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# Invasive aspergillosis in the intensive care unit

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**Invasive aspergillosis is a devastating infection affecting severely immunocompromised patients, most frequently with hematologic malignancies. In recent years, a surge in the incidence of invasive aspergillosis has been reported in critically ill patients without the classical risk factors. The mortality of the disease is equally high in the group of intensive care unit (ICU) patients, while the clinical signs and symptoms are nonspecific and the diagnosis remains a challenge. New noninvasive diagnostic methods in combination with better tolerated antifungal drugs aim for early diagnosis and improved prognosis of invasive aspergillosis. In this review, we discuss the epidemiology, the diagnostic strategy and algorithms, and the therapeutic choices of this severe infection in critically ill patients.**

**Keywords:** aspergillosis; diagnosis; treatment

## Introduction

Systemic infections are a common cause of death in critically ill patients, and pulmonary aspergillosis (PA) ranks among the most lethal and difficult diagnosed infections in this population. *Aspergillus* spp. can cause a broad spectrum of lung infections, manifesting as acute invasive pulmonary aspergillosis (IA), local necrosis with cavitation, mycetoma, and tracheobronchitis of allergic bronchopulmonary aspergillosis (ABPA).[1] Among these entities, IA is one of the most devastating forms of *Aspergillus* infection, with increasing frequency over the last decades. Invasive pulmonary aspergillosis (IPA), characterized by invasion and necrosis of lung parenchyma by *Aspergillus*, affects not only immunocompromised but also critically ill patients who do not have classical risk factors (for example, hematological malignancies or allogeneic hematopoietic stem cell transplantion (HSCT).[2] New risk factors that predispose to IPA in critically ill patients include chronic obstructive pulmonary disease (COPD), chronic use of systemic and inhaled corticosteroid, cirrhosis, solid-organ transplantation, and severe sepsi.[3,4] The mortality rate is high in the classic risk groups, exceeding 50% in neutropenic patients and 90% in HSCT recipients.[5,6] Interestingly, in less immunocompromised ICU patients, the mortality rate is equally high, reaching 90%.[7]

The clinical presentation of IA is nonspecific, and the diagnostic criteria are poorly defined in critically ill populations, rendering the diagnosis of the disease challenging. A high index of clinical suspicion is necessary; for example, the isolation of *Aspergillus* spp. in lower respiratory tract samples should not always be considered to indicate colonization.[8] New diagnostic tests have been introduced in daily practice in order to maximize the chance for therapy. This review covers the epidemiology, risk factors, diagnostic procedures and algorithms, and therapeutic choices of invasive aspergillosis in ICU patients.

## Epidemiology and risk factors

*Aspergillus fumigatus, A. flavus, A. niger, A. terreus*, and *A. nidulans* are the most common isolated *Aspergillus* spp. in humans.[9,10] The first description of IA as an opportunistic infection was published in 1953.[4] An increasing incidence has been observed during the past two decades and attributed to the use of chemotherapeutic and immunosuppressive drugs. The most important risk factors for

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**Table 1. Reported incidence of aspergillosis in the ICU setting**

| Author | Year | Incidence (%) |
|---|---|---|
| Roosen | 2000 | 15 |
| Valles | 2002 | 19 |
| Meersseman | 2003 | 5.8 |
| Dimopoulos | 2004 | 3.7 |
| Garnacho-Montero | 2005 | 1.1 |
| Kumar | 2006 | 0.7 |
| Vandewoude | 2006 | 0.3 |

IPA are the various types of immunodeficiency, including HSCT, prolonged and severe neutropenia (more than three weeks with neutrophils $< 500/\mu L$), hematologic malignancies, corticosteroid therapy, and HIV infection.[11] Neutropenia is the most significant risk factor, since neutrophils and alveolar macrophages are the most important host defenses against *Aspergillus*.[12] Several cases of IPA following HSCT have recently been described. Patients after allogeneic HSCT are in higher risk for IPA comparing to autologous HSCT (frequencies 2.3–15% and 0.5–4%, respectively).[2]

