Epidemiology, diagnosis and treatment of fungal respiratory infections in the critically ill patient

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ABSTRACT

Objective. To elaborate practical recommendations based on scientific evidence, when available, or on expert opinions for the diagnosis, treatment and prevention of fungal respiratory infections in the critically ill patient, including solid organ transplant recipients.

Methods. Twelve experts from two scientific societies (The Spanish Society for Chemotherapy and The Spanish Society of Intensive Care and Coronary Units) reviewed in a meeting held in March 2012 epidemiological issues and risk factors as basis for a document about prevention, diagnosis and treatment of respiratory fungal infections caused by *Candida* spp., *Aspergillus* spp or Zygomycetes.

Results. Despite the frequent isolation of *Candida* spp. from respiratory tract samples, antifungal treatment is not recommended since pneumonia by this fungal species is exceptional in non-neutropenic patients. In the case of *Aspergillus* spp., approximately 50% isolates from the ICU represent colonization, and the remaining 50% cases are linked to invasive pulmonary aspergillosis (IPA), an infection of high mortality. Main risk factors for invasive disease in the ICU are previous treatment with steroids and chronic obstructive pulmonary disease (COPD). Collection of BAL sample is recommended for culture and galactomannan determination. Voriconazole and liposomal amphotericin B have the indication as primary therapy while caspofungin has the indication as salvage therapy. Although there is no solid data supporting scientific evidence, the group of experts recommends combination therapy in the critically ill patient with sepsis or severe respiratory failure. Zygomycetes cause respiratory infection mainly in neutropenic patients, and liposomal amphotericin B is the elective therapy.

Conclusions. Presence of fungi in respiratory samples from critically ill patients drives to different diagnostic and clinical management approaches. IPA is the most frequent infection and with high mortality.

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Epidemiología, diagnóstico y tratamiento de las infecciones fúngicas respiratorias en el paciente crítico

RESUMEN

Objetivos. Elaborar unas recomendaciones prácticas basadas en la evidencia científica, cuando esté disponible, o en opiniones de expertos para el diagnóstico, tratamiento y prevención de infecciones fúngicas respiratorias en el paciente crítico incluyendo a pacientes trasplantados de órgano sólido.

Metodos. Doce expertos pertenecientes a dos Sociedades Científicas (Sociedad Española de Quimioterapia y Sociedad Española de Medicina Intensiva, Crítica y Unidades Coronarias) revisaron en una reunión celebrada en Marzo de 2012 los aspectos epidemiológicos y factores de riesgo como base para generar un documento para la prevención, diagnóstico y tratamiento de infecciones fúngicas respiratorias causadas por *Candida* spp., *Aspergillus* spp. o Zigomicetos.

Results. A pesar del frecuente aislamiento de *Candida* spp. del tracto respiratorio, el tratamiento antifúngico no está recomendado debido a que una neumonía por éstas especies es excepcional en pacientes no neutropénicos. En el caso de *Aspergillus* spp., aproximadamente el 50% de los aislamientos en UCI indican colonización y el otro 50% de los casos están asociados a aspergilosis pulmonar invasora (API), una infección con una alta mortalidad. Los principales factores de riesgo de una infección fúngica invasora en la UCI son el tratamiento previo con esteroides y enfermedad pulmonar obstructiva crónica (EPOC). La recogida de muestras mediante lavado broncoalveolar está recomendada para el cultivo y la determina-

ción de galactomanano. Voriconazol y anfotericina liposomal B presentan la indicación como tratamiento de primera línea mientras que caspofungina está indicada en la terapia de rescate. Aunque no hay datos sólidos que apoyen la evidencia científica, el grupo de expertos recomiendan la terapia combinada en el paciente crítico con sepsis o fallo respiratorio severo. Los zigomicetos causan infección respiratoria principalmente en pacientes neutropénicos, y anfotericina liposomal B es la terapia de elección.

Conclusiones. La presencia de hongos en muestras respiratorias de pacientes críticos conlleva diferentes enfoques de diagnóstico y manejo clínico. API es la infección más frecuente y presenta una alta mortalidad.

INTRODUCTION

Isolation of *Candida* spp. and, at a lower rate, *Aspergillus* spp. from respiratory samples in patients admitted in the Intensive Care Unit (ICU) is frequent[1]. Isolation of other filamentous fungi as Mucorales, *Scedosporium* o *Fusarium* is by far less frequent, but these fungi are associated with high mortality in critically ill patients.

Fungal pulmonary involvement presents particular characteristics that complicate the patient's management. Presence of fungi may represent a true infection although frequently it only implies colonization of the respiratory tract, leading to a very different management and prognosis. Discrimination between colonization and infection is not easy and, frequently, antifungal treatment is initiated associated with an increase in adverse events and costs. On the other hand, fungal infections are associated with high mortality in those cases where treatment initiation is delayed. In last years, new antifungals and new routes of administration for some of them have been introduced in the clinical practice, thus complicating treatment election by treating clinicians. Lastly, most available information regarding diagnosis and treatment of fungal respiratory infections (especially in the case of *Aspergillus* spp.) is referred to neutropenic onco-hematological patients and cannot always be extrapolated to the critically ill patient.

This document summarizes conclusions from a meeting held in Seville (Spain) on March 2-3, 2012 with participanting experts from two scientific societies (The Spanish Society for Chemotherapy and The Spanish Society of Intensive Care and Coronary Units). Approaches to critical issues as epidemiology, diagnosis, discrimination between colonization and infection, treatment and prevention of fungal respiratory infections were addressed. Each participant presented a review of a critical issue, which afterwards was discussed by all participants that approved the consensus recommendation. No grades of the quality of the evidence or strength of recommendations were used.

The objective was to elaborate practical recommendations based on scientific evidence, when available, or on expert opinions for the management of fungal respiratory infections caused by *Candida* spp., *Aspergillus* spp. and Zygomycetes in the critically ill patient, including solid organ transplant recipients.

EPIDEMIOLOGY OF FUNGAL RESPIRATORY INFECTIONS IN THE CRITICALLY ILL PATIENT

None of the available epidemiological studies on fungal infections in critically ill patients has been specifically focused on respiratory infections. However, valuable information can be obtained from epidemiological studies focused on fungal infections in general. Two multicentre Spanish studies (EPIFUCI[2] and ENVIN-UCI[3]) and one Italian study[4] in critically ill patients showed that while *Candida* was isolated from multiple body sites, nearly all *Aspergillus* spp. came from respiratory samples. In the last study, the incidence of aspergillosis was 6.3/1,000 admissions[4].

