CONSENSUS STATEMENT

Clinical practice guideline for the management of invasive diseases caused by *Aspergillus*: 2018 Update by the GEMICOMED-SEIMC/REIPI

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ABSTRACT

Aspergillus infection is a significant cause of morbi-mortality in an at-risk population. The Study Group of Fungal Infections (GEMICOMED) from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) has reviewed announcements made in invasive aspergillosis management. We have organized our recommendations in such a way as to provide a guide in resolving different clinical situations concerning the entire spectrum of invasive diseases caused by *Aspergillus* in various populations. Diagnostic approach, treatment and preventions strategies are outlined. It is not our aim that these guidelines supplant clinical judgment with respect to specific patients; however, it is our objective to perform a comprehensive summary of quality of care evidence for invasive aspergillosis management in different settings.
Keywords: Aspergillosis, treatment, isavuconazole, voriconazole, therapeutic drug monitoring

Resumen ejecutivo del documento de consenso del GEMICOMED perteneciente a la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) sobre el tratamiento de las infecciones invasoras producidas por *Aspergillus*

RESUMEN

Las infecciones causadas por *Aspergillus* causan una elevada morbimortalidad en la población susceptible. EL Grupo de Estudio de Micología Médica de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (GEMICOMED/SEIMC) ha revisado las novedades más importantes sobre el manejo de las infecciones invasoras causadas por *Aspergillus*. Hemos organizado nuestras recomendaciones en tres apartados: diagnóstico, tratamiento y profilaxis en diferentes grupos de pacientes susceptibles de padecer estas infecciones. Se revisan distintas situaciones clínicas que pueden estar causadas por este hongo. Nuestro objetivo no es que estas guías de tratamiento suplanten el juicio clínico de los médicos ante un determinado paciente; sin embargo, sí deseamos poder ofrecer un resumen comprensible sobre las evidencias que existen para realizar un óptimo manejo de la infección invasora causada por *Aspergillus* en diferentes situaciones clínicas.

Palabras clave: Aspergilosis, tratamiento, manejo clínico, isavuconazol, voriconazol, monitorización de noveles

EXECUTIVE SUMMARY

Background

The relationship between *Aspergillus* and the host ranges from saprophytic colonization to life-threatening infections, mainly affecting immunocompromised hosts. Advances in oncohematological patient care have increased long-term survival of such patients, and the new immunosuppressive drugs for different populations have lead to span the spectrum of populations at risk of this infection so the incidence of aspergillosis is expected to rise in next years. Consequently, physicians from different specialties face the challenge of treating these patients.

New diagnostic tools and treatment for this infection have been recently published. For that reason, and considering the relevance of the infections caused by *Aspergillus*, the Study Group of Fungal Infections (GEMICOMED) from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) has decided to perform a new document with the main objective to provide update recommendations on the management of aspergillosis. The guidelines have been divided into three sections: definitions and diagnostic, treatment, and prophylaxis of acute and chronic forms of invasive diseases caused by *Aspergillus*. These guidelines are addressed to professionals of infectious diseases specialists, microbiologist, hematologist, pediatricians and all other health professionals responsible of treating fungal infections. The 2018 revised recommendations for the management of these infections are summarized below.

Methods

A multidisciplinary panel of experts in the management of patients with aspergillosis from the GEMICOMED was selected to review the literature, evaluate the evidence and give clinical recommendations to treat patients with invasive aspergillosis.

Authors have been divided into three-person expert teams to answer selected questions provided by the coordinators (CGV, AA, MCE). Literature searching for relevant scientific publications was performed using medline database through PubMed (*http://www.ncbi.nlm.nih.gov/pubmed*). The keywords used are shown in each question. Only papers published in full text in English or Spanish were selected. No specific period of inclusion was defined but authors were encorauged to report mainly the latest literature evidence. The full text has been debated and latter approved by all the

authors. The criteria to evaluate the strength of recommendation and quality of evidence are summarized in table 1. The potential conflicts of interest of all members of the expert panel are listed at the end of the paper.