However, there are several reports of IA in immunocompetent patients who are critically ill without neutropenia, hematological malignancy, or HSCT. The incidence of IA in a typical ICU varies from 0.33% to 6.9% (Table 1). Interestingly, histopathological or microbiological evidence of IA has been observed in 6.9% of ICU patients in a retrospective autopsy-controlled study, although most (70%) did not have a diagnosis of hematological malignancy, a classical risk factor of IA and the reported mortality was 90%.[7] According to other studies, IA is one of the most frequently undiagnosed infections in the critically ill.[13] Mortality rates in ICU patients with proven or probable IPA (Fig. 1) vary between 59% and 95%, depending on the relevant study (Fig. 1).[14]

The risk factors for invasive aspergillosis among patients admitted to the ICU can be classified in three categories: *high risk* includes patients with neutropenia, hematological malignancy, or allogeneic HSCT; *intermediate risk* includes patients undergoing prolonged treatment with corticosteroids, those who have undergone autologous HSCT, and patients with COPD, liver chirrosis, solid-organ cancer, HIV infection, lung transplantation, or systemic immunosuppresive therapy; and *low risk* consists of patients with severe burns, solid-organ transplantation, steroid treatment (more than seven days), a prolonged ICU stay (more than 21 days), multiple organ dysfunction syndrome, malnutrition, postcardiac surgery, and near drowning.[1] In addition to the classical high-risk groups, three nonclassical risk groups have also been recognized for IP: patients with COPD, liver cirrhosis, or disturbed immunoregulation due to critical illness. It is well known that the systematic inflammatory response syndrome (SIRS) leads to anti-inflammatory response, characterized by a decrease of innate and adaptive immunity, a decrease of all cellular immune functions, and a decrease of the number of myeloid dendritic cells.[15]

The incidence of IPA in COPD patients is 1.3%, according to a review of 50 studies.[16] According to Bulpa *et al.*, IPA in this group of patients is defined as proven, probable, possible, or colonization based on histopathological, clinical, and radiological criteria (Table-slide 13 in Bulpa). This classification is based on the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institutes of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) for the diagnosis of invasive fungal infections in immunocompromised patients.[17] The emergence of IPA in this group of patients has been mainly attributed to the prolonged administration of high doses of corticosteroids.[18] Nevertheless, IPA has been described in COPD patients who have never received steroids, while some viral infections may play a causal role.[19] Guinea *et al.* retrospectively classified, according to Bulpa

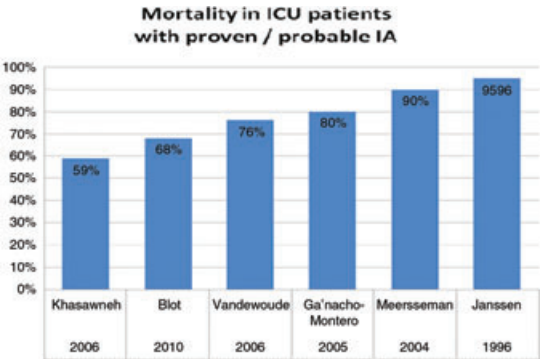


**Figure 1.** Mortality rates in ICU patients with proven/probable IA.

criteria, a large series of COPD patients with isolation of *Aspergillus* from respiratory samples.[20] They reported probable IPA in at least 22.1% of these patients, and logistic regression showed the following variables as independent predictors of IPA: admission to the ICU, chronic heart failure, recent antibiotic treatment, and administration of high doses of steroids.