The role of other fungi as Zygomycetes, *Fusarium* spp. or *Scedosporium* spp. is much less relevant in the critically ill patient, representing less than 1% of all isolates from the ICU[1,5]. Among them, mucormycosis is the most frequent infection and its management is also addressed in the present document.

Candida spp.

C. albicans is the most frequent species isolated from respiratory samples (approx. 50%) followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Despite the frequent isolation of *Candida* spp. from respiratory samples, isolation in non-neutropenic patients is not considered diagnosis of pneumonia regardless the species isolated[6,7].

Aspergillus spp.

In contrast to *Candida*, the genus *Aspergillus* acquires relevance in the respiratory infection of the critically ill patient. *Aspergillus fumigatus* is the most frequent species (80-90% cases) causing invasive pulmonary aspergillosis (IPA), although its frequency seems to be declining in last years with an increase in cases by other no-*fumigatus* species, especially *Aspergillus flavus* or *Aspergillus terreus*[5,8]. Conidia of *Aspergillus* are easily aerosolized, being transmission by air nearly universal. *A fumigatus* presents rapid replication and small size conidia, thus favoring its frequency as etiological agent of IPA. Humans continuously inhale *Aspergillus* conidia but, in general, they are efficiently eliminated by the immune system[9]. Isolation of *Aspergillus* is more frequent when there are renovation works in hospitals[10], with reported outbreaks in the ICU linked to isolation of this fungus in air conditioning systems[11].

Principal risk factors for development of IPA are summarized in table 1 and can be divided in high-, intermediate- and low- risk factors[12]. The main risk factor for IPA development is neutropenia; increasing the risk when neutropenia is prolonged and when its magnitude increases. Classically, the highest IPA incidence is observed in patients with hematological malignancies, especially in allogeneic hematopoietic progenitor cell transplant recipients[13,14]. Other immunocompromised patients, especially those under prolonged steroid treatment, have high risk for developing IPA[8]. Nowadays, severe chronic obstructive

Table 1 Risk factors for invasive fungal infection in critically ill patients

High risk	Intermediate risk	Low risk
- Neutropenia (< 500 mm^3)	- Prolonged steroid treatment prior to ICU admission	- Severe burns
- Hematological malignancy	- Autologous hematopoietic progenitor cell transplantation	- Solid organ transplantation
- Allogeneic hematopoietic progenitor cell transplantation	- COPD, especially under inhaled steroid treatment	- Treatment with steroids for <7 days
- Lung transplantation without prophylaxis	- Hepatic cirrhosis	- Prolonged ICU stay (>21 days).
	- Solid organ malignancy	- Malnutrition
	- HIV infection	- Cardiac post-surgery
	- Lung transplantation with prophylaxis	- Near drowning
	- Systemic treatment with immunosuppressants	- Multiorgan dysfunction syndrome (situation of immune paralysis)
		- Influenza A (H1N1) infection

pulmonary disease (COPD) treated with steroids is the most frequent comorbidity in hospitalized patients with IPA[15,16].

In the ICU, only 10-15% patients with IPA present neutropenia. Around 50% of IPA cases in the ICU occur in COPD patients, nearly all of them under prolonged steroid treatment[17,18,19]. In a published study including 1,753 patients admitted in 73 Spanish ICUs, the two factors that were significantly associated with isolation of *Aspergillus* spp. in the multivariate analysis were steroid treatment (OR: 4.5, 95% CI: 1,73-11), and COPD (OR: 2.9, 95% CI: 1,06-8,08)[17]. Steroids alter distribution and function of neutrophils and macrophages, and directly stimulate the growth of *A. fumigatus in vitro*[20]. The immune response is impaired in critically ill patients, with depressed monocyte/macrophage function, especially in the late phase of multiorgan dysfunction that can be considered a low-risk factor for IPA development (table 1)[21,22].

Currently, frequency of *Aspergillus* isolation from lower respiratory tract samples is 16.3 cases per 1,000 hospitalized COPD patients, with an increase from 7 (year 2000) to 13 cases (year 2007) per 1,000 admissions of COPD patients. In these patients IPA was associated with heart insufficiency, antibiotic treatment within 3 months prior to admission, accumulated steroid dose >700 mg (prednisone equivalent) within 3 months prior to admission or from admission to *Aspergillus* isolation, and ICU admission[23,24].

Hepatic transplantations, with an incidence of 1-9%, and mainly pulmonary transplantation, with an incidence of 5-20%, are the solid organ transplantations that present, with the highest frequency, IPA as complication[25]. Risk factors for IPA in different solid organ transplant recipients are shown in table 2. Specifically, risk factors as retransplantation, renal insufficiency, transplantation due to fulminant hepatic failure and citomegalovirus (CMV) infection have been identified in the case of hepatic transplantation[26,27].

Incidence of aspergillosis in lung transplant recipients varies according to presentation: 30%-60% for colonization post-transplantation, 8%-12% for respiratory tract infection and 6%-16% for invasive/disseminated infection[28,29]. In the early post-transplantation period, infection mainly occurs in the bronchial suture area. In late post-transplantation periods, invasive and disseminated presentations are the most frequent and severe. Risk factors for early and late IPA presentations in lung transplant recipients are summarized in table 2[27,30,31].

Order Mucorales

Mucormycosis is an opportunistic acute infection caused by fungi from the order Mucorales of the Zygomycetes class. Within this family, most frequent genera are *Rhizopus*, *Mucor* and *Lichtheimia* (before *Absidia*)[5]. Spores of these microorganisms enter the organism through inhalation or through open wounds. The most frequent clinical presentation is rhinocerebral mucormycosis followed by pulmonary infection[32].

Risk factors include neutropenia, onco-hematological diseases, inadequately controlled diabetes mellitus, severe trauma, burns and treatment with deferoxamine in dialyzed patients[33]. Prolonged treatments with voriconazole, that is not active against these fungi, have been associated with an increased incidence of these infections[34,35]. Among organ solid transplantations, liver and lung transplant recipients are the most affected, with an estimated incidence of 1.5%[32].

Conclusions: *Aspergillus* spp. is the main responsible for fungal respiratory infections in the critically ill patient followed by far by fungi from the order Mucorales. In the critically ill patient, main risk factors for IPA are COPD and use of steroids. *Aspergillus* spp. is also the principal fungus causing respiratory infection in solid organ transplant recipients, mainly affecting lung transplant recipients followed by liver transplant recipients. Although risk factors may vary according to the type of transplantation, reintervention, need for renal replacement therapy or CMV infection are risk factors for IPA following solid organ transplantation. Pulmonary infection by Zygomycetes mainly affects neutropenic patients.