QUESTIONS

DIAGNOSIS

1.- What are the diseases caused by *Aspergillus* and how is invasive pulmonary aspergillosis (IPA) defined?

Searched key words: Aspergillosis, *Aspergillus*, Invasive aspergillosis

Executive summary

1.- Different forms of aspergillosis have been described and associated with different clinical symptoms (See table 2).

2.- Invasive pulmonary aspergillosis (IPA) is a systemic infection caused by *Aspergillus* that occurs in immunocompromised patients. It is the most severe form of aspergillosis.

Evidence summary

Aspergillus is widely found in nature, both in outdoor and indoor air. On a daily basis, we inhale hundreds of *Aspergillus* conidia that a healthy immune system is able to regularly remove them. However, different underlying diseases allow the development of *Aspergillus* infections, referred to as aspergillosis[1]. Table 2 summarizes characteristics of the disease caused by *Aspergillus*.

2.- What microbiological methods can be used to diagnose invasive aspergillosis (IA)? Are all diagnostic methods useful in all populations?

Searched key words: Diagnosis, Aspergillosis, *Aspergillus* diagnostic tests, Galactomannan, Glucan, BDG, *Aspergillus* LFD, *Aspergillus* PCR.

Executive summary

1.- Diagnosis of IA in patients with suspected infection is mainly based on culture (***AI***), galactomannan antigen quantification (GM) (***AII***) and tecniques based on the amplification of

fungal DNA by the polymerase chain reaction (PCR) (***AII***).

2.- Sensitivity of cultures is usually low but provides information on epidemiology and antifungal susceptibility (***AII***).

3.- GM serum quantification is recommended in neutropenic and haematological patients who are not in prophylaxis (***AII***).

4.- The PCR-based techniques have been extensively used and might improve diagnosis in haematological and ICU patients (***AII***) albeit an effort in standardization and harmonization of the techniques is still needed.

Evidence summary

IA diagnosis relys on a combination of clinical, radiological, microscopic and microbiological data. Current microbiology methods used in clinical practice to diagnose IA are direct detection, culture, the detection of different fungal components (GM and BDG) and the detection of fungal DNA (PCR).

For direct detection, fast stains can be performed on specimens by using microscopy and optically brighter methods such as Calcofluor white or Blankophor. A positive culture also allows for identification and susceptibility testing of the isolates. Culture of usually sterile samples or those obtained from deep sites together with histopathological detection of the fungus remain the gold standard for the diagnosis of invasive infections[2], however, they lack sufficient sensitivity[3].

Galactomannan (GM) is a water-soluble cell wall polysaccharide that is released by *Aspergillus* species during fungal growth. GM can be detected in different body fluids by a commercially available ELISA technique (Platelia *Aspergillus*®, Bio-Rad, Marnes-La-Coquette, France). Due to its diagnosis accuracy, this test has been included as a mycological criterion within the European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus definitions[2]. Sensitivity, specificity, negative and positive predictive values of the test vary with the population studied, body fluid used, and cut off value used. Serum GM may helps to diagnose IA in patients in whom hyphae produce angioinvasion (neutropenic population)[4-6]. Two serial determinations with an index $\geq$ 0.5 have very good sensitivity and negative predictive value in neutropenic and haematological patients[7,8]. The European Conference on Infections in Leukaemia (ECIL) guidelines recommends a single value of ≥0.7 or consecutive values of ≥0.5 in serum samples in the absence of anti-mould prophylaxis as a cut-off[9]. In those patients with mould antifungal prophylaxis, the load of GM in blood is probably lower and positive determinations are usually false-positive results. Consequently, GM in serum is not recommended unles there is a high clinical

suspicion of the infection[10]. In other populations, with other forms of pulmonary aspergillosis, GM in BAL (bronchoalveolar lavage), interpreted together with clinical and radiological findings, is the tool of choice, being 1.0 the current cut-off value recommended as positive diagnostic result[7].