Patients with severe liver disease are also at high risk for IA, with mortality rates exceeding 70%.[21] These patients, though apparently immunocompetent, are prone to bacterial or fungal infections as both humoral and cell-mediated immunity are depressed by liver dysfunction, opsonization is decreased due to complement deficiencies, neutrophil migration and phagocytosis are defected, and the oxidative burst activity of neutrophils is impaired.[13,22–24] The immune system of these patients is additionally weakened because of probable corticosteroid therapy, transfusions of allogeneic blood products, hemodialysis, and sepsis.[13] Critically ill patients are prone to IPA in the absence of hematologic malignancy, neutropenia, COPD, or liver disease, as many critical illnesses have concomitant disturbances in immunoregulation characterized by macrophage deactivation and an altered cellular immune response termed *immunoparalysis*.[25] Moreover, other predisposing factors such as hyperglycemia, corticosteroid use, renal failure and continuous renal replacement therapy, and broad spectrum antibiotic treatment may lead to invasive fungal disease.[26,27] Concerning the pathogenesis of IPA, *Aspergillus* has a sporulating capacity ranging from 1 to 100 spores/mm$^3$ in the normal air.[28,29] The conidia released from the spores are small enough (2–3 μm of diameter) to reach the alveoli by inhalation, thus the respiratory tract is the main source of entry. The impaired immunologic response of the host may lead to *Aspergillus* colonization of the respiratory system and subsequently to invasive aspergillosis.

## Diagnosis

The diagnosis of IPA is challenging, especially in the ICU setting. The diagnostic methods can be classified as laboratory and high technology. Laboratory methods are the microscopic examination of various samples, culture and identification of the fungus, histopathological examination of tissues, antibody detection, antigen detection, and PCR.[30] High technology methods include the detection of antibodies and metabolites of fungus, detection of fungal cell wall components, and fungal PCR. The gold standard of diagnosis remains the histopathological examination of invasive lung tissue sampling (thoracoscopic or open lung biopsy).[31] The presence of nonpigmented septate hyphae with repeated dichotomous branching and a culture positive for *Aspergillus* from the same site is diagnostic. However, a proven diagnosis in ICU patients is very difficult, since coagulopathy and hemodynamic instability render invasive procedures rather difficult. Therefore, several alternative diagnostic methods and algorithms have been developed.

In recent years there is an increasing interest in the detection of *Aspergillus* spp. antigens or DNA in body fluids (e.g., serum, bronchoalveolar lavage (BAL), and cerebrospinal fluid (CSF)). Galactomannan and (1,3)-β-D-glucan are fungal cellular wall components frequently used for the IA diagnosis. Galactomannan (GM), a polysaccharide released in body fluids during *Aspergillus* growth, can be detected in serum and BAL by a double-sandwich ELISA technique several days before clinical and radiological signs of the disease become apparent.[2] Maertens *et al.* compared the accuracy of the galactomannan assay at multiple cutoffs and concluded that consecutive positive results at a threshold of 0.5 ng/mL had best sensitivity and specificity, but that a single result of $>0.8$ was also basis for therapy, with sensitivity of 96% and specificity of 97%.[32] According to other reports, GM detection had a sensitivity of 71% and specificity of 89% for IPA in patients with a hematological malignancy or HSCT, but the method is less useful in nonneutropenic patients.[33]

Meersseman *et al.* reported the diagnostic significance of GM measurement in BAL of critically ill patients;[7] in this study 110 patients were included, 36 with hematologic malignancy and 74 with other immunocompromising factors. The sensitivity of GM detection was 88% with a cut off value of 0.5, compared to 42% for the respective measurement in serum, while the specificity of GM in both BAL and serum was 86%. The low sensitivity of serum GM levels for the detection of IP, especially in nonneutropenic patients, is attributed to the clearing of GM by neutrophils from the circulation.[34] Additionally the serum of BAL GM levels may be falsely elevated in patients receiving either dietary sources of GM

(cereals, pasta) or several antibiotics (for example, piperacillin-tazobactam).[34,35]

Another fungal cell wall constituent, highly sensitive and specific for invasive mycosis, is (1,3)-β-D-glucan. The sensitivity of this assay for IPA diagnosis varies from 55% to 100% and the specificity from 52% to 100%, with false positive results observed in patients with bacterial infections, liver disease, hemodialysis, abdominal surgery, and antibiotics.[9] The same antigen might be present in other fungal infections (e.g., candidiasis). The combination of galactomannan and (1,3)-β-D-glucan might improve specificity and positive predictive value of each separate test.[36] Fungal DNA can be detected in blood, serum, tissue biopsies, BAL, and CSF by application of molecular techniques, in particular PCR. The sensitivity and specificity of the method for BAL are 67–100% and 55–95%, respectively, while the respective values for serum are 100% and 65–92%.[37,38] The main problem with PCR is that specimens can be contaminated; in addition, PCR cannot discriminate colonization from true infection. Although PCR facilitates earlier the possible diagnosis of IPA, it is not a routine clinical assay; and no study has proven that it has a significant impact on patient management or outcome.

In the early stages of IPA, chest radiograph is usually negative or shows nonspecific changes. Chest high-resolution computer tomography (HRCT) is useful for the early diagnosis of IPA. Early lesions include multiple nodules and the characteristic halo sign, which appears mainly in neutropenic patients as a zone of low attenuation due to coagulation necrosis with decreased blood flow in the center that is surrounded by a translucent ground-glass halo.[39] Later, in the course of the disease after the recovery of neutrophil function, the air crescent sign, or central cavitation, is apparent and characterized by coagulation necrosis of an original nodule.[40] None of these signs is pathognomonic for IPA, and according to Vanderwoude *et al.* the most common radiological findings in ICU patients with IPA are nonspecific infiltrates and consolidation.[8] The halo sign can also be found in infections caused by *Nocardia* spp, Mucorales, *Trichosporon* spp, and *Fusarium* spp, and in patients with metastases, eosinophilic pneumonia, or bronchoalveolar carcinoma.[41,42]

According to the EORTC/MSG criteria, the definition of proven aspergillosis includes the identification of *Aspergillus* by histopathological examination or culture. Probable disease requires at least one immunocompromising host factor, one clinical criterion, and mycological evidence (culture or antigen). In the absence of mycological evidence, IA is defined as possible.[43] These definitions, although expanded to include nonimmunocompromised hosts, have poor diagnostic accuracy in ICU patients.[8] The isolation of *Aspergillus* from respiratory samples in critically ill patients is associated with IPA in 10–80% of them, depending on the study.[44,45] According to Perfect *et al.*, 12% of patients with *Aspergillus* species isolated from respiratory samples developed invasive aspergillosis. Furthermore, the isolation of *Aspergillus* from respiratory samples carried a poor prognosis, as only 38% of the patients were alive three months after diagnosis.[46] Dimopoulos *et al.* reported that 2.7% of 222 ICU patients had evidence of disseminated aspergillosis at autopsy; *A. fumigatus* was isolated from respiratory samples of all these patients premortem and considered to be colonization.[26] Interestingly, Khasawneh *et al.* performed a retrospective review of medical reports to determine the clinical significance of isolation of *Aspergillus* from respiratory samples of critically ill patients and found that 28% of them developed probable or definite IA, and 78% of them were *Aspergillus* colonization cases.[14] However, Khasawneh *et al.* concluded that the isolation of the fungus is a poor prognostic marker irrespective of invasive disease or colonization.

Given all these diagnostic problems, Vandewoude *et al.* developed an adapted clinical algorithm based on the EORTC/MSG criteria in order to discriminate *Aspergillus* colonization from invasive disease in critically ill patients, with isolation of the fungus from endotracheal aspirates: 48% of the patients were classified as IPA and 52% as colonization.[8] The available histopathology data from 26 patients confirmed the diagnosis based upon the clinical algorithm. Blot *et al.* externally validated this clinical diagnostic algorithm in order to discriminate colonization from IPA in mechanically ventilated critically ill patients, with *Aspergillus* isolated from endotracheal aspirates (Table 2).[47] It is worth noting that the evaluated algorithm accepted any radiological abnormality, since radiological lesions are nonspecific in ICU patients. Additionally, the algorithm contained a culture and microscopy positive BAL as an alternative criterion for host factors, since IPA

**Table 2. Criteria proposed for the diagnosis of probable IPA in the ICU setting[48]**

**1.** *Aspergillus (+)* in lower respiratory tract specimen culture (entry criterion)
**2.** Compatible signs and symptoms (one of the following)
- Fever refractory to at least three days of appropriate antibiotic therapy
- Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause
- Pleuritic chest pain
- Pleuritic rub
- Dyspnea
- Hemoptysis
- Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support

**3.** Abnormal medical imaging by portable chest X-ray or CT scan of the lungs
**4.** Either
**4a.** Host risk factors (one of the following conditions)
- Neutropenia (absolute neutrophil count less then 500/mm$^3$) preceding or at the time of ICU admission
- Underlying hematological or oncological malignancy treated with cytotoxic agents
- Glucocorticoid treatment (prednisone or equivalent, >20 mg/day)
- Congenital or acquired immunodeficiency

OR
**4b.** Semiquantitative *Aspergillus*-positive
- Culture of BAL fluid (+ or ++) without bacterial growth together with a positive cytological smear showing branching hyphae

Probably invasive pulmonary aspergillosis: 1 + 2 + 3 + either 4a or 4b
When ≥1 criterion is not met, the case is classified as *Aspergillus* colonization

may develop in patients without apparent risk factors. IPA was defined as probable, if all the criteria were fulfilled. The case was defined as colonization if one or more criteria were not met. The judgment, based upon the algorithm in each case, was compared to the results of histopathology examination on lung biopsy or autopsy. The algorithm demonstrated 61% specificity and 91% sensitivity for the discrimination of IPA from colonization in mechanically ventilated patients.

## Treatment

Treatment of IPA remains difficult, although new antifungal agents have been introduced in clinical practice. Amphotericin B has been the standard therapy of IPA for decades. However, many studies have shown reduced efficacy of the drug, partially due to the development of resistance among *Aspergillus* subspecies and the serious adverse effects associated with its use, in particular nephrotoxicity, electrolyte disturbances, and hypersensitivity reactions.[48,49] Newer lipid-based preparations of amphotericin with reduced nephrotoxicity have been introduced in the treatment of fungal infections. Nevertheless, these agents are more expensive and higher doses may be needed for the same antifungal therapeutic result.[50]

The treatment of choice for IA is voriconazole, a broad-spectrum triazole.[51,52] In a large, prospective, multicenter trial Herbrecht *et al.* compared voriconazole to amphotericin B for the treatment of IPA in patients with hematologic diseases.[53] At week 12 a significantly higher rate of favorable response was observed in patients receiving voriconazole (53%) compared to patients receiving amphotericin B (32%), as well as a higher 12-week survival (71% vs. 58%). Fewer drug-related side effects were reported with voriconazole. The drug is available in intravenous (i.v.) and oral formulations, with a recommended dose of 6 mg/kg twice daily i.v. on day 1 and then 4 mg/kg twice daily. The clearance of the drug is hepatic by the

cytochrome P450, so the possibility of drug–drug interaction is considerable: voriconazole interacts with many drugs, included, but not limited to, cyclosporine, warfarin, carbamazepine, and statins, and it reduces the clearance of midazolame.[51] Additionally, fatal interaction with highly active antiretroviral therapy (HAART) has been reported in patients with HIV infection.[54] However, voriconazole is generally well tolerated. The most commonly described adverse effect is visual disturbances, such as blurred vision, photophobia, and altered color perception. Less frequent toxicities include liver function test abnormalities and dermatologic reactions.[2] The intravenous form of the drug includes the solvent vehicle sulfobutylether beta cyclodextrin sodium,[55] which accumulates in cases of moderate and severe renal impairment with possible toxic effects on kidney and liver. Therefore, it is recommended that only the oral form of voriconazole be used in cases of renal dysfunction. However, in critically ill patients the oral administration of the drug may lead to insufficient intestinal absorption.[56] Monitoring of therapeutic blood levels of voriconazole may reduce the unnecessary discontinuation of the drug due to adverse effects and improve the therapeutic response in invasive aspergillosis.[57]

Echinocandins, such as caspofungin, micafungin, and anidulafungin, could be alternative drugs for the treatment of IPA in refractory cases. Caspofungin has proven to be effective in the treatment of IPA in patients who cannot tolerate the first-line agents or in cases of IPA refractory to standard treatment.[58] Caspofungin as a first-line treatment for IPA, in contrast, has not been compared to voriconazole. Echinocandins inhibit the (1,3)-β-D-glucan component of the fungal cell wall, while azoles target the cell membrane. Therefore, combination therapy might be considered in refractory cases of IPA.[59] Potential advantages could be the more rapid fungicidal activity, the reduction of the doses of individual agents, and the prevention of emergence of resistance.[60] There are few clinical data concerning combination antifungal therapy and most of the studies have important limitations. Nevertheless, studies do not support the combination of voriconazole with amphotericin B.[61] Additionally the inhibition of ergosterol synthesis by azoles deprives amphotericin B of its target, leading to an antagonistic effect of the combination.[62] The combination of caspofungin and liposomal amphotericin B showed a response rate of 42%, while a survival advantage of voriconazole plus caspofungin, compared to voriconazole alone, was found in a retrospective analysis of IPA treatment.[63,64] Particularly in the critically ill, the combination of echinocandin with a lipid formulation of amphotericin B or azole might be useful, but randomized controlled studies are needed for further evaluation of the treatment.[64] Nevertheless, combination treatment might be considered in refractory cases of IPA or in breakthrough infections.[65]

Failure of antifungal treatment could be due to intrinsic or secondary antifungal resistance. Intrinsic resistance to various antifungals agents has been reported particularly for non-*fumingatus Aspergillus* species. *A. terreus* shows primary resistance to amphotericin B, while there are also recent reports of resistance in several species of the *Fumigati* group.[66,67] Acquired resistance in *Aspergillus* species to azoles is rarely reported, occuring mainly in *A. fumigatus*.[68] There are few reports of resistance to echinocandin, in particular of echinocandin-exposed patients.[69] The clinical and radiological response of each patient to antifungal treatment will determine the duration of therapy,[70] which can be very prolonged, ranging from several months to more than one year. The clinical and radiological resolution, including the absence of fever and the decrease of thoracic aspergillary lesions on CT,[71] are prerequisites for the cessation of antifungal therapy. Regarding laboratory indices, Boutboul *et al.*, studied the kinetics of serum *Aspergillus* GM in HSCT patients and concluded that an increase of GM level 1.0 over baseline during the first week of therapy was predictive of treatment failure (with sensitivity of 44% and specificity of 87%).[72]

## Environmental sources and prevention of IPA

Environmental factors combined with host factors may play a significant role in the development of IPA. The most frequent source of infection is inhalation of *Aspergillus* spores that are found in soil, decomposing plant matter, household dust, and some building materials.[73] Construction or renovation work near hospitals has been associated with nosocomial outbreaks of IPA[74,75] due to *Aspergillus* spores contaminating the hospital air. According to other reports, *A. fumigatus* can contaminate hospital water, with potential implications on patients'

health.[76] However, the likelihood of hospital water as an environmental source for IPA is not clear; large trials with molecular techniques are required in order to determine its importance.

Environmental control measures are necessary in order to protect high-risk patients from exposure to *Aspergillus* spores. During hospital building work the following barrier protection methods have been used:[77] portable high-efficiency particulate air (HEPA) filter air purifier units installed in rooms housing immunocompromised individuals; a copper-8 quinolinolate formulation applied to surfaces in the rooms and above the false ceilings; windows sealed; regular cleaning of the rooms; and patients moved to other areas of the hospital until the implementation of containment measures. Other control strategies include disinfection of water supplies and methods to decrease patient exposure. However, the cost-effectiveness of these measures has to be examined, and large epidemiological studies are required in order to lead to effective prevention strategies against this lethal disease.

## Conclusions

IA is an infection with poor prognosis, probably fatal in the case of delayed diagnosis. ICU patients lacking the classical risk factors can develop IA, particularly if COPD or severe hepatic failure is present. The diagnosis of the disease is rather difficult because biopsies are not easily performed in such patients. Therefore, the finding of *Aspergillus* in respiratory tract samples should raise the suspicion of IA. Critical care providers need algorithms in order to make therapeutic decisions, given the availability of new and effective drugs. Randomized controlled strategies are necessary for the validation of these algorithms and the subsequent development of guidelines and recommendations.

## Conflicts of interest

The authors declare no conflicts of interest.

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