IMAGING, MICROBIOLOGICAL AND CLINICAL DIAGNOSIS OF RESPIRATORY INVASIVE FUNGAL INFECTIONS (IFI)

a) Diagnosis of respiratory IFI by *Candida*

The number or colonies in cultures of respiratory tract samples, even if samples had been collected by fibrobronchoscopy, is not valuable for the diagnosis of pneumonia by *Candida*. To diagnose pneumonia by *Candida*, it is required biopsy and demonstration of tissue invasion. While colonization of the respiratory tract by *Candida* is very frequent in critically ill patients with mechanical ventilation, pneumonia by *Candida* is extremely infrequent since the innate mechanisms of defense of lungs make them relatively resistant to *Candida* invasion[36].

b) Diagnosis of respiratory IFI by *Aspergillus*

In the critically ill patient, IPA generally presents a nonspecific symptomatology with fever and respiratory insufficiency. Thus, IPA should be suspected in patients with risk factors and respiratory symptoms in the presence of nodules or pulmonary infiltrates[25].

Aspergillar tracheobronchitis is a non frequent presentation, mostly affecting lung transplant recipients (where it can cause bronchial suture dehiscence) although it can also be found in other immunocompromised patients[37]. It has also been described in critically ill patients, and pseudomembranous tracheobronchitis can produce airway obstruction[38].

Thorax x-ray is nonspecific and usually shows bilateral infiltrates with nodules in some cases. CT scan has also low utility in the critically ill patient since characteristic signs of IPA as the halo sign and the air crescent sign are not frequent, around 5%[18,23,39], a very low rate compared with 80% in neutropenic patients.

Among respiratory tract samples, BAL is the sample showing the highest sensitivity and specificity, which increase if *Aspergillus* colony count is performed[18]. In addition, it has been reported the increase in the probability of IPA by the number of positive cultures to *Aspergillus*: 5.9% (1 culture), 18.4% (2 cultures) and 38.2% (≥3 cultures)[15]. However, 61% patients with confirmed IPA presented only one positive culture and only 18% patients presented three or more[14].

It is important to highlight that 30-50% patients with IPA also present bacterial isolation in respiratory tract cultures[17,23,40], a fact associated with a worse prognosis[40]. The presence of *Aspergillus* in blood culture, perhaps with the exception of *A. terreus*[41], is not considered diagnostic since it means contamination usually.

Cultures of respiratory samples, including those obtained by BAL, are positive in only 50% patients with IPA. Diagnostic meth-

Table 2 Risk factors for development of invasive pulmonary aspergillosis (IPA) in solid organ transplant recipients

	Early IPA	Late IPA (>90 days post-transplantation)
Lung transplantation	Respiratory tract ischemia	Single lung transplantation
	Recurrent bacterial infections	Endobronchial prosthesis
	CMV infection	Renal insufficiency
	Previous airway colonization	Chronic rejection
	Renal failure	
	Renal replacement therapy	
Liver transplantation	Retransplantation	>6 g of prednisone in the 3rd month post-transplantation
	Renal insufficiency, especially if hemodialysis is required post-transplantation	Hemodialysis post-transplantation
		Renal insufficiency post-transplantation
	Liver transplantation due to fulminant hepatic failure	Post-transplantation leukopenia (<500/mm3)
	CMV infection	
	Complicated surgery or reintervention	
Heart transplantation	Isolation of *Aspergillus* spp. in respiratory tract cultures	Re-admission to the ICU
		Renal insufficiency post-transplantation
	Surgical reintervention	Concentrations of tacrolimus >15 ng/mL or of cyclosporine >500 ng/mL in the 3rd month post-transplantation
	CMV infection	
	Hemodyalisis post-transplantation	>2 episodes of acute rejection
Renal transplantation	Graft rejection	
	Hemodialysis	
	High and prolonged steroid doses	

ods different from culture may increase sensitivity, allowing an earlier diagnosis[25]. Diagnosis of aspergillar tracheobronchitis is performed by fibrobronchoscopy with biopsy and culture[42].

Galactomannan (GM)

The galactomannan is a component of the cell wall of *Aspergillus* that is released during tissue invasion and can be detected in serum, BAL, urine or cerebrospinal fluid. The most common technique uses the monoclonal antibody EBA-2 (Platelia Aspergillus®, Bio-Rad). False positives have been described with betalactam treatment, mainly piperacillin-tazobactam, reducing the test specificity[43]. Positivity is considered when the index is >0.7 in a single sample or >0.5 in two consecutive determinations[44]. Validity as diagnosis depends on the type of patient, being the highest in the neutropenic patient: 85% sensitivity and 95% specificity. In patients with hematological malignancies sensitivity is 70%, in those with bone marrow transplantation it is of 80%, and lower in the case of solid organ transplantation (25-50%)[25,45]. In ICU patients admitted due to COPD and IPA, positivity of two serum determinations presents a sensitivity of 41.7% and a specificity of 93.5%[46].

Quantification of galactomannan in BAL (but not in more accessible respiratory samples) is becoming of great utility, with an adequate diagnostic value in onco-hematological patients with neutropenia[47,48] and in critically ill patients. In this sense, in 110 critically ill patients (22% with neutropenia), using a cut-off value of 0.5, sensitivity and specificity in BAL was 88 and 87%, respectively, while sensitivity of galactomannan determination in serum was only 42%. In 11 out of the 26 cases with proven IPA, both BAL culture and galactomannan in serum were negative while the galactomannan in BAL was positive[49]. Similarly, in a Spanish study including 51 critically ill patients with a low number of neutropenic patients (11%), the most adequate cut-off value was ≥1, with 100% sensitivity and 89.36% specificity for proven IPA, and of 80% and 87.5%, respectively, for proven and probable IPA cases. In addition, galactomannan positivity anticipated a mean of 4.3 days the positivity of culture to *Aspergillus* spp[50].

Galactomannan determination in BAL has also been assessed in two risk populations as critically ill patients with COPD[51] or solid organ transplant recipients[52], being its diagnostic value higher than that of the serum determination.