BDG is a polysaccharide component of the cell wall of many pathogenic fungi (*Aspergillus* spp., *Fusarium* spp., *Candida* spp. or *Pneumocystis jirovecii*) but not of Mucorales or *Cryptococcus.* Of the four commercially available assays only Fungitell® (Associates of Cape Cod, Inc., East Falmouth, MA, USA) has been approved by European Agencies for the presumptive diagnosis of invasive fungal infection, and has been included in the EORTC/MSG definitions of invasive fungal disease. Reports on the screening performance of BDG are limited and strength of recommendation must be lower than that for GM and PCR tecniques. BDG technique has good sensitivity and negative predictive values, but poor specificity and positive predictive values for diagnosing IA due to a high rate of false positive results[11].

An *Aspergillus*-specific LFD (OLM Diagnostics, Newcastle, United Kingdom) has recently been developed as a point-of-care test for the diagnosis of invasive aspergillosis and poses an up-and-coming alternative to GM determination. Using a monoclonal antibody (JF5), LFD is an immunochromatographic assay that detects an extracellular glycoprotein secreted during active growth of *Aspergillus* in serum and BAL fluid. LFD is a rapid test (15 min) with easy manipulation that can be performed easily without need for specific laboratory equipment. Several studies, including a meta-analysis, have been published with this assay, demonstrating a good sensitivity and specificity, especially in BAL[12-15]; however, this technique is not available in many countries.

Aspergillus DNA detection assays by PCR have very high sensitivity and can be performed on any kind of specimen, including body fluids and tissue samples[16]. Combined with other biomarkers, DNA detection assays by PCR improve the diagnosis of IA[17-19]. Currently, PCR has not been included in the EORTC/MSG consensus definitions as a reliable microbiological marker[20], and will most likely change once procedure standardization becomes available. Most of the authors in these guidelines have commonly been using different formats of *Aspergillus* DNA detection. Most likely, in the near future, the implementation of commercialized PCR assays (alone or in combination with other tests) in clinical setting will help us to standardize this tool, which would be a key point test in IA diagnosis. So far today, detection of BDG or the use of an *Aspergillus*-specific LFD do not have a main role for routine and systematic diagnosis of IA.

3.- What is the recommended diagnostic approach for patients with suspicion of IA…

a) …in oncogical and hematological patients?

Searched key words: Invasive Aspergillosis, Oncologic patients, Haematologic patients, Neutropenia, Diagnostic.

Executive summary

1.- In neutropenic patients with suspicion of IPA the recommended approach is: a) chest computed tomography (CT) scan (***AII*****),** b) serum and/or BAL GM detection (***AII***), and c) bronchoscopy (***AII***). Bronchoscopy allows for performing fungal culture, GM and PCR determinations that increase the probability of ruling in IPA diagnosis and ruling out other infections (***AI***).

2.- Histopathologic examination of tissues and fluid specimens are recommended whenever possible (***AI***), particularly to rule out other infections or diseases in patients with pulmonary nodules and negative biomarkers results (***BII***).

3.- For oncologic, non-neutropenic patients, specially those with solid lung tumors or pulmonary metastatic disease in whom chronic form of aspergillosis would be rule out, the recommended approach is: *a)* to take into account nonspecific clinical presentation, *b)* chest CT scan, and *c)* bronchoscopy with fungal culture and BAL GM detection (***AIII***).

Evidence summary

Histopathologic examination of the infected tissue remains the gold standard for diagnosis of IA by demonstrating the presence of characteristic invasive branching septate hyphae[2]. Moreover, biopsy samples might help to rule out alternative diseases. Unfortunately, most of the times, the biopsy procedure is an invasive method that cannot be regularlly performed due to the risk for the patients.

