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Diagnostic and therapeutic approach to fungal pneumonia in the critically ill patient

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ABSTRACT

Aspergillus spp. is the fungus most frequently producing ventilator-associated pneumonia (VAP), constituting 8% of them. This risk is significantly increased in onco-hematological patients: solid organ transplant recipients, chronic obstructive pulmonary disease (COPD), corticotherapy, cirrhosis, solid cancer, or viral pneumonias. The European Organization for Research and Treatment of Cancer Mycoses (EORTC/MSG criteria) developed for onco-hematological patients with angioinvasive forms of aspergillosis have important limitations for broncho-pulmonary forms, such as aspergillosis cases in the ICU. In recent years, new diagnostic criteria were developed to have a greater role in broncho-alveolar lavage, especially GM and lateral flow assay (LFA). Voriconazole and isavuconazole are the first treatment option. However, drug-drug interaction, level requirements, toxicity, and QT-interval modification are limitations that may favor isavuconazole or liposomal amphotericin B in the ICU.

Keywords: *Aspergillus*, broncho-alveolar lavage, voriconazole, isavuconazole

EPIDEMIOLOGY OF FUNGAL PNEUMONIA IN THE INTENSIVE CARE UNIT

Ventilator-associated pneumonia (VAP) is the most frequent infection in patients admitted to the intensive care unit (ICU), usually occurring in one third of them. According to the latest national surveillance study of nosocomial infection in ICUs in Spain [1], *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are the etiologic agents most frequently implicated, as all of them produce 44% of VAP [1].

In this registry, 10% of VAP are of fungal origin, mainly caused by *Aspergillus* sp. which accounts for 8% in ICU patients [1]. The role of other molds is anecdotal: zygomycosis infections are currently restricted to patients with hematological malignancies, primarily with prolonged neutropenia and solid organ transplanted patients with a degree of immunosuppression. In contrast, there has been a significant decrease in recent years in diabetic ketoacidosis as a zygomycosis risk, associated with improved management of diabetes in the general population [2]. Infections from agents such as *Fusarium*, *Scedosporium*, and *Loomentospora*, while other molds are limited to prolonged neutropenias and similar behavior to invasive aspergillosis. *Pneumocystis jirovecii* pneumonia, restricted to patients with cellular immunosuppression and endemic fungal infections (*Histoplasma* spp. and others) are in certain geographical areas, and need special consideration.

Candida spp. as a causative agent of VAP is controversial. Some authors exclude it as an etiological agent and others estimate its incidence below 1%, related to risk factors such as severe immunosuppression, malnutrition, high fungal load (e.g., diabetes, alcoholism, gastroesophageal reflux, presence of esophageal diverticula), or broad-spectrum antibiotic therapy [3]. Regardless of causality, a recent meta-analysis associated airway colonization by *Candida* spp. in ICU patients with a longer duration of intubation, higher ICU mortality, and a higher 28-day mortality rate [4].

RISK FACTORS FOR INVASIVE PULMONARY ASPERGILLOSIS (IPA) IN ADMISSIONS TO THE ICU

By the number of patients, ICU admission alone is the largest risk factor for IPA, above classic factors such as the onco-hematologic patient or transplant recipients, as they comprise most IPA diagnoses in a general hospital [5].

Bassetti et al. categorized patients in the ICU according to IPA risk (Table 1) [6]. Isolation of *Aspergillus* spp. in respiratory

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specimens is associated with high mortality in the critically ill patient. Invasive forms can be associated with mortality between 69–77%, but colonization in the absence of infection is also associated with a mortality of 38%, as demonstrated in a recent study on 563 patients from 30 ICUs in eight countries [7]. The following independent mortality factors were observed: age, hematopoietic progenitor transplantation, mechanical ventilation, high SOFA score (Sequential Organ Failure Assessment) and dialysis at diagnosis – which are associated with invasion vs. colonization in cancer patients (including hematologic) or solid organ transplantation [7].

Patients with severe chronic obstructive pulmonary disease (COPD) receiving broad-spectrum antibiotics and corticosteroids are becoming one of the main risk groups for IPA in ICU [8]. Guinea et al. analyzed the risk of IPA in COPD patients and confirmed the following: admission to the ICU, chronic heart failure, antibiotic treatment in the 3 months prior to admission, accumulated dosage of corticosteroids equivalent to > 700 mg prednisone in the 3 months prior to admission, and a similar accumulated dosage of corticosteroids from admission to the first clinical isolation of *Aspergillus* [8].

Table 1	Risk Factors for IPA in ICU Patients
1. High risk	Neutropenia (500/mm ³) Hematological malignancy Allogeneic HSCT
2. Intermediate risk	Prolonged treatment with corticosteroids before admission to the ICU Autologous HSCT COPD Liver cirrhosis Solid organ cancer HIV infection Lung transplantation Systemic immunosuppressive therapy
3. Low risk	Severe burns Solid organ transplant Steroid treatment for > 7 days Prolonged stay in the ICU (> 21 days) Malnutrition Post cardiac surgery Near drowning

COPD chronic obstructive pulmonary disease, HIV human immunodeficiency virus, HSCT hematopoietic stem cell transplantation, ICU intensive care unit, and IPA invasive pulmonary aspergillosis. Modified from reference [6].

The use of certain biologics is associated with an increased risk of IPA in clinical practice; in recent years, this includes ibrutinib –used to treat chronic lymphocytic leukemia, mantle lymphoma, and Waldenström's disease; an elevated risk of IPA was observed when used in combination or after other immunosuppressive treatment, especially Janus kinase inhibitors (JAK) (ruxolitinib) or idelalisib [9].

Controversy exists about ECMO (extracorporeal membrane oxygenation) as a risk factor for IPA in ICU patients. Some studies attribute a 7% risk of IPA to ECMO patients [10]. However, a study conducted in over 20,000 patients in 300 centers in the American Extracorporeal Life Support Organization Registry found that 1.4% was the risk of IPA among ECMO patients, higher in onco-hematology, solid organ transplantation, or influenza [11].

In recent years, certain respiratory viral infections, such as influenza or SARS-CoV-2, appear as important risk factors for IPA. A study performed in 7 ICUs in Belgium and the Netherlands, confirmed an incidence of IPA in 19% of those admitted with influenza to the ICU; this reached 32% in immunocompromised patients, with an associated overall mortality of 51% [12]. Coinciding with the SARS-CoV-2 pandemic, an increased incidence of IPA is described, especially in patients admitted to the ICU, estimated at 20–35% in certain national series [13].

DIAGNOSIS OF ASPERGILLUS PNEUMONIA: PECULIARITIES OF THE CRITICALLY ILL PATIENT

Regardless of the type of clinical form or baseline condition, documentation of hyphae on biopsy, or isolation of *Aspergillus* spp. in a culture of a sterile specimen constitutes a diagnosis of proved infection. However, this can only be confirmed in a minority of patients.

The positive predictive value of *Aspergillus* isolation in sputum is generally low, depending on patient type and risk; it may not exceed 10% in COPD patients, or even reach 50% in liver transplant recipients, or exceed 80% in hematopoietic stem cell transplant patients. The EORTC and Mycoses study groups (MSGs) developed well-validated criteria in onco-hematological patients [14,15]. As such, a diagnosis of probable aspergillosis includes the intersection of 3 factors: a) host at risk (e.g., prolonged neutropenia, GVHD, or solid organ transplantation), b) image: the presence of at least one of the following four patterns: dense, well-circumscribed lesions with or without a halo sign, air crescent sign cavity, wedge-shaped and segmental, or a lobar consolidation, and c) microbiologic: *Aspergillus* spp. isolation in respiratory samples, a positive galactomannan (GM) in serum or bronchoalveolar lavage (BAL), or a positive direct test (cytology, direct microscopy). Positive PCR was not considered diagnostic of IPA in previous consensus criteria [14] but was incorporated in the last version [15]. The presence of only 2 factors: a) host and b) image, in the absence of a microbiological confirmation, can only be considered possible invasive fungal infection.

Application of these criteria to a patient admitted to the

Table 2 Algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients

Proven Invasive Pulmonary Aspergillosis Idem EORTC/MSG criteria

Putative Invasive Pulmonary Aspergillosis (All four criteria must be met):

1. *Aspergillus*-positive lower respiratory tract specimen culture (= entry criterion)
2. Compatible signs and symptoms (one of the following):
 - *Fever refractory to at least 3 d of appropriate antibiotic therapy
 - *Recrudescence 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 despite appropriate antibiotic therapy and ventilatory support
3. Abnormal medical imaging by portable chest X-ray or CT lung scan
4. Either 4a or 4b
 - 4a. Host risk factors (one of the following):
 - *Underlying hematological / oncological malignancy treated with cytotoxic agents
 - *Neutropenia (absolute neutrophil count, $500/\text{mm}^3$) preceding or same time as ICU admission
 - *Glucocorticoid treatment (prednisone equivalent, $> 20 \text{ mg/d}$)
 - *Congenital or acquired immunodeficiency
 - 4b. Semiquantitative *Aspergillus*-positive culture of BAL fluid, without bacterial growth, together with a positive cytological smear showing branching hyphae

Aspergillus respiratory tract colonizationIf more than 1 criteria are missing for a diagnosis of putative IPA, the case is classified as *Aspergillus* colonization

Definition of abbreviations: BAL: bronchoalveolar lavage; CT: computed tomography; EORTC/MSG: European Organization for the Research and Treatment of Cancer/ Mycosis Study Group; ICU: intensive care unit. Modified from reference [19].

ICU is challenging. Different studies confirmed that the sensitivity of these criteria decreases significantly in non-onco-hematological patients; in these patients, according with a bronchopulmonary origin, the most frequent radiological infiltrates are peribronchial consolidation or a tree-in-bud pattern, differing from the typical signs observed in onco-hematological patients (re: halo sign or air crescent sign cavity) [16].

In addition to low specificity of IPA infiltrates in the non-onco-hematologic patient is the lower cost-effectiveness of serum GM with bronchopulmonary forms of IPA, the case with most ICU patients. Serum GM has a lower sensitivity in patients with immunosuppressive conditions and in COPD patients vs. hematological patients. We previously confirmed a sensitivity of only 56% in the diagnosis of IPA in liver recipients [17], with a sensitivity $< 50\%$ reported in a systematic review of the literature for non-hematology-oncology patients [18]. The value of serum GM in patients with COPD, and risk of IPA, was evaluated in several studies, with sensitivity ranging between 30% and 60% [18]. Reduced sensitivity has been linked to two factors: increased clearance of GM by circulating neutrophils and lower angio-invasiveness of *Aspergillus* spp. [18].

To overcome these problems in ICU patients, new criteria for probable or putative aspergillosis are proposed (AspICU) and extensively validated in prospective cohorts [19] (Table

2). These criteria require a 'sine qua non' condition or isolation of *Aspergillus* spp. in respiratory samples (sputum or broncho-aspirated sample). Thereafter, for AspICU criteria, the following combination was required: a) compatible signs and symptoms, b) abnormal medical imaging by portable chest X-ray or CT scan of the lungs (not limited to accepted in onco-hematologic patients), and c) host risk factors or positive cytological smear. For diagnosis of tracheobronchitis, the presence of tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar on a bronchoscopic analysis with visualization of hyphae in biopsy or isolation of *Aspergillus* in culture, is required [19].

These discrepancies in diagnostic criteria led to considerable confusion in ICU patients. The description in recent years of IPA in patients with influenza, and more recently in patients infected by SARS-CoV-2, allowed these diagnostic criteria to be reevaluated. Table 3 shows criteria applied for diagnosis of IPA with influenza (IAPA) for patients admitted to the ICU [20]. These criteria, as in AspICU criteria, accept the presence of "pulmonary infiltrates" without specificity of EORTC/MSG infiltrates (halo sign, air crescent sign cavity...). To acquire specificity, it is compensated with a more demanding microbiological criterion, such as isolation of *Aspergillus* spp. or a GM > 1.0 in BAL, or the presence of positive GM in blood (> 0.5); yet, as already mentioned, this technique is not sensitive in non-onco-hematological patients [20]. In the absence of BAL, the iso-

Table 3		Proposed case definition for IAPA in ICU patients	
Entry criteria: influenza-like illness + positive influenza PCR or antigen + temporally relationship			
		<i>Aspergillus tracheobronchitis</i>	IAPA in patients without documented <i>Aspergillus tracheobronchitis</i>
Proven	Biopsy or brush specimen of airway plaque, pseudomembrane or ulcer showing hyphal elements and <i>Aspergillus</i> growth on culture or positive <i>Aspergillus</i> PCR in tissue		Lung biopsy showing invasive fungal elements and <i>Aspergillus</i> growth on culture or positive <i>Aspergillus</i> PCR in tissue
Probable	Airway plaque, pseudomembrane or ulcer and one of the following: Serum GM index >0.5 or BAL GM index \geq 1.0 or Positive BAL culture or Positive tracheal aspirate culture or Positive sputum culture or Hyphae consistent with <i>Aspergillus</i>	A: Pulmonary infiltrate and at least one of the following: Serum GM index >0.5 or BAL GM index \geq 1.0 or Positive BAL culture OR B: Cavitating infiltrate (unattributed to another cause) and at least one of the following: Positive sputum culture or Positive tracheal aspirate culture	

Modified from reference [20]

lation of *Aspergillus* in sputum, tracheal aspirate, or bronchial aspirate (sample with a higher risk of colonization) is compensated by a pulmonary infiltrate of IPA, such as cavitory infiltrate, which would not be justified with any other cause [20].

In the absence of characteristic radiological images, most diagnostic criteria in the ICU and non-onco-hematological patients employs the bronchoalveolar lavage, primarily GM in BAL, as the principal tool for IPA diagnosis. In a recent multicenter study to analyze the role of GM in bronchoalveolar lavage fluid for diagnosis of IPA in non-hematological patients including ICU and COPD patients a global sensitivity of BALF GM (optical density index [ODI] \geq 1.0) of 77.4% was confirmed; sensitivity was higher in patients with immunosuppressive conditions than those with COPD (81.8% vs 66.7%; p : 0.38) [16]. In COPD patients, the best performance was obtained for BALF GM (ODI \geq 0.5). The sensitivity of GM in serum was very poor in both populations (36.4% and 11.6%, respectively) [16].

The recently published criteria for IPA in patients with SARS-CoV-2 infection (CAPA) include the same criteria as IAPA for proven and probable aspergillosis, although for probable aspergillosis, they also consider diagnostic criteria as the visualization of hyphae on BAL cytology (or fungal stain) and PCR amplification of *Aspergillus* spp. in blood ($\times 2$) or BAL, provided that such amplification occurs in advance of cycle 36 [21]. In addition, the CAPA criteria also include a category of possible aspergillosis that maintains the same clinical and imaging criteria as probable aspergillosis, but it allows for a microbiological "non-bronchoscopic lavage" specimen [21]. The visualization of hyphae in non-bronchoscopic lavage, or in isolation in culture or a high titer (> 4 GM), or > 2 for a determination when accompanied by isolation in culture - are all considered diagnostic. Detection of GM in non-bronchoscopic lavage is

seen as evidence of CAPA; on the other hand, proposed cutoff values are based on a single study and need further validation [22]. Authors propose that although classification of possible CAPA will likely be sufficient to initiate antifungal therapy, in line with other consensus statements, it is not recommended for enrolling patients in clinical trials [21].

Lateral flow devices (LFD) to detect fungal antigens are not novel [23,24]. The first LFD for IA was described in 2008 but generated pooled sensitivity and specificity [23]. The recent release of the IMMY Sona *Aspergillus* GM lateral flow assay (LFA) incorporates two monoclonal antibodies (Mab), one novel Mab and one targeting a similar GM epitope to the Bio-Rad Platelia *Aspergillus* Antigen Assay (Hercules, CA, USA), has the potential to improve performance as demonstrated with cryptococcal LFD [23]. Comparison to the OLM LFD when testing BAL fluid showed the LFA as providing significantly better sensitivity (83% vs. 69%, $p = 0.008$), while maintaining specificity (87%) for proven or probable IPA [23]. An automated digital cube reader for quantification of results was recently added to the test kits. Diagnostic performance of the LFA is improved when utilizing a higher cutoff of 1.0 or 1.5 ODI, vs. the currently recommended cutoff of 0.5 ODI, which showed limited specificity [24].

IPA THERAPY IN ICU

We recommend either voriconazole or isavuconazole as first-line treatment for possible, probable, or proven aspergillosis in the ICU patient. Since Herbrecht's study [25], all guidelines include voriconazole as the first option; however, the critically ill patient has some characteristics that may limit its use, or the possibility of interactions with other drugs metabo-

Azole	CYP2C8	CYP2C9	CYP2C19	CYP3A4
Fluconazole	++	++	+	++
Itraconazole	+	+	-	+++
Voriconazole	++	++	+++	++
Posaconazole	-	-	-	+++
Isavuconazole	-	-	-	+ / ++

Notes: -, no inhibition; +, mild inhibition; ++, moderate inhibition; +++, strong inhibition.

Modified from reference [30].

olized at the cytochrome p450 level, despite the need to have levels available, given the frequency with which voriconazole cannot reach therapeutic levels. This makes isavuconazole or liposomal amphotericin B important alternatives in many patients in ICU. Voriconazole levels below 1 mg/L are associated with therapeutic failure in up to 46% of cases; response improves when this level is reached [26]. However, several studies, especially in the ICU, confirmed that approximately half the patients do not achieve serum therapeutic levels [27]. This difficulty with voriconazole occurs even with its intravenous formulation, as related to the individual-dependent bioavailability characteristic of this drug. Yet, the therapeutic range of voriconazole is narrow and serum levels > 5 mg/L are associated with hepatic and encephalopathic toxicity [26]. The presence of high levels has been documented in the ICU in up to 10% of patients.

The SECURE study conducted mainly in onco-hematological patients, but not including critically ill patients, confirmed the non-inferiority of isavuconazole vs. voriconazole in IPA, but with a significant reduction in hepatic, cutaneous, and ocular toxicity [28]. A recently published randomized, double-blind study also confirmed the non-inferiority of posaconazole to voriconazole, mainly in onco-hematological patients [29]. In the case of posaconazole, in addition to intravenous administration, the oral tablet formulation also significantly improved the pharmacokinetics and absorption of the oral solution formulation [29]. Among the three azole drugs mentioned, isavuconazole has a lower degree of interactions, conferring a substantial advantage in the critically ill patient [30]. Isavuconazole is a moderate CYP3A4 inhibitor, while other azoles, especially voriconazole, in addition to inhibiting CYP3A4, also inhibit CYP2C8, CYP2C9, and CYP2C19 (Table 4). This indicates that the use of certain drugs, such as lopinavir, prednisone, estradiol, atorvastatin, or midazolam, do not require adjustment when administered with isavuconazole, only with voriconazole; others, such as cyclosporine, tacrolimus or sirolimus, where use may be contraindicated in the presence of voriconazole, they can be used with caution with isavuconazole [30]. Unlike other QT-prolonging azoles (voriconazole, posaconazole), isavuconazole reduces QT, favoring isavuconazole in the ICU. In favor of their use are post hoc results of the SECURE study, in which > 95% of patients receiving isavuconazole had

levels > 1.5 mg/L, and determinations > 7 mg/L occurring in < 10%: these were not associated with increased toxicity, so except under special conditions, level monitoring was not required.

These arguments were also used by other authors to justify the use of liposomal amphotericin to the detriment of voriconazole. Garnacho et al. in a consensus of ICUs in Spain, justified the use of amphotericin B to the detriment of voriconazole in the management of aspergillosis in ICU patients, if the following circumstances were present: a) concomitant treatment with drugs metabolized by CYP3A4 or 2C9, b) treatment with drugs that can prolong QT, c) severe liver failure (Child C), or d) glomerular filtration rate < 50 mL/min [31].

Most guidelines advise against the use of combination therapy to treat invasive aspergillosis, due to lack of scientific evidence in published studies. In addition to *in vitro* and experimental studies, with diverse and contradictory results, the only prospective randomized clinical trial for a mainly onco-hematological population, analyzing the superiority of voriconazole and anidulafungin over voriconazole monotherapy did not confirm a significant reduction in mortality at 12 weeks, except in some subgroups [32]. In general, echinocandins are not recommended for use as monotherapy in primary invasive aspergillosis [20,21]. Despite this study, with some methodological limitations (long recruitment period and large number of losses, among others), many experts still consider the use of combination therapy in severe patients, especially given high expected mortality, such as ICU patients.

Optimal duration of therapy is unknown, as radiological lung imaging may not be a helpful tool, but the expert panel suggests 6-12 weeks as a treatment course. It seems reasonable to include follow-up lung CT imaging to document the resolution of infiltrates before termination of treatment. In patients who are immunocompromised, longer treatment might be necessary. Following the GM-index in serum as a measure of therapeutic response may be limited by its poor sensitivity if testing serum in non-neutropenic patients. However, follow-up respiratory samples, such as GM testing, could be useful in determining efficacy in patients who are GM-positive, which may similarly help determine treatment duration [20,21].

CONFLICTS OF INTEREST

Author declares no conflicts of interest

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