1,3 β-D- glucan (BG)

BG is a component of the cell wall of most fungi (with the exception of *Cryptococcus* spp. and Zygomycetes). The Fungitell® test (Associates of Cape Cod Inc., Falmouth, USA) has been approved with cut-off values of <60 pg/ml and >80 pg/ml for negativity and positivity, respectively[53]. False positives have been described in patients under hemodialysis, in those treated with amoxicillin/clavulanic acid, azithromycin, pentamidin, immunoglobulins, albumin or glucans, with the use of cellulose filters for intravenous administration and in gram-positive bacteremia. In addition, it is also positive in infections by other fungi containing 1,3 β-D- glucan in the cell wall as *Candida* spp.[54,55].

The use of BG detection has provided acceptable diagnostic values in onco-hematological patients with neutropenia[55,56,57]. However, its utility for the diagnosis of IPA in immunocompromised critical patients is limited and lower than that of the galactomannan determination in BAL[50,58].

Nucleic acids

Detection of nucleic acids by the polymerase chain reaction (PCR) presents 88% sensitivity and 75% specificity for IPA diagnosis. The lack of a standardized method is the reason for discrepancies in the literature, however, sensitivity of DNA detection in BAL may be higher than in serum, more even if antifungal treatment has been initiated[59,60].

Conclusions: Utility for IPA diagnosis of imaging techniques, including CT scan, is low in the critically ill patient due to the low frequency of the presence of characteristic signs in non-neutropenic patients. Facing suspicion of IPA, determination of galactomannan in serum should be requested and, if possible, fibrobronchoscopy with BAL for culture and galactomannan determination since in this sample the accuracy of the test is higher than in serum.

c) Diagnosis of respiratory IFI by fungi from the order Mucorales

Pulmonary mucormycosis is characterized by a high degree of necrosis due to invasion of the bronchial wall, peribronchial tissue and blood vessels, producing thrombosis and pulmonary infarction with progressive pneumonia that progresses to cavitations. Clinical presentation does not differ from other bacterial or fungal pneumonias. The CT scan usually shows multiple nodular images and the so-called "reverse halo sign"[61]. Definitive diagnosis requires demonstration of tissue invasion by the characteristic non septated hyphas. Serological tests are not useful for diagnosis of mucormycosis, and its identification by PCR is not standardized[62].

Conclusions: A high degree of mucormycosis suspicion is required for initiating empirical antifungal treatment in the absence of isolation from respiratory samples, since neither clinical signs nor complementary tests are suggestive for diagnosis.

COLONIZATION VS. INFECTION: GREAT DILEMMA FOR TREATMENT DECISION MAKING IN RESPIRATORY IFI

Differentiation between fungal infection and colonization is one of the major challenges for clinicians.

a) *Candida* spp.

Pneumonia by *Candida* spp. is exceptional in non-neutropenic patients[63,64]. This was confirmed in a recent study including 135 ICU patients with evidence of pneumonia in necropsies (57% of them presenting BAL or bronchoaspirate cultures

positive to *Candida* spp. in the two previous weeks) where definitive diagnosis of pneumonia by *Candida* spp. was 0%[6].

However, *Candida* spp. colonization of the respiratory tract may have clinical significance since colonized patients present significantly higher length of stay and mortality[6], and is a risk factor for development of pneumonia by *Pseudomonas aeruginosa* and, in general, by multiresistant bacteria[65,66]. It should be taken into account that presence of *Candida* spp. in respiratory samples can be part of multifocal colonization that, in the presence of risk factors, is associated with a high incidence of invasive candidiasis[67].

Conclusions: Isolation of *Candida* from respiratory samples does not imply diagnosis of pneumonia by this fungus, which is exceptional in the non-neutropenic patient.

b) *Aspergillus* spp.

Identification of *Aspergillus* in respiratory samples may represent a simple colonization or be suggestive of IPA. The probability of being a true infection depends on the type of patient: 72% for patients with neutropenia[14,68], 55% for solid organ transplant recipients[14] and 22% for COPD patients[23]. When prospectively analyzing the significance of *Aspergillus* isolation from respiratory samples in all patients admitted in a general hospital, only 10% cases corresponded to true IPA[69]. A recent Spanish series has demonstrated that colonized patients are older and present higher number of comorbidities than those presenting IPA[70]. In the case of patients admitted to the ICU, it depends on the type of patients, ranging from 25% to 70%[17,18,19,71].

In any case, isolation of *Aspergillus* from respiratory samples in a patient admitted to the ICU is a marker of bad prognosis, regardless colonization or infection[71]. With respect to the implicated species, *A. terreus* seems to produce true infection more frequently than other species[10].

Criteria from the *European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group* (EORTC/MSG) continues to be the basis for diagnosis of IPA, classifying it as possible, probable or proven[72]. However, its utility in the ICU is limited: absence in many cases of classical risk factors, of typical signs in CT scan or frequent negative results in the serum galactomannan test.

For this reason, several scales have been described as tools for IPA diagnosis and subsequent decision making. Bouza et al.[15] published an scale, based on relative risk values for significant variables in a multivariate analysis, but since it was constructed with data from a general hospitalized population, the scale may be not appropriate for critically ill patients.

Vandewoude et al.[18] analyzed 172 critically ill patients with isolation of *Aspergillus* spp. from respiratory samples and proposed a diagnostic algorithm shown in table 3. In 26 cases, the diagnosis based on this clinical algorithm was confirmed by histological data. Applicability and generalization of diagnostic algorithms requires prospective validation[73].

In this sense, a recent multicentre study has validated the clinical algorithm proposed by Vandewoude et al. for IPA diagnosis. In that study, 524 critically ill patients with at least one endotracheal aspirate culture positive to *Aspergillus* spp. were included, 115 of them with histological data[74]. Globally, positive and negative predictive values were 61% and 92%, respectively. When only COPD patients receiving prolonged steroid therapy were considered, positive and negative predictive values were 45% and 100%, respectively, for an IPA prevalence of 20% among patients with positive endotracheal aspirate culture, and of 77% and 100%, respectively, for a prevalence of 50%. In any case, the diagnostic utility of this algorithm was higher than the EORTC/MSG criteria.

In COPD patients, the high mortality of IPA is due, among others, to the difficulty for diagnosis and to the absence of unequivocal diagnosis criteria leading to treatment delay[75]. Bulpa et al. established diagnostic criteria based on a revision of the literature on COPD patients with IPA[76] that have not been validated in prospective series.

Conclusions: There are several clinical algorithms that may help clinicians in discriminating patients with IPA from those presenting only colonization by *Aspergillus*. Up to now, only the one proposed by Vandewoude has been validated in critically ill patients and can be used for decision making when facing *Aspergillus* spp. isolation from respiratory samples. One of the problems of the algorithm is the requirement of a positive culture of a respiratory sample, because IPA may be present in the absence of positive culture. Presence of species as *A. terreus* should be considered indicative of high probability of infection.

Figure 1 summarizes the scheme of actions to follow in the case of isolation of *Aspergillus* spp. from respiratory samples in patients with respiratory insufficiency that present high-risk or intermediate-risk factors (table 1).

c) Mucorales

Definitive diagnosis of mucormycosis requires histological demonstration of tissue invasion. However, its isolation in the critically patient, especially if there are risk factors and compatible radiological image, should always lead to initiation of antifungal treatment[5].

Conclusions: Isolation of Zygomycetes in a patient with risk factors should be considered an infection and antifungal treatment should be initiated. Considering the high mortality of this infection, colonization should only be considered in the case of isolation in a patient without risk factors, lack of clinical signs/symptoms and with a thorax CT scan showing absence of compatible alterations. In these cases collection of new respiratory samples is recommended. In case of a new positive culture, the possibility of antifungal treatment should be reconsidered.

Table 3 Diagnostic criteria for invasive pulmonary aspergillosis in critically ill patients

Definitive invasive pulmonary aspergillosis
A) Positive result of histological testing and positive result of culture from lung tissue obtained by biopsy or autopsy
B) Positive result of culture of a specimen from a normally sterile site by use of aseptic invasive technique
Probable pulmonary aspergillosis
1. *Aspergillus*-positive lower respiratory tract specimen culture
2. Compatible signs and symptoms:
Fever refractory to at least 3 days of appropriate antibiotic therapy
Recrudescent fever after a period of defervescence of at least 48 hours while still on antibiotics and without other apparent cause
Pleuritic chest pain
Pleuritic rub
Dyspnoea
Hemoptysis
Worsening respiratory insufficiency in spite of appropriate respiratory therapy and ventilatory support
3. Abnormal medical imaging by portable chest x-ray or CT scan of the lungs
4. Either:
a) Host risk factors: one of the following conditions
Neutropenia (absolute neutrophil count <500 /mm^3) preceding or at the time of ICU admission
Underlying haematological or oncological malignancy treated with cytotoxic agents
Glucocorticoid treatment (prednisone or equivalent, >20 mg/day)
Congenital or acquired immunodeficiency
OR
b) Semiquantitative *Aspergillus*-positive culture of BAL (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae
***Aspergillus* colonization**
Not fulfilling the criteria for proven or probable invasive pulmonary aspergillosis

Taken from Vandewoude et al[18]

THERAPEUTIC ARSENAL, INDICATIONS FOR TREATMENT, DRUGS OF CHOICE AND POTENTIAL ROUTES OF ADMINISTRATION

Three antifungal classes are available for the treatment of fungal infections: polyenes, azoles and echinocandins. Polyenes, mainly amphotericin B, are fungicidal and present the widest spectrum of activity, with resistance to these agents only reported in *Candida lusitaniae* and *A. terreus*[77]. The traditional formulation of amphotericin B deoxycholate has been replaced by lipid-based formulations: liposomal amphotericin B, amphotericin B lipid complex and amphotericin B colloidal dispersion, being the two first mentioned commercially available in our country. Liposomal amphotericin B improves the pharmacokinetic profile, increasing the Cmax/MIC value, the pharmacodynamic parameter associated with efficacy against *Aspergillus*[78], with a value of the area under the concentration-time curve higher for liposomal amphotericin B than for other lipid-based presentations[79].

Lipid formulations of amphotericin B have shown similar efficacy than the conventional formulation, with lower toxicity[80]. Among them, the liposomal formulation is the best tolerated with a lower incidence of infusion-related adverse reactions (fever, chills) and with lower rate of renal failure[81]. In a double-blind clinical trial comparing liposomal amphotericin B (at 3 and 5 mg/kg) with amphotericin B lipid complex (5 mg/kg) in the treatment of febrile neutropenia, no differences in efficacy were found between the two formulations, but the rate of adverse events was significantly higher for amphotericin B lipid complex: fever (23.5% and 19.8% vs. 57.7% on day 1 for liposomal amphotericin B at 3 mg/kg, liposomal amphotericin B at 5 mg/kg vs. amphotericin B lipid complex; p<0.001), chills (18.8% and 23.5% vs. 79.5% on day 1; p<0.001) and especially, nephrotoxicity (14.1% and 14.8% vs. 42.3%; p<0.01)[82]. Another study comparing these two lipid formulations in the treatment of invasive aspergillosis reported 21.2% nephrotoxicity with amphotericin B lipid complex versus only 2.8% with liposomal amphotericin B (p<0.001)[83]. Development of renal

dysfunction with liposomal amphotericin B is minimal, even in critically ill patients with previous renal impairment[84].

Azoles are fungistatic and despite their similar mechanism of action, differences in their chemical structure lead to drug-dependent activity profiles. All are active against yeasts, but while fluconazole is not active against filamentous fungi, itraconazole, voriconazole and posaconazole are active against *Aspergillus*[77]. Posaconazole, only available by oral route, is the unique azole active against Mucorales.

Echinocandins are active against *Aspergillus* and *Candida*, without activity against Mucorales[85]. They exhibit fungistatic activity against *Aspergillus*, being the ratio Cmax/MIC (minimum inhibitory concentration) the pharmacodynamic parameter predicting efficacy with a value of 10-20[78]. In Europe, only caspofungin has been approved for the treatment of aspergillosis as salvage therapy.

a) *Candida* spp.

Expert recommendations and clinical practice guidelines do not recommend antifungal treatment facing isolation of *Candida* spp in respiratory samples regardless the number of positive samples or the sample type[86,87].

b) *Aspergillus* spp.

Prompt initiation of antifungal therapy has demonstrated benefits in terms of mortality in patients with IPA. In this sense, a secondary analysis of the study *Ambiload* showed that survival was significantly higher when treatment was initiated in case of possible IPA when compared to probable or proven cases[88]. More recently, a retrospective study that evaluated 412 ICU patients with IPA has demonstrated that a delay in the initiation of antifungal therapy implicates an increment of hospital length of the stay with the corresponding increase of hospital cost[89]. Thus, it is recommended early initiation of antifungal treatment, often empirically[90]. In critically ill patients, the need for immediate initiation of antifungal treatment in patients with respiratory insufficiency is determined by the presence of high-risk factors (figure 1).

Current recommendations from scientific societies for the treatment of IPA are summarized in table 4[90,91,92]. Regarding treatment in critically ill patients and transplant recipients, it should be highlighted that clinical trials carried out to obtain the indication for IPA treatment did not include this type of patients[93].

A randomized, open clinical trial showed the superiority of voriconazole versus amphotericin B deoxycholate for the treatment of IPA, with a 12-week survival rate significantly higher for voriconazole (70.8% vs. 57.9%)[94]. Although important limitations of this clinical trial were evidenced[95], voriconazole is indicated as primary therapy in all current guidelines[90,91,92]. Other observational studies have confirmed the clinical utility of voriconazole in the treatment of IPA[96,97]. A retrospective study in critically ill hematological patients with IPA requiring mechanical ventilation concluded that treatment with voriconazole was associated with a lower mortality rate[40].

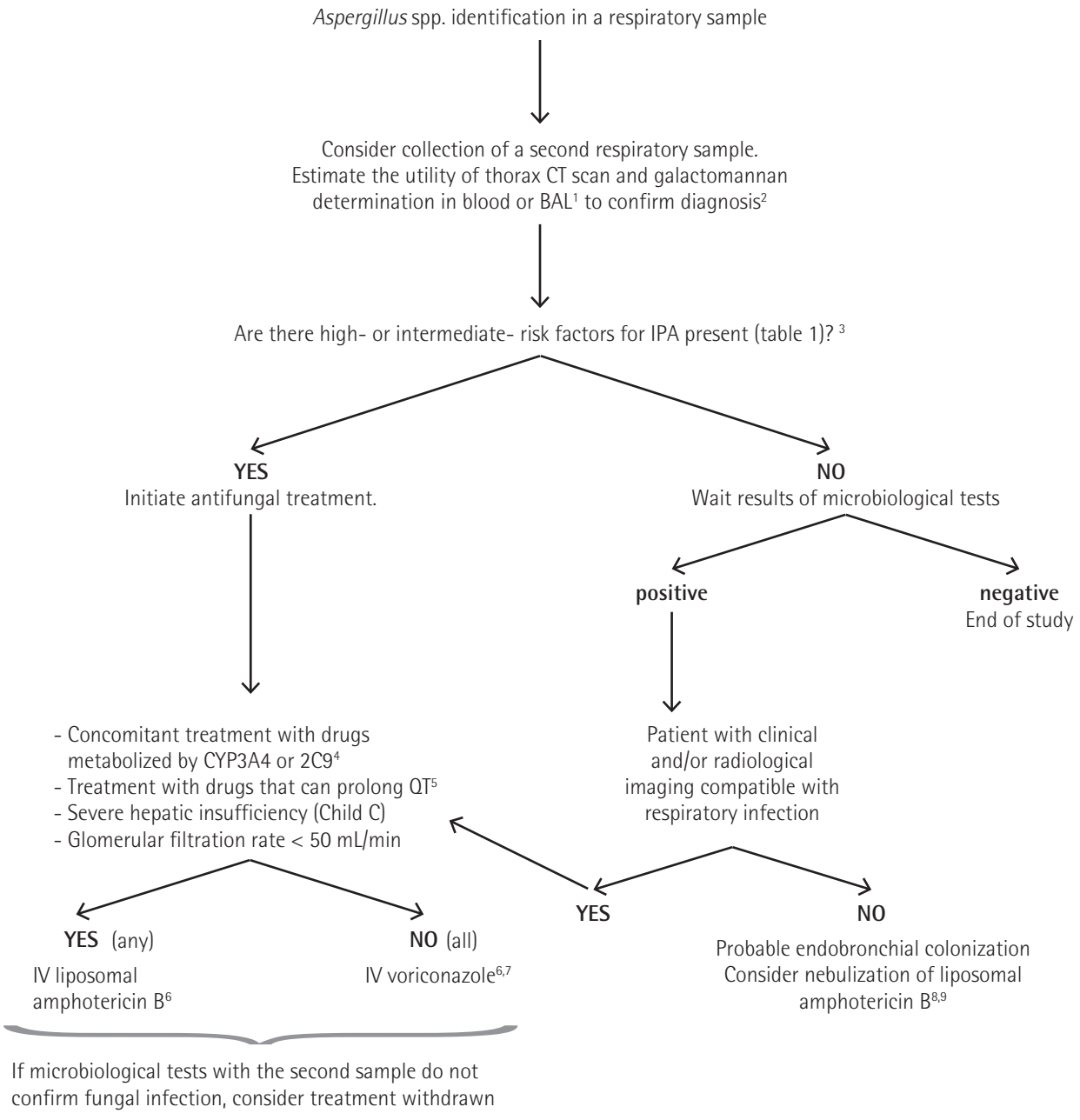
However, it should be taken into account that voriconazole presents interactions with an elevated number of drugs. Voriconazole is metabolized by, and inhibits, enzymes of cytochrome P450: CYP2C19, CYP2C9 and CYP3A4[98]. This affects a high number of drugs (table 5), a fact of critical importance especially in transplant recipients. In addition, there is a great inter-subject variability in serum voriconazole concentrations in relation to age, dose, underlying diseases, hepatic function and the genetic polymorphism of CYP2C19[99,100], making necessary monitoring of plasmatic concentrations. In critically ill patients there is a great variability of voriconazole serum concentrations, with concentrations ≤1 mg/L associated with therapeutic failure and those ≥5.5 mg/L with toxicity[99]. In a recent clinical trial randomizing patients with fungal infection to serum levels monitoring from the 4th day on or to fixed standard treatment, the group of patients with monitoring serum concentrations showed higher clinical response rate and a significantly lower rate of treatment discontinuation[101].

Cyclodextrin sodium is the vehicle used for the intravenous formulation of voriconazole, a compound mainly associated with neurological toxicity that can be accumulated in patients with renal insufficiency, without an adequate clearance by renal replacement therapies[102]. For this reason, in the case of renal insufficiency (creatinine clearance <50 ml/min) careful assessment of the risk-benefit of its administration is necessary. Voriconazole produces hepatic toxicity in up to 45% of patients with previous hepatic impairment compared with 10.3% produced by liposomal amphotericin B, with a clear correlation between the loading dose and the degree of hepatic impairment[103].

With respect to amphotericin B, the liposomal formulation has also demonstrated utility in the treatment of IPA. In this sense, in the *Ambiload* study, 201 patients (93% with hematological malignancies and 73% with neutropenia) were included and two liposomal amphotericin B doses (3 mg/kg and 10 mg/kg) compared, with similar efficacy but lower toxicity in the 3 mg/kg arm[104]. There is no published clinical trial comparing lipid amphotericin B formulations in the treatment of IPA. Nevertheless, in a retrospective study no differences in efficacy (clinical cure and mortality) were found, but the frequency of adverse events was significantly lower with liposomal amphotericin B[105]. No information on the utility of nebulized amphotericin B in the treatment of IPA is available.

Caspofungin was approved as salvage therapy after intolerance or failure of conventional therapy[106]. Afterwards, one study assessed the use of caspofungin as primary treatment of IPA in high-risk neutropenic patients, obtaining a clinical cure of 33% and 12-week survival of 53%[107]. Recently, a dose-escalation study using doses up to 200 mg/day in the treatment of IPA showed good tolerance but clinical cure rates were similar to those obtained with voriconazole or liposomal amphotericin B[108].

Other antifungals with approved indication for the treatment of IPA are amphotericin B lipid complex, itraconazole and posaconazole. These drugs receive low strength of recommendation in current guidelines and its clinical utility in the treatment of IPA in critically ill patients is limited.



[1] Thorax CT scan and galactomannan determination in serum and BAL are indicated in all patients with clinical suspicion.
[2] It is advisable to confirm diagnosis by means of examination of a second respiratory sample, preferably obtained by bronchoalveolar lavage. Visualization of hyphaes with calcofluor staining and/or presence of several colonies in cultures from more than one sample suggests the possibility of infection/colonization rather than accidental contamination.
[3] In high- or intermediate- risk patients presenting sepsis or septic shock criteria without other apparent infectious focus, antifungal treatment should be immediately initiated.
[4] Carbamazepine, barbiturics, rifamicins, phenytoin, phenobarbital, among others.
[5] Citalopram, diphenhydramine, fluoxetine, foscarnet, granisetron, metronidazole, nortriptiline, ondansetron, macrolides, among others.
[6] If the radiological image is bilateral and/or extensive, there is severe respiratory insufficiency, severe sepsis or unfavorable evolution, the use of two antifungals is recommended (voriconazole or liposomal amphotericin B or caspofungin) and consider addition of nebulized liposomal amphotericin B.
[7] When using voriconazole, monitor serum concentrations from the 3rd-5th day on.
[8] Use jet nebulizers with a high flow compressor.
[9] More published data with liposomal amphotericn B, but a randomized trial on efficacy is lacking.

Figure 1 | **Management of ICU patients with respiratory insufficiency and isolation of *Aspergillus* spp. from respiratory samples**

Table 4 Current recommendations from scientific societies for the treatment of invasive pulmonary aspergillosis

Drug	IDSA	ATS	ECIL	SEIMC
Voriconazole	Primary therapy A-I	Primary therapy A-II	Primary therapy A-I	Primary therapy A-I
Liposomal amphotericin B	Alternative treatment A-I	Primary therapy A-II	Primary therapy B-I	Primary therapy A-I
Amphotericin B lipid complex	Alternative treatment A-II	-	Primary therapy B-II	-
Caspofungin	Alternative treatment B-II	Alternative treatment C-II	Alternative treatment C-II	Alternative treatment C-II
Micafungin	Alternative treatment B-II	Alternative treatment -	-	-
Combination therapy	Salvage therapy B-II	Salvage therapy C-II	Primary therapy D-III Salvage therapy C-II	Salvage therapy C-III

IDSA: Infectious Diseases Society of America; ATS: American Thoracic Society; ECIL: European Conference on Infections in Leukemia; SEIMC: Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica

Nowadays, there is insufficient clinical support to recommend combination therapy as primary therapy in the treatment of IPA and current guidelines recommend it as salvage therapy[90,91]. Although some studies reported a decrease in mortality with combination therapy[109,110], a recently published meta-analysis including eight studies in onco-hematological patients mainly treated with liposomal amphotericin B plus caspofungin or voriconazole plus caspofungin concluded that cumulative evidence on combination therapy is moderate and controversial[111]. However, it should be highlighted that in observational studies on IPA, approximately 30-50% patients are treated with combination therapy and up to 30% of patients in the ICU require salvage therapy due to therapeutic failure of the primary treatment[23,40,70,98].

Duration of treatment for IPA should be established on a patient-basis according to the clinical and radiological evolution of the patient. In general, 6 to 12 weeks are recommended for non-neutropenic patients and longer periods in the onco-hematological patient if neutropenia persists. If combination therapy is used, one of the drugs should be withdrawn when the clinical situation improves and even change to oral therapy until the end of treatment. For aspergillar tracheobronchitis, antifungal treatment should be administered by intravenous route as for the treatment of IPA[92]. A recent review of the literature evidenced that 35.9% cases of aspergillar tracheobronchitis received combination therapy, a percentage increasing to 48.5% in last decade[42]. Nebulized amphotericin B would be a logical therapeutic alternative, but only scarce cases have been reported[112], not allowing establishing recommendations.

Conclusions: When IPA is suspected, diagnostic procedures and prompt initiation of systemic antifungal treatment should be performed (figure 1). When possible, associated risk factors should be minimized; in this sense, doses of immunosuppressant agents and specially steroids should be reduced or withdrawn.

In cases of IPA requiring ICU admission or developed during ICU stay with respiratory failure or criteria of severe sepsis, the group recommends combination therapy. Similarly, if the patient under antifungal monotherapy is admitted to the ICU due to clinical worsening, it is recommended initiation of combination therapy. Drug election will depend on pre-existing renal or hepatic failure, need for other drugs that may interact with the antifungal (especially voriconazole) and the *Aspergillus* species, if known. Those stable patients with IPA treated with monotherapy that require admission to the ICU for other reasons, can be maintained under treatment with the same antifungal if signs/symptoms of disease progression and adverse events attributable to the antifungal are not present. If the patient is diagnosed of IPA during ICU stay but does not present criteria of severe sepsis or respiratory failure, monotherapy should be used, being voriconazole the first option. When this azole is used, monitoring of serum concentrations is always required from the 3rd-5th day on and 1-2 times a week. However, as shown in figure 1, if certain situations are present the initial option would be liposomal amphotericin B.

When combination therapy is used, one of the drugs should be withdrawn when the clinical situation improves or the risk factor (mainly, use of steroids or neutropenia) disappears and, if possible, change to oral route until the end of treatment. Obviously, if treatment is empirically initiated and diagnostic procedures disregard IPA, treatment should be withdrawn.

Table 5 Voriconazole interactions

Type of interaction	Recommendation
The drug decreases voriconazole concentrations	
Carbamazepine	Contraindicated
Rifampicin	Contraindicated
Phenobarbital	Contraindicated
Ritonavir	Contraindicated
Voriconazole increases drug concentrations	
Astemizole	Contraindicated
Cisapride	Contraindicated
Cyclosporine	Reduce dose by half and monitor concentrations
Ergotamine alkaloids	Contraindicated
Omeprazole	Reduce dose by half
Quinidine	Contraindicated
Sirolimus	Contraindicated
Everolimus	Reduce dose
Tacrolimus	Reduce dose by two third and monitor concentrations
Terfenadine	Contraindicated
Warfarin	Monitor prothrombin time
The drug decreases voriconazole concentrations and voriconazole increases drug concentrations	
Rifabutin	Contraindicated
Phenytoin	Double voriconazole dose and monitor phenytoin concentrations
Voriconazole probably increases drug concentrations	
Statins	Consider reduction
Sulphonylureas	
Calcium channel blockers	
Benzodiazepines	

c) Mucormycosis

As in other fungal infections, it has been demonstrated in patients with mucormycosis that delays in initiation of antifungal treatment are associated with higher mortality rates[113]. Amphotericin B is the drug of choice and due to its lower toxicity, the use of liposomal amphotericin B is recommended[90,114,115]. Doses to be used should range from 5 to 10 mg/kg/day[5,115,116]. Treatment duration is not defined and should be individually chosen in any case.

Posaconazole may be an alternative in combination with liposomal amphotericin B in case of therapeutic failure or intolerance. Monotherapy with this azole is not recommended, monitoring of serum concentrations is required and existing interactions with a high number of compounds should be taken into account[117].

Echinocandins do not exhibit *in vitro* activity against Mucorales, although some technical reasons and experimental data suggest that when combined with amphotericin B, they may be of clinical utility[118]. A retrospective series of patients with rhinocerebral mucormycosis suggested that the combination of caspofungin and a lipid formulation of amphotericin B reduces mortality[119]. This therapeutic strategy has not been reported for pulmonary mucormycosis.

Conclusions: When pulmonary mucormycosis is suspected, antifungal treatment should be initiated without delay. Liposomal amphotericin B at doses of at least 5 mg/kg/day is recommended. Its use as empirical treatment has the added value of effectively covering a possible IPA, an infection with very similar clinical and radiological presentations to mucormycosis.

PROPHYLAXIS OF RESPIRATORY FUNGAL INFECTIONS

There are different strategies of demonstrated utility for

the prevention of invasive fungal infections in high-risk patients with systemic antifungal administration, but its review is out of the scope of this document. On the other hand, review of available data on nebulized antifungal administration, an attractive option for IPA prevention is addressed. Up to now, this route of administration has only been employed for amphotericin B and its lipid formulations, with clinical experience mainly in lung transplant recipients and neutropenic patients. It should be highlighted that although it is common practice and supported by extensive scientific literature, none of the amphotericin B formulations is approved for use via nebulization.

In lung transplant recipients, two studies carried out in our country demonstrated the lack of systemic absorption of nebulized liposomal amphotericin B and demonstrated persistence of therapeutic concentrations in the epithelial lining fluid (ELF) for 14 days[120,121]. Recently, therapeutic concentrations in ELF following nebulization of amphotericin B lipid complex have been confirmed, but using higher doses and shorter dosing intervals[122]. With regard to its effect in preventing IPA, a Spanish study retrospectively reviewed 60 cases of lung transplant recipients followed during 6 months. Nebulization of amphotericin B lipid complex was efficacious since only one patient developed a probable IPA[123]. A clinical trial including 100 lung transplant recipients randomized to amphotericin B deoxycholate or amphotericin B lipid complex as aerosolized prophylaxis showed similar efficacy of both compounds in the prevention of infection by *Aspergillus*[124].

Two clinical trials compared nebulized liposomal amphotericin B versus placebo in onco-hematological patients with neutropenia present for more than 10 days, and showed a significant decrease in IPA episodes without association with severe adverse events[125,126]. In fact, the *European Conference on Infections in Leukemia* (ECIL) recommends (B-II) as prophylaxis of invasive fungal infections, nebulization of liposomal amphotericin B (together with intravenous fluconazole) in the neutropenia phase of the allogeneic hematopoietic progenitor cell transplantation. In the same circumstance, nebulization of conventional amphotericin B is not recommended (D-I)[127].

Nebulized liposomal amphotericin B at the standard 25 mg dose does not alter the pulmonary function nor the composition of the pulmonary surfactant[121,128]. It is well known that, in lung transplant recipients, there is a dysfunction of the pulmonary surfactant associated with a dysfunction of the graft, and for this reason, it is important that none exogenous agent alters the composition of the surfactant[129].

A jet nebulizer with a high flow compressor should be used to obtain an adequate particle size (from 3 to 5μ)[130]. When choosing the nebulizer, the compound to be nebulized should be considered since doses of the two available lipid formulations are different and thus, the volume to be nebulized: 25 mg (5 ml) liposomal amphotericin B or 50 mg (10 ml) amphotericin B lipid complex. Some authors recommend loading doses during four consecutive days, doubling doses in intubated patients. Afterwards, it can be administered 2-3 times/week. An exhaustive bronchial hygiene prior to nebulization is crucial. Contamination of nebulization systems can be the origin of respiratory infections and thus, strict disinfection protocols should be followed. In addition, obstructions of ventilator filters may occur and this possibility should be closely monitored[131].

Conclusions: The use of amphotericin B is a strategy with demonstrated utility for prevention of IPA in high-risk patients, especially in lung transplant recipients and onco-hematological patients with prolonged neutropenia, being the liposomal formulation recommended in clinical practice guidelines. Although nowadays there is not a single compound approved for administration via nebulization, its use has become common practice in high-risk patients. Advantages of liposomal amphotericin B nebulization are easy administration, undetectable serum concentrations, excellent tolerance with mild and well tolerated adverse events, low risk of infection by emergent molds and cost-effectiveness.

However, its use as prophylaxis in the critically ill patient has not been established yet. A theoretical possibility would be its use in COPD patients treated with high doses of steroids admitted to the ICU. With current data, no formal recommendation for use can be done and responsible clinicians should examine cases individually.

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