The proposed approach for neutropneic patients with suspction of IA is based on the results of several studies. Those patients usually have acute IPA as clinical syndrome. Consequently, most patients presented with halo sign and/or macronodules in chest CT scan[21]. Some radiological features might help to differentiate between IPA and other mold infections[22]. Serum GM is strongly recommended as an accurate marker for the diagnosis of IPA in hematological patients with high-quality evidence[23]. Persistent GM positive antigenemias during antifungal therapy are surrogate marker of poor prognosis and should trigger a prompt clinical reassessment[24]. In those patients receiving anti-mold prophylaxis serum GM values can be difficult to interpret. Standardized BAL specimens are strongly recommended for direct stain examination, fungal culture, and

GM/*Aspergillus* DNA detection.

Detection of GM in BAL samples is particularly useful in patients with high clinical suspicion of invasive aspergillosis. The optical density index cutoff of GM for a positive result in this population should be >=1 although the cut-off is under debate at present[23,25]. Nucleic acid testing by PCR should be considered in combination with other diagnostic tests in the clinical context of patients but keeping in mind the lack of standardization.

Oncologic, non-neutropenic patients usually have chronic clinical forms of aspergillosis. The diagnosis requires a combination of a CT scan images (commonly negative in this setting), positive cultures in BAL or an immunological response to *Aspergillus* spp (26). The clinical features of patients usually are subacute (at least 3 months).

b) …in solid organ transplantation (SOT)?

Searched key words: Aspergillosis, Diagnosis, Non-neutropenic, SOT (lung, renal, kidney, liver, intestinal).

Executive summary

1.- Bronchoscopy plays a key role to approach IA diagnosis. Check visual images on bronchoscopy, BAL fungal culture and GM in BAL are strong recommended (***AII***).

2.- CT scan has a limited value as most of the classic radiological findings are rarely found in these patients (***BII***).

3.- Serum GM detection has less sensitivity than in BAL (***BIII***).

Evidence summary

Aspergillosis in SOT, especially lung transplantation, faces a wide spectrum of clinical syndromes. The approach to aspergillosis diagnosis in patients with different type of organ transplant should be different. Positive cultures from the airway samples in SOT other from lung transplants are infrequent but have a high positive predictive value for the development of IA. In lung transplantation, positive cultures in airway samples preclude a bronchoscopic examination to exclude tracheobronchitis.

The detection of GM in serum[5,27] or BAL samples[28] is recommended, albeit their role is much more limited than their role in the setting of neutropenic patients. Sensitivity of the GM for IA diagnosis would be higher in testing BAL with a reported sensitivity of 100% and specificity of 91% at the

index cutoff value of ≥1 in SOT recipients[29]. In liver and lung transplantation, up to 13% and 20%, respectively, of false positive GM have been described[30,31]. The role of PCR-based procedures in this population remains to be clarified.

Classic radiological findings for IA in CT scan (halo sign and air crescent) are rarely found in these patients but the development of pulmonary nodules in the early posttransplant period is highly suggestive of invasive fungal infection in lung and heart transplant recipients[32].

c) …in patients receiving mould antifungal prophylaxis?

Searched key words: Aspergillosis, Diagnosis antifungal prophylaxis, Breakthrough fungal infection.

Executive summary

1.- The following investigation (some combined or all as clinically indicated) has suggested: CT scanning (***AIII***), bronchoscopy with culture, GM and PCR on BAL fluid (***AIII***).

2.- The use of serum GM or PCR is not recommended in patients receiving antifungal prophylaxis (***BII***).

3.- If any abnormality is detected on CT scan and all microbiologic tools are negative, biopsy is recommended for IA diagnosis and ruling out other diseases (***AIII***).

Evidence summary

IA may occur in patients receiving antifungal agents (prophylaxis or therapy) and diagnostic performance is known to be negatively affected under these circumstances[33-35], due to the impact in the fungal burden and the low positive predictive value. Therefore, combined procedures are recommended for diagnosing IA in high-risk patients on antifungal prophylaxis[33]. Ruling out other infections, even other co-fungal infections, is mandatory.

d)…in intensive care unit (ICU) patiens?

Searched key words: IA, Diagnostic approach for IA, ICU patients.

Executive summary

1.- We recommend performing a bronchoscopy with fungal cultures and GM in BAL in critically ill

patients with suspicion of IA (***AII***).

2.- A CT scan may be done. For the diagnosis of IA in non-neutropenic critically ill patients typical signs (halo and air crescent signs) are rarely observed (***CIII***).

3.- Serum galactomannan (GM) is of little value for the diagnosis of IA in non-neutropenic critically ill patients (***CIII***).

Evidence summary

The diagnosis of IA in the ICU setting is a real challenge because of the non-specific clinical presentation in critically ill patients. Moreover, clinical forms of the infection in this population are not well-described and might vary depending on the host. The diagnostic approach for a hematological patient admitted in ICU should probably be quite different from that of a patient with major lung comorbidity. In the real world, the diagnosis of aspergillosis in non-severe immunosuppresed patients in ICU is often suspected when *Aspergillus* is isolated from tracheal or bronchial aspirates. However, differentiation of true IA from colonization is difficult and worrisome for clinicians. Besides, current EORTC/MSG definitions of probable or possible IA are not useful for the diagnosis of IA in non-immunosuppressed critically ill patients. Although several algorithms have been proposed to solve this problem only the one has been prospectively validated[36]. The term “putative aspergillosis” has been coined in an attempt to determine the significance of an *Aspergillus*-positive lower respiratory tract specimen culture in a critically ill patient (Table 3). This clinical algorithm exhibits a better performance for the diagnosis of IA in ICU patients than the EORTC/MSG criteria. *Aspergillus* respiratory tract colonization is set when one or more criteria necessary for a diagnosis of putative IA are absent. The main weakness of this approach is that cultures of respiratory samples, including those obtained by BAL, are positive for *Aspergillus* spp. in only 50% of critically ill patients with the final diagnosis of IA[37,38].

Measurement of serum GM is of little aid for the diagnosis of IA in non-neutropenic critically ill patients. Conversely, quantification of GM in BAL is of great utility in critically ill patients. In this sense, in 110 critically ill patients, sensitivity and specificity in BAL was 88 and 87%, respectively, while sensitivity of GM determination in serum was only 42%. In 11 out of the 26 cases with proven IA, both BAL culture and GM in serum were negative while the GM in BAL was positive[38]. 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 IA, and 80% and 87.5%, respectively, for proven and probable IA cases. For IA cases (proven and probable) diagnostic accuracy for GM in BAL was significantly higher than GM

and BDG in serum. GM determination in BAL has also been assessed in critically ill patients with chronic obstructive pulmonary disease (COPD) with its diagnostic value higher than that of the serum determination[39]. Other more accessible respiratory samples such as tracheal or bronchial aspirates are not validated for GM quantification and must not be used for diagnosis of IA[40].

The presence of BDG in serum indicates the presence of fungal invasion but it is not specific for *Aspergillus* species. Several studies carried out in critically ill patients coincide that the diagnostic accuracy of this test is inferior to the reliability of BAL GM and comparable to serum GM[14,41,42].

The diagnostic performance of PCR in BAL fluid is acceptable and comparable to that of GM in BAL fluid[25]. Moreover, the combination of the GM test and PCR in BAL increases the sensitive and specific diagnosis of IA[14].

Aspergillus LFD is a novel test that detects this filamentous fungus in BAL. This diagnostic technique has been evaluated especially in critically ill with sensitivity and specificity comparable to BAL GM testing[37]. This point-of-test can be performed easily and provides rapid results (< 15 minutes).

The CT scan findings of in non-neutropenic critically ill patients are not specific[40] at all and a CT scan is not always feasible especially in patients with severe hypoxemia. As such, this diagnostic tool is not recommended in this population.

4.- When should we use a IA diagnosis-driven approach?

Searched terms: Diagnostic-driven Antifungal therapy, Galactomannan screening, *Aspergillus* DNA detection

Executive summary

1.- The application of a diagnostic-driven approach may be considered only as an alternative strategy for high-risk hematological patients unable to receive anti-mold prophylaxis (***BIII***). There is no evidence to support such an approach in other high-risk populations, such as SOT recipients (***BII***).

2.- Diagnostic-driven antifungal therapy may be based on the screening (at least on a twice-a-week basis) for serum GM antigen or *Aspergillus* DNA detection at regular intervals throughout the entire at-risk period (***AII***). This surveillance should be initiated at the start of the high-risk period

(i.e., first cycle of chemotherapy) and continued until no longer at risk. If patient enters subsequent high-risk periods, the surveillance strategy should be reinitiated (***AII***).

3.- Screening for serum GM antigen or *Aspergillus* DNA detection should not be routinely performed in asymptomatic high-risk patients receiving anti-mold prophylaxis (***AII***).

Evidence summary

Diagnostic-driven (also called pre-emptive or biomarker-driven) antifungal therapy is based on the scheduled monitoring for surrogate markers of IA throughout the entire at-risk period to guide the initiation of antifungal treatment in otherwise asymptomatic patients. This approach emerged about one decade ago as a logical alternative to empirical therapy, and is ultimately aimed at optimizing antifungal therapy and avoiding the potential consequences of over-treatment[43]. The first studies that tested the efficacy and safety of such strategy were performed in high-risk hematological patients, and were based on serum GM test. A positive result prompted a thoracic CT scan examination and initiation of antifungal therapy[43]. One open-label randomized controlled trial demonstrated that a diagnostic-driven strategy did not affect overall survival when the use of antifungal drugs decreased, as compared to the use of such with a classical empirical approach[44].

Diagnostic-driven approach is conceptually founded on the assumption that the baseline incidence of IA among high-risk patients (pretest probability) is high enough to guarantee a reasonable positive predictive value for the presence of a positive serum GM. Nevertheless, the advent of broad-spectrum triazoles with good oral bioavailability and excellent anti-*Aspergillus* activity has dramatically changed this scenario. The rate of breakthrough IFD among high-risk patients receiving prophylaxis with posaconazole or voriconazole has been consistently found to be very low (usually below 5%)[10,45-47]. Therefore, the diagnostic performance of screening strategies for serum GM antigen or *Aspergillus* PCR results deeply impacted by the very low pretest probability of IA[48]. Thus, the application of a diagnostic-driven antifungal therapy approach should be currently restricted to the unlikely scenario of high-risk hematological patients not receiving any form of anti-mold prophylaxis[49,50].

More recent studies have evaluated the role of *Aspergillus* PCR testing as a biomarker to guide the use of therapy, suggesting that DNA detection may offer advantages over the sole testing for serum GM in the early diagnosis and preemptive therapy of IA[17,51]. A combined monitoring strategy based on serum GM and *Aspergillus* DNA was associated with earlier diagnosis in high-risk hematological patients in a recent controlled randomized trial[17].

On the other hand, diagnostic-driven strategies has not been proven useful in non-neutropenic

patients at risk of IA, such as SOT recipients. A *post-hoc* analysis of a multicenter randomized trial involving high-risk liver transplant recipients reported that serum GM or BDG testing had limited clinical utility for the diagnosis of post-transplant invasive fungal disease (including IA)[27]. In addition, it has been reported a high rate of false positive results for serum GM screening after liver transplantation, particularly within the very early post-transplant period, which appears to be associated to the concurrent use of β-lactams antibiotics such as ampicillin[52].

4.- How should we improve the growth of *Aspergillus* in culture?

Searched key words: Specimen collection, *Aspergillus* growth, Calcofluor, *Aspergillus* culture, *Aspergillus* culture media.

Executive summary

1.- The growth of bacteria presenting in respiratory and other non-sterile samples must be reduced by processing the sample within 2-4 hours (or refrigerate until processing) and using antibiotic-supplemented media (***AII***).

2.- Microscopic examination of sterile samples and BAL fluid by optical brightener methods (calcofluor or Blankophor) is recommended (***AI***).

3.- In patients with neutropenia or leukemia the isolation of *Aspergillus* is highly predictive of invasive pulmonary aspergillosis (***AI***).

4.- An incubation temperature of 35-37ºC and use of specific media (cornmeal, oatmeal, potato dextrose and Czapek-Dox agar) can encourage growth, sporulation and may permit identification of *Aspergillus* (***AII***).

EVIDENCE SUMMARY

Appropriate collection of the clinical specimen is essential to improve the *Aspergillus* isolation in culture. Specimens should be received at the laboratory within 2-4 hours, and if delayed, should be refrigerated in order to prevent overgrowth of commensal bacteria. Interpretation of respiratory specimens should take into account the possible isolation of commensal flora or contaminating fungal spores[53]. Sputum should be collected after deep expectoration (ideally in the early morning, resulting from a deep cough -not saliva- or induced by aerosol), but bronchial specimens and BAL are more representative of lung infection. In cases of invasive pulmonary aspergillosis, it has been found that the submission of three specimens was adequate to detect 91% of cases[54].

Underlying disease is critical in selecting patients in whom sputum cultures may be useful. Although cultures from respiratory secretions present a low sensitivity and uncertain specificity for aspergillosis, in patients with neutropenia or leukemia the isolation of *Aspergillus* is highly predictive of invasive pulmonary aspergillosis[55]. A tissue biopsy is a precious diagnostic specimen, allowing immediate microscopic diagnosis of IA infection; subsequent culture may yield the pathogen for identification and antifungal susceptibility testing.

Whenever possible, with BAL and other body fluids or aspirates, a centrifugation step (1500 g for 5-10 min) is necessary to concentrate fungal inoculum and the sediment must be resuspended in a reasonable small volume (100-250 µL). For dense specimens, such as sputum, a liquefaction step with mucolytic agents (0.5% pancreatin, 0.5% N-acetyl-Lcysteine or Sputolysin) is necessary to improve the diagnostic utility of the sample, following a centrifugation step to concentrate the fungi. Tissues should be chopped carefully into smaller pieces but not ground or homogenised. Besides culture, microscopic examination of sterile samples and BAL fluid by optical brightener methods (calcofluor or Blankophor) is recommended. A study of respiratory samples (mostly BAL) from transplant recipients and neutropenic patients found a sensitivity of 88% and a specificity of 99% for detection of *Aspergillus*-like elements by Blankophor in comparison with a 76% sensitivity for culture[56].

The growth of bacteria presenting in respiratory and other non-sterile samples must be reduced using antibiotic-supplemented media (i.e. chloramphenicol). Media routinely used for primary isolation are glucose peptone agar (Sabouraud's chloramphenicol agar). Petri dishes provide adequate conditions for the recovery of *Aspergillus*, since a larger inoculum can be cultured and a greater and better-aerated area is offered than in tube or bottle slants. The tendency for media in plates to dry out during prolonged incubation can be minimized by placing the plates into oxygen permeable cellophane bags. The overall culture isolation rate of *Aspergillus* from BAL or bronchial washing specimens is much higher than that from surgical and biopsy tissue specimens[57]. It has been reported that only 54% of patients with haematological malignancies had positive cultures for *Aspergillus* from lung tissue specimens with histological evidence of aspergillosis[58]. Although the optimum growth temperature for the main pathogenic moulds is 30ºC, an incubation temperature of 35-37ºC may speed up the growth of *Aspergillus* species. Moulds grow best in rich media (as Sabouraud's glucose agar), but the overproduction of mycelium may result in loss of sporulation, not allowing for their microscopic identification. A subculture to a less rich medium can encourage sporulation and may permit identification. Common media used for this purpose are cornmeal, oatmeal, potato dextrose and Czapek-Dox agar, which are neutral and permits

moderate to vigorous growth of *Aspergillus*.

5.- When should *Aspergillus* resistance to antifungals be suspected and what are the recommended methods to assess antifungal drug susceptibility?

Searched key words: *Aspergillus* resistance, Azole resistance, Antifungal resistance,

Executive summary

1.- *Aspergillus* resistance to antifungal drugs should be suspected in every therapeutic failure scenario and when cryptic species are identified as causative agents of invasive aspergillosis. However, we recommend testing for antifungal resistance in every isolate coming from an invasive infection for epidemiological and antifungal resistance purposes (***AII***).

2.- Commercially available test that have been standardized in multicenter studies can be used in clinical laboratories to screen for resistance; however, European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical Laboratory Standards Institute (CLSI) reference methods should be used to confirm antifungal resistance (**AII**).

Evidence summary

Antifungal, mainly azole, resistance in *Aspergillus* species has been increasingly reported in the last decade in Northen Europe[59]. Standardized methods for susceptibility testing and associated clinical breakpoints and epidemiological cutoff values are available nowadays. However, the true rates of global antifungal resistance in these pathogens are unknown but purportedly low in Spain[60]. Resistance rates in *A. fumigatus* range from 0 to over 20% depending on the country and the type of study performed[61]. In addition, a shift in the etiology of aspergillosis and the emergence of cryptic and rare *Aspergillus* species that can display intrinsic resistance to antifungals[62] have been observed[63,64]. They have been associated with refractory cases of invasive aspergillosis. Unfortunately, they cannot be distinguished by classic identification methods and molecular tools, such as DNA sequencing (β-tubulin or calmodulin) are required for identification of species level in *Aspergillus*. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) could also be an alternative as it has also shown to present good results[65].

Aspergillus resistance to antifungal drugs should be suspected in every therapeutic failure scenario (regardless of the isolated species) and when cryptic species are identified as causative agents of invasive aspergillosis. Every isolate coming from an invasive infection should be tested for

antifungal resistance. Methods to test *Aspergillus* susceptibility are summarized in table 4.

Currently, antifungal susceptibility testing (AFST) is still the most reliable procedure in determining the best clinically active antifungal agent and also detecting resistance in *Aspergillus*. Both the CLSI and the EUCAST have developed microdilution methods that are recommended for detecting *in vitro* resistance in filamentous fungi. Clinical breakpoints or epidemiological cut-off values (ECV) have been established for amphotericin B, itraconazole, voriconazole and posaconazole and, just recently, isavuconazole. However, AFST for *Aspergillus* is not performed routinely in many microbiological laboratories worldwide, constituting a main gap in detecting azole resistance. The use of commercially available systems, as Sensititre® YeastOne™ or Etest®, is recommended in clinical laboratories. Sensititre® YeastOne™ (TREK Diagnostics Systems, Ohio, USA) is a microtitre broth dilution method that presents good correlation with CLSI M38-A with *Aspergillus* species[66]. Etest® (bioMerieux) is an agar diffusion method using a strip with a predefined concentration gradient of the antimicrobial agent. Individual antifungals may be tested and there is reasonable agreement with the standard CLSI M38-A methodology[67]. A simple agar-based screening method containing four-well plate (Vipcheck™) has also been used to detect azole-resistant strains[68]. The incorporation of this screening approach in clinical laboratories may result in separating potential resistant isolates that could be sent to referral laboratories for antifungal susceptibility testing and identification of resistant mechanisms.

Direct detection of *A. fumigatus* azole-related mutations in culture-negative clinical samples using real time PCR assays has been reported in limited studies. A commercialized multiplex real-time PCR for detection of two environmentally associated resistance mechanisms (TR34/L98H and TR46/Y121F/T289A) and *Aspergillus* species has become available recently (AsperGenius®, PathoNostics). This technique allows identification of only two mechanisms of resistance; as such a negative test result does not rule out the presence of azole resistance[69]. Nevertheless, resistance detection by molecular methods, also in culture-negative specimens, cannot be recommended as a screening method and warrant further standardