospective trial showed that the pre-emptive antifungal approach was also safe for high-risk neutropenic patients (in comparison to the empirical approach), concerning overall survival at day 42. There was no difference in IFD rates, and this approach reduces the use of antifungals.[185] Serial biweekly screening of serum GM in high-risk patients not on mold-active prophylaxis is typically part of the diagnostic-driven approach.[82,119,186]

Secondary Prophylaxis

Secondary prophylaxis is the initiation or continuation of antifungals to prevent recurrence of IA in patients with a history of successfully treated IA and entering a subsequent risk period of immunosuppression. The ESCMIDD-ECMM-ERS guidelines recommend secondary prophylaxis with the antifungal proven to be previously effective.[82] Typically, the primary therapy that was successful in the particular patient is continued as secondary prophylaxis. Voriconazole tends to be a good option in allogeneic HSCT recipients,[187] and there are limited data for caspofungin and liposomal AmB in these patients.[188,189]

Response

Response Criteria

The EORTC/MSGERC also established response criteria for IFD. However, these criteria date from 2008[190] and are under revision because there are definitely shortcomings in the definitions, e.g., considering "stable disease" as failure in highly immunocompromised patients.[191]

Response is undivided in success (complete response, partial response) and failure (stable disease, progressive disease, death) and is also based on clinical, radiological, and mycological data. These response criteria are summarized in ►**Table 4.**

Table 4 Responses to antifungal therapy in patients with invasive mold disease

Outcome, response	Criteria
Success	
Complete response	Survival and resolution of all attributable symptoms and signs of disease; plus
	Resolution of radiological lesion(s); persistence of only a scar or postoperative changes can be equated with a complete radiological response; plus
	Documented clearance of infected sites that are accessible to repeated sampling (e.g., mold disease involving the palate, sinuses, or cutaneous lesions)
Partial response	Survival and improvement of attributable symptoms and signs of disease; plus
	At least 25% reduction in diameter of radiological lesion(s); plus
	Documented clearance of infected sites that are accessible to repeated sampling (e.g., mold disease involving the palate, sinuses, or cutaneous lesions)
	In cases of radiological stabilization (defined as a 0–25% reduction in the diameter of the lesion), resolution of all attributable symptoms and signs of fungal disease can be equated with a partial response
	In cases of radiological stabilization, biopsy of an infected site (e.g., lung biopsy) showing no evidence of hyphae and negative culture results can be equated with a partial response
Failure	
Stable response	Survival and minor or no improvement in attributable symptoms and signs of disease; plus
	Radiological stabilization (defined as a 0–25% reduction in the diameter of the lesion); or
	Persistent isolation of mold or histological presence of invasive hyphae in infected sites
Progression of disease	Worsening clinical symptoms or signs of disease; plus
	New sites of disease or radiological worsening of pre-existing lesions; or
	Persistent isolation of mold species from infected sites
Death	Death during the prespecified period of evaluation regardless of attribution

Source: Adapted from Segal et al 2008[190].

Follow-Up of Treatment

Duration of Antifungal Therapy

The duration of antifungal therapy is not well defined. The IDSA guidelines[83] recommend a minimum of 6 to 12 weeks depending on patient characteristics and clinical factors of the disease, such as clinical response, immune reconstitution, and recovery from GVHD.[82] Probably, antifungal treatment duration in the setting of VAPA might be shorter, although this needs to be confirmed in prospective clinical trials.

When to Assess Response?

The ESCMID-ECMM-ERS guidelines suggest to assess response after 2 weeks of treatment. While the ECIL-6 nor the IDSA-guidelines give clear recommendations.[83,147] An expert panel has recommended reasons for changing first-line antifungal treatment, as shown in ►**Table 5**.[191]

Follow-Up Imaging

The same applies to the timing of follow-up CT imaging in IPA, which is definitely best decided on a patient-by-patient basis.[83] Generally, it is accepted that follow-up CT-scan is performed at the earliest 2 weeks after the start of therapy, because it is known that IPA usually worsens during the first week of treatment.[91,192] Also, immune reconstitution[**] after neutrophil recovery or tapering of immunosuppression can result in an increase of lesions.[193] Earlier imaging may be considered in cases of clinical deterioration, suspected alternative diagnosis, or complications such as invasion of the great vessels.[97]

[**]Immune reconstitution inflammatory syndrome can be defined as clinical or radiological worsening concerning progressive IA, temporally related to neutrophil recovery and no change in antifungal therapy.

Biomarkers

Future methods of monitoring response are being investigated. As serial mycological examinations tend to be impractical or too invasive for the patient, new indirect tests are emerging for both diagnosis and monitoring of IPA response. While the GM assay is already implemented in the revised and updated EORTC/MSGERC criteria as a mycological criterium for diagnosis of IPA,[2] serum GM is also a promising biomarker to monitor therapeutic response and could be used as a prognostic tool, especially in hematological

Table 5 Reasons for changing first-line antifungal treatment

Days since initiation of therapy	Clinical and diagnostic findings compared with baseline
At any time	
	Identification of a pathogen resistant to primary antifungal therapy
8–14	
	On the basis of changes in GM:
	(i) Serum: the serum GM index has not fallen by either 1 unit or to <0.5 unit based on measurements taken at least 7 days apart (ii) BAL: positive GM from BAL in a patient with a previous BAL test that did not meet the definition of positive (too low or entirely negative) without regard for the interval of time between samples. Note that there is not a definition for rising GM index values from BAL as these values are subject to sampling error Or
	Clinical deterioration consistent with persisting or progressive invasive fungal disease with no other identifiable etiology Or
	New distinct site of infection detected clinically or radiologically
≥15	
	Any of the above criteria Or
	Progression of original lesions on CT (or other imaging) based on >25% growth of initial lesions in the context of no change in immune status

Abbreviations: BAL, bronchoalveolar lavage; GM, galactomannan.
Source: Adapted from Slavin et al 2022[191].

patients.[194–200] Serum GM kinetics assessed at IPA diagnosis and after 1 week of antifungal therapy correlate well with mortality at 6 and 12 weeks.[200] Both initial serum GM and decrease of GM in response to antifungal treatment are important parameters for survival.[201] However, despite the lower sensitivity of serum GM for the diagnosis of IA in nonneutropenic patients, solid organ-transplant recipients, and patients on mold-active antifungal prophylaxis, prognostic properties of serum GM tend not to be influenced in these populations.[194]

Conclusion

IPA is a life-threatening fungal infection, caused by invasion of *Aspergillus* species in the lung. While IPA is well-known in immunocompromised patients with the presence of host risk factors as defined by the EORTC/MSGERC, IPA is increasingly recognized in critically ill patients. Clinicians must be aware and should be sufficiently aggressive in their search for the fungus. After all, the clinical presentation is highly nonspecific, and diagnosis is easily missed. Early bronchoscopy with BAL for both culture- and nonculture-based mycological tests are crucial in diagnosis, especially in critically ill patients. First-choice antifungal drugs are triazoles and the choice of drug should be made on an individual basis. Early and reliable diagnosis and subsequent rapid initiation of appropriate antifungal therapy are crucial for this disease, which still has a high mortality in both immunocompromised and nonimmunocompromised patients.

Conflict of Interest
None declared.

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