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## Management of invasive pulmonary aspergillosis in non-neutropenic critically ill patients

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**Abstract** During recent years, a rising incidence of invasive pulmonary aspergillosis (IPA) in non-neutropenic critically ill patients has been reported. Critically ill patients are prone to develop disturbances in immunoregulation during their stay in the ICU, which render them more vulnerable for fungal infections. Risk factors such as chronic obstructive pulmonary disease (COPD), prolonged use of steroids, advanced liver disease, chronic renal replacement therapy, near-drowning and diabetes mellitus have been described. Diagnosis of IPA may be difficult and obtaining histo- or cytopathological demonstration of the fungus in order to meet the gold standard for IPA is not always feasible in these patients. Laboratory markers used as a non-invasive diagnostic tool, such as the

galactomannan antigen test (GM), 1,3- $\beta$ -glucan, and *Aspergillus* PCR, show varying results. Antifungal therapy might be considered in patients with persistent pulmonary infection who exhibit risk factors together with positive cultures or sequentially positive GM and *Aspergillus* PCR in serum, in whom voriconazole is the drug of choice. The benefit of combination antifungal therapy lacks sufficient evidence so far, but this treatment might be considered in patients with breakthrough infections or refractory disease.

**Keywords** Invasive pulmonary aspergillosis · Non-neutropenic critically ill · Antifungal therapy · Serological markers

### Introduction

Invasive pulmonary aspergillosis (IPA) has emerged as an important cause of morbidity and mortality in patients receiving intensive chemotherapy, allogeneic stem cell transplantation, and solid organ transplantation. However, during recent years, several reports have described a rising incidence of IPA in critically ill patients admitted to the intensive care unit (ICU), even in the absence of an apparent predisposing immunodeficiency [1–6]. The incidence of IPA in the ICU ranges from 0.3% to as much as 5.8% [2, 3, 6] and it carries an overall mortality rate exceeding 80%, with an attributable mortality of

almost 20% [4, 5]. This high mortality is at least partially related to difficulties in timely diagnosis, caused by insensitive and non-specific clinical signs and lack of unequivocal diagnostic criteria.

In this review, we will describe the pathophysiological mechanisms and risk factors for IPA in non-neutropenic critically ill patients, limitations and advances in the diagnostic process, and alterations in treatment with antifungal therapy. A Medline/PubMed search was performed for all articles about IPA in critically ill patients in relation to risk factors, diagnosis and antifungal therapy. All publication types of human studies in the English language were searched and an extraction of relevant articles was made for the purpose of this narrative review.

## Pathophysiological mechanisms and risk factors

*Aspergillus* spp. are opportunistic moulds that cause both allergic and invasive syndromes. The genus *Aspergillus* contains approximately 175 species, only a minority of them have been associated with human disease. Infections are caused mostly by *Aspergillus fumigatus*; next in line are *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus nidulans* [7]. *Aspergillus* is found in soil, water, food, and in the air and grows on a wide variety of organic material, such as decaying vegetation. The conidia (spores) are easily aerosolised. The route of transmission is by air. Although exposure is universal, invasive infection occurs almost exclusively in immunocompromised individuals. Infections have frequently been described in patients with haematological malignancies and solid organ transplant recipients, but also in patients undergoing chronic intermittent haemodialysis in whom these infections were associated with hospital construction and/or ventilation systems contaminated with *Aspergillus* spp. [8]. Even hospital water is a frequently overlooked source of nosocomial aspergillosis [9, 10].

Natural antifungal defence in humans is based on normal mucosal barriers and an intact macrophage and neutrophil function. Alveolar macrophages form the first line of defence against inhaled *Aspergillus* conidia that reach the alveoli. Macrophages normally are capable of killing the conidia and preventing germination, by releasing cytokines such as tumour necrosis factor (TNF)- $\alpha$  and macrophage inflammatory protein (MIP)-1 $\alpha$  [11]. During neutropenia, TNF- $\alpha$  and MIP-1 $\alpha$  synthesis is reduced and the conidia can germinate to form hyphae. T-cell mediated acquired immunity also has an important role in protecting against fungal infections, as *Aspergillus* antigens are able to induce T-helper (Th)-1 and Th-2 type reactivity [12, 13]. Th-1 reactivity is displayed by an increase of interferon- $\gamma$  and interleukin (IL)-12 and has protective effects against infection. In contrast, Th-2 reactivity is characterised by production of IL-4 and IL-10 and leads to disease progression, at least in a murine model of IPA [12, 14, 15].

Critically ill patients in ICU exhibit a complex change in immune function characterised by deactivation of macrophages and an altered cellular response due to the severity of illness which is also termed “immunopar-

alysis” [16, 17]. This immunologic derangement might explain why *Aspergillus* infections are able to develop in critically ill patients who do not display the predisposing classical risk factors [17, 18]. Many other factors will negatively influence the immune function during critical illness, such as (acute) hyperglycaemia [19] and the use of corticosteroids [20–23]. Corticosteroids have profound effects on the distribution and function of neutrophils, monocytes, and lymphocytes and they directly stimulate the growth of *Aspergillus fumigatus* in vitro possibly via sterol binding proteins in the fungus [21]. In particular intravenous corticosteroids treatment in patients with chronic obstructive pulmonary disease (COPD), is associated with a rising incidence of IPA [1, 24–26]. Also broad-spectrum antibiotics, which affect the distribution of normal flora, have been described as a risk factor [27]. However, not every critically ill patient in the ICU is at risk for developing invasive fungal infections. Apparently, other specific – patient-related – predisposing conditions seem to be associated with the development of IPA, in which COPD and other chronic lung disease [1–4, 28–32], diabetes mellitus [2, 31, 33], acute liver failure/advanced liver cirrhosis [2–4, 34], chronic renal failure [8, 35], and near drowning [4, 36–38] have been described (Table 1). In these, mainly retrospective, studies a mean in-hospital mortality of 80% was found in patients with highly suspected or proven IPA in the presence of at least one of these underlying conditions, despite antifungal therapy. Remarkably, patients who were suspected to be colonised only with *Aspergillus* spp. (i. e. no signs of pulmonary infection) demonstrated even a high in-hospital mortality rate [3, 31], which might suggest that colonisation should be considered as a potentially important finding.

## Diagnostic features

A few years ago, a consensus for standard definitions and diagnosis for invasive fungal infections in immunocompromised patients with cancer and recipients of haematopoietic stem cell transplants was established by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) in which three levels of probability for invasive fun-

**Table 1** Risk factors for IPA in non-neutropenic critically ill patients in the ICU

Risk factor	Reference
COPD in combination with prolonged corticosteroid use	[1, 24–26]
High-dose systemic corticosteroids > 3 weeks (e. g. prednisone equivalent > 20 mg/day)	[2, 31, 33]
Chronic renal failure with RRT	[8, 35]
Liver cirrhosis/acute hepatic failure	[2–4, 34]
Near-drowning	[4, 36–38]
Diabetes mellitus	[2, 3, 31, 33]

COPD, Chronic obstructive pulmonary disease; RRT, renal replacement therapy

gal infections are proposed: “proven”, “probable” and “possible” [39]. However, these guidelines are intended for use in clinical trials and for epidemiologic research and, moreover, are focused on patients with classical risk factors such as neutropenia, malignancies and after haematopoietic stem cell transplantation, and it may not be possible to extrapolate them to the non-neutropenic critically ill patient.

The diagnosis of IPA in non-neutropenic critically ill patients is difficult because signs and symptoms are non-specific, and the initiation of additional diagnostic examinations is often delayed because of a low clinical suspicion. For the timing of suspicion of IPA in these patients, the combination of persistent or rapid developing infiltrative abnormalities on thoracic imaging and/or a persistent pulmonary infection despite broad spectrum antibiotics accompanied by one or more predisposing conditions (Table 1) might be the moment for triggering further diagnostic exploration. Although histopathological evidence of IPA is defined as the gold standard due to a very high tropism for blood vessels [39], trans-bronchial biopsy or surgical lung biopsy via mini-thoracotomy may not justify the risk of this invasive procedure in critically ill patients on mechanical ventilation with, sometimes, severe bleeding diathesis. Therefore, the diagnostic process will contain thoracic imaging and microbiological examination by means of direct microscopy and culture of sputum or broncho-alveolar lavage fluid (BALF). Excluding the possibility of contamination during the pre-analytical phase of a sample, isolation of *Aspergillus* spp. in the respiratory tract may represent three clinical situations: (1) evidence of current disease, (2) true colonisation, or (3) a marker for the future development of invasive disease. In the immunocompetent host, cultures of *Aspergillus* in respiratory secretions are usually a result of colonisation [32]; in the immunocompromised host, however, they may indicate invasive disease [6, 7, 39]. The positive predictive value of cultures in general is as high as 80–90% [2, 6, 40], although in some groups of patients (e. g. after lung transplantation) specificity is much lower [41]. Sensitivity of cultures in the diagnosis of IPA is poor [2, 6, 40]. Direct microscopic examination of sputum or BAL, stained with specific fluorescent stain for chitin (a fungal cell wall component) is easy to perform, rapid to read, and improves the sensitivity of microbiological examination [40, 42]. Direct visualisation of the hyphae makes it possible to discriminate between septate (e. g. *Aspergillus*, *Fusarium* and *Scedosporium*) and non-septate (e. g. *Mucorales*) moulds. Ubiquitous moulds of the order *Mucorales* cause serious infection in immunocompromised patients and, in contrast to *Aspergillus*, *Fusarium* and *Scedosporium* spp., they are not susceptible to voriconazole, which is an important factor when choosing a pre-emptive antifungal drug. The extra value of a positive culture is that growing of the fungus enables identification and susceptibility testing

to antifungal drugs. This is important in view of the antifungal resistance of – for example – *Aspergillus fumigatus* isolates to voriconazole [43]. Nonetheless, reliance on microscopy and/or culture alone results in substantial underdiagnosis due to the low sensitivity. Fibre-bronchoscopy with inspection of the tracheo-bronchial tree, sampling of deep airway secretions and BAL can be helpful; the macroscopic finding of ulcerative lesions and/or pseudomembranes together with a positive microscopy and/or culture is highly suggestive for *Aspergillus*-related tracheobronchitis.

Chest computerised tomography (CT) has proved to be an important tool for the diagnosis of IPA in neutropenic, severely immunocompromised patients, even in the absence of evident lesions on a conventional chest X-ray. Radiological findings might include nodules with rapid growth and/or cavitations. A ‘halo sign’ (a pulmonary mass surrounded by a zone of lower attenuation with ground-glass opacification produced by adjacent haemorrhage) and/or the ‘air crescent sign’ (crescentic radiolucencies around a nodular area of consolidation) may be present [44–50]. The frequency of the halo sign in patients with IPA is relatively high in the early stages of the disease, but becomes progressively lower with the passage of time [51]. Combining the halo sign and the air crescent sign, the sensitivity for IPA is more than 80% as specificity reaches 60–98% [47]. However, thoracic imaging in mechanically ventilated ICU patients is less helpful due to many confounding factors such as atelectasis and, sometimes major, pleural effusions. A lower sensitivity (5–24%) of the halo sign and air crescent sign in non-neutropenic patients has been reported in the literature [3, 6, 52]. Due to the high tropism for blood vessels, IPA might be complicated by localisations in the central nervous system. Therefore, in patients with documented or highly suspected IPA, CT scanning or magnetic resonance imaging (MRI) of the brain should be considered to exclude dissemination to the brain.

In the past decade, non-invasive diagnostic tests, serological and molecular, have focused on the detection of surrogate markers for *Aspergillus* spp., such as the galactomannan (GM) antigen, 1,3- $\beta$ -glucan and the detection of *Aspergillus* DNA by PCR. GM is a major *Aspergillus* cell-wall component that is released during the growth phase of the fungus, and detection of GM would be indicative for invasive disease [53]. Many studies have been done in order to investigate the value of the commercial Platelia *Aspergillus* assay (BioRad™, Marnes-La-Coquette, France) as a diagnostic tool for IPA, but mainly in patients with haematological malignancies [54–57]. The specificity of the GM assay for diagnosing IPA is at least 85%, as demonstrated by these studies, but the sensitivity of the assay varied considerably between 29% and 100% depending on the cut-off value. The most important finding of these studies was that in around two-third of patients, circulating antigen could be

detected at a mean of 8 days before a probable diagnosis was made by a combination of radiographic findings and *Aspergillus* isolation [53, 54]. In a recent meta-analysis, 27 studies were included regarding the value of the GM serum assay for surveillance of IPA in high-risk patients. The median sensitivity for proven cases was 71% (specificity 89%); for proven or probable cases, median sensitivity and specificity were 61% and 93%, respectively [58]. Specificity increased to 95% using a cut-off value of 1.5 in cases with proven or probable IPA. Because GM is a water-soluble carbohydrate, it can also be detected in BALF. Although the Platelia ELISA (enzyme-linked immunosorbent assay) is not validated for detection of GM in this fluid, there is an increasing tendency to use these samples for diagnosis of IPA. In small clinical studies among patients with haematological malignancies and in solid organ transplant recipients, the sensitivity of the GM EIA (enzyme immuno-assay) applied to BALF ranges from 85% to 100% with a high index cut-off ( $> 1.5$ ) to define positivity [59–62]. However, there are several clinical circumstances that might influence the diagnostic performance of the GM antigen test in either serum or BALF. First, the false-positive reactivity which might be caused by gastro-intestinal translocation of fungal GM from contaminated food or drink, as demonstrated in small children [63], and the use of the intravenous antibiotics piperacillin–tazobactam and amoxicillin–clavulanic acid, which is associated with serum ELISA reactivity in patients without evidence of IPA [64–67]. Second, an important factor that affects the release of GM antigens is antifungal drug therapy. Different animal and human studies have shown decreased sensitivity of the GM assay when (prophylactic) antifungal drugs were used [63, 68, 69]. In several prospective studies that assessed the performance of antigen detection, patients received antifungal prophylaxis with itraconazole, which might have a significant effect on the sensitivity of the assay [53, 54]. Third, when the Platelia *Aspergillus* ELISA kit was launched in Europe about a decade ago, another cut-off serum ratio was recommended than at present. Over the past years, several studies suggested lower cut-off values, ranging from 0.5 to 1 [39, 53, 54, 70–72]. It is clear that alterations in cut-off level will change the performance of the assay. Finally, the studies show that monitoring GM levels is crucial in order to diagnose (and eventually monitor treatment outcome) correctly, which means that the assay has to be performed twice weekly, preferably on receipt of the specimen. In critically ill patients without classical risk factors for IPA, the diagnostic value of the GM assay has been investigated only in one retrospective study and demonstrated a sensitivity of only 53% in patients with proven or probable IPA (cut-off value 1.0) [3]. Thus, it has to be stressed that the available data from patients with (haematological) malignancies and after solid organ transplantation can not be extrapolated to the critically ill

patient in general. In the meantime, due to lack of more reliable, non-invasive diagnostic tests, the GM assay could be used as an additive tool in the diagnostic work-up of IPA.

The 1,3- $\beta$ -glucan is a cell wall component of many filamentous fungi and yeasts, including *Aspergillus* spp. and *Candida* spp. Reproducible assay results, with high specificity and a high positive predictive value, demonstrated that use of an assay to detect serum 1,3- $\beta$ -glucan derived from fungal cell walls is a useful diagnostic adjunct for invasive fungal infection [73]. In addition, false-positive tests have been found in patients after haemodialysis, cardiopulmonary bypass surgery, high-dose immunoglobulin treatment, and after exposure to glucan-containing gauze [33]. Furthermore, in a recent small prospective study among ICU patients, serum glucan levels did not appear to be specific for fungal infections, as serum glucan levels were also elevated in bacterial infections [74]. Hence, the usefulness of 1,3- $\beta$ -glucan in the diagnosis of IPA has to be further evaluated.

Amplification of nucleic acid by PCR technology for the diagnosis of IPA is being increasingly studied. It can be applied to serum and BAL specimens [75–79]. Experience is limited to patients with haematological malignancies. White et al. evaluated the performance of a real-time PCR in whole blood in a group of patients with haematological malignancies and showed sensitivity of 92.3% and specificity of 94.6% for the diagnosis of IPA with good agreement of the GM ELISA [80]. They concluded that a negative PCR obtained twice weekly allowed a wait-and-see approach concerning starting antifungal treatment. However, comparable to the GM antigen test, there are a number of factors that potentially have an impact upon the clinical sensitivity of PCR. The magnitude of the quantitative PCR signal falls with antifungal therapy, thereby causing false-negative PCR results [81] while the (transient) colonising presence of *Aspergillus* in the respiratory tract may suggest a low positive predictive value [78]. Furthermore, patients at risk for IPA are often prescribed a multitude of drugs and fluids, all of which may act as non-specific inhibitors of the PCR. For example, anticoagulants inhibit PCR, thereby limiting its sensitivity [82].

One might conclude that the use of GM antigen test, 1,3- $\beta$ -glucan, and *Aspergillus* PCR as serological and molecular markers cannot be advocated for routine use in critically ill patients, and caution is warranted in the interpretation of positive test results in patients without a clinical suspicion of pulmonary infection as well as negative test results in patients with persisting pneumonia. However, the finding of sequentially positive GM tests in serum or BALF – using higher cut-off values – together with a positive *Aspergillus* PCR, in a patient with persisting pulmonary infection who carries one or more risk factors, is highly indicative for IPA and might justify treatment with antifungal therapy.

## Antifungal therapy

There have been important developments in antifungal drugs in the past few years, although amphotericin B deoxycholate has been the standard therapy for IPA for decades. However, multiple studies have now established not only its lack of efficacy due to an increasing antifungal resistance but also demonstrated unacceptable toxicity of this compound, in particular nephrotoxicity [83, 84]. Continuous infusion of amphotericin B deoxycholate over 24 h may reduce its nephrotoxicity [85, 86], although the efficacy may be reduced due to lower peak serum levels [87]. The use of lipid formulations may also reduce toxicity and have been studied extensively for empirical use in febrile neutropenia with the same efficacy rate as conventional amphotericin B [88]. However, lipid formulations are more expensive, and the initial use of higher doses does not improve efficacy and is associated with greater toxicity than lower doses, which suggests that high doses may not be routinely warranted.

Among the triazoles, itraconazole has activity against *Aspergillus*, but its clinical utility in critically ill patients with IPA has been limited by drug interactions and toxicity as well as erratic bioavailability of the oral suspensions [89]. Furthermore, strains of *Aspergillus fumigatus* resistant to itraconazole have already been described [90]. A large multicentre randomised trial established that voriconazole provides higher response rates and better survival than amphotericin B in the treatment of "probable or proven" IPA among patients with haematological diseases with fewer drug-related adverse events [91]. As a result of this study, voriconazole is increasingly recommended as initial therapy for IPA [92]. Voriconazole is available for intravenous and oral use. It is rapidly absorbed within 2 h after oral administration and the oral bioavailability is over 90% [93]. Clearance is hepatic via N-oxidation by the hepatic cytochrome P450 (CYP) isoenzymes, CYP2C19, CYP2C9 and CYP3A4 [93], which makes the potential for drug interactions considerable. For instance, voriconazole considerably reduces the clearance of intravenous midazolam [94], and fatal interactions with highly active antiretroviral therapy (HAART) have been described [95]. As voriconazole has limited aqueous solubility, the intravenous form includes the solvent vehicle sulfobutylether beta cyclodextrin sodium [96]. The clearance of sulfobutylether beta cyclodextrin sodium is linearly related to creatinine clearance and accumulation has been described in subjects with moderate to severe renal impairment [96], although in animal experiments the frequency of acute toxicity of sulfobutylether beta cyclodextrin sodium is low. Target organs for toxic effects are the kidney and liver, causing obstruction of renal tubules and necrosis in the liver respectively [96]. Because of this potential toxicity, it is recommended to treat patients with moderate to severe renal failure and who are on renal replacement therapy only with

the oral form of voriconazole, if feasible. However, in critically ill patients safe oral administration of drugs is difficult to accomplish as, for instance, gastric reflux, gastro-intestinal bleeding and impaired function of the intestine are frequent co-morbidities, leading to potentially insufficient intestinal absorption of the drug. Furthermore, it is important to recognise that some patients may have inadequate levels of the oral drug, particularly if a standard dose of 200 mg twice daily is used rather than the recommended 4 mg/kg twice daily dose which has been studied for the intravenous formulation [97]. At present, no clinical data are available regarding the bioavailability of the oral form of voriconazole in critically ill patients. It might be considered that the fear of potential adverse effects from intravenous solutions does not justify the risk of insufficient treatment by oral solutions. Moreover, limited clinical data showed no obvious toxicity in patients undergoing intermittent haemodialysis who were treated with daily 400–800 mg of intravenous voriconazole during 2 weeks [96], and the pharmacokinetics of voriconazole appears not to be affected by continuous renal replacement therapy (CRRT). On the basis of pharmacokinetics, dose reduction is not recommended in patients receiving CRRT [98, 99]. Regarding the efficacy of voriconazole, there is a potential concern with (long-term) voriconazole therapy as occasional breakthrough infections with yeasts and moulds, with decreased susceptibility to voriconazole, have been reported [43, 100, 101].

Posaconazole is a promising new triazole with broad-spectrum antifungal profile and has shown activity for salvage treatment of IPA in patients who are refractory to or intolerant of conventional therapy. A recent multicentre, prospective study among haematological and non-neutropenic patients with refractory IPA demonstrated a 42% overall success rate for posaconazole recipients versus 26% for control subjects [102]. Posaconazole is, however, only available for oral administration, which makes it probably less applicable in critically ill patients who are susceptible to impaired drug absorption in the digestive tract.

Echinocandins are a novel class of parenterally administered semi-synthetic lipopeptides with a pathogen-specific mechanism for non-competitive inhibition of biosynthesis of the fungus cell-wall enzyme complex 1,3- $\beta$ -D-glucan [103]. The echinocandins have documented in vivo activity against *Candida* spp. and *Aspergillus* spp. At present, there are three approved echinocandins, caspofungin, anidulafungin and micafungin, of which particularly caspofungin has demonstrated efficacy for the treatment of IPA. The first clinical trial to document the efficacy of caspofungin was among patients with "proven or probable" IPA who had treatment failure with (liposomal) amphotericin B, itraconazole or voriconazole, or who were intolerant to these antifungal drugs [104]. Caspofungin seems to be as effective as and generally better tolerated than liposomal amphotericin B when given

**Table 2** Treatment options with antifungal drugs for IPA in critically ill patients in the ICU

Setting	First choice	Alternatives
Primary therapy of IPA	Voriconazole 6 mg/kg q 12 h i.v. on day 1, then 4 mg/kg q 12 h i.v. or Voriconazole 400 mg q 12 h oral on day 1, then 200 mg q 12 h oral <sup>a</sup>	Liposomal amphotericin B 3-5 mg/kg/day i. v.  or Amphotericin B deoxycholate 1 mg/kg/day i. v.  or Caspofungin 70 mg i.v. on day 1, then 50 mg/day i. v. <sup>b</sup>

<sup>a</sup> Oral administration is recommended only in patients with intact intestinal absorption; <sup>b</sup> In patients with moderate to severe hepatic failure, dose reduction is recommended to 35 mg/day i. v.

as empirical antifungal therapy in patients with persistent fever and neutropenia [105]. However, prospective randomised clinical trials aimed at the treatment of IPA in (non-neutropenic) critically ill patients are lacking.

In general, there is no conclusive evidence that extended-spectrum triazoles are superior to echinocandins or polyenes, or vice versa, for monotherapy of IPA in ICU patients. We need prospective randomised controlled trials to solve this issue in critically ill patients. Table 2 gives an overview of treatment options with antifungal drugs for IPA.

### Combination therapy

Because the efficacy of antifungal therapy for IPA is poor, with more than 50% of all patients experiencing failure of first-line therapies [106, 107] empirical administration of combination antifungal regimens for proven or probable IPA may be an important strategy to improve outcome.

Theoretically, there are several foreseeable advantages of combination therapy, such as a widened spectrum and potency of drug reactivity, more rapid antifungal effect, synergy, and a reduced risk of antifungal resistance [108]. The available antifungal drugs target four different cell functions: cell membrane integrity (polyenes), ergosterol biosynthesis (azoles, allylamines), DNA synthesis (pyrimidine analogues) and cell-wall integrity (echinocandins). Although antifungal drugs are targeted against specific cell functions, many drugs also have pleiotropic effects that may inhibit other elements of fungal homeostasis [108]. For instance, azoles inhibit many cytochrome-dependent enzymes of the fungal respiration chain and amphotericin B generates oxidative species that damage fungal mitochondrial function and enhance macrophage fungal killing [109]. These subtle effects could be enhanced when one antifungal is applied together with a second drug, resulting in synergy. Conversely, the combination could act antagonistically, e. g. when one antifungal agent affects the targets of the other one.

Only a few clinical studies with small numbers of patients have tried to address the need for combination an-

tifungal therapy [110–113]. These studies have important limitations because they lack a control group and because of many other uncontrolled factors including the choice of combination, the duration of therapy, concomitant antibacterial or antiviral treatment, the lack of adequate follow-up to estimate relapses, and no discrimination between primary and sequential therapy. Results from those studies should therefore be viewed with caution. Large, adequately powered, prospective clinical trials are needed but they might be difficult to perform because of inadequate enrolment, differences in providing benefits, difficulty in documenting fungal infection, controversy regarding endpoints, the lack of surrogate markers to correlate in-vitro evidence to outcome prognosis, and the associated costs [114]. Nevertheless, combination antifungal therapy might be considered for certain clinical conditions such as refractory disease or breakthrough infections [115, 116].

### Conclusion

Recent data indicate that IPA may be an underestimated opportunistic fungal infection in critically ill patients, even in the absence of severe pre-existent immunological disorders, and carries a high mortality rate. A decrease in immune function or dysregulation of the immune system due to the severity of illness, together with specific underlying risk factors such as COPD, diabetes mellitus, chronic renal replacement therapy, advanced liver disease, and long-term use of steroids, might explain the relatively high occurrence of IPA among these patients. The high mortality rate is partially related to difficulties in timely diagnosis because of non-specific signs and symptoms, low clinical suspicion, and time delay due to high risks for invasive procedures to obtain histopathological evidence for diagnosing IPA. The presence of a persistent pulmonary infection despite broad-spectrum antibiotics or abnormal thoracic imaging by CT scanning together with one of these risk factors should trigger further diagnostic exploration by collecting respiratory secretions and/or laboratory markers. Meeting the gold standard alone

should not be the threshold for starting antifungal therapy, considering the high mortality rate. Invasive infection in patients with negative cultures might be supported by positive serological and molecular markers such as galactomannan antigen testing and *Aspergillus* PCR, which requires at least two sequentially positive samples. Antifungal therapy might be considered when persistent pneumonia with positive cultures for *Aspergillus* spp. or sequentially positive GM and *Aspergillus* PCR are present, accompanied with one of those risk factors for which voriconazole appears to be the first-line treatment. Combination therapy might be considered in breakthrough infections with moulds or yeasts or in refractory disease, although clear evidence is lacking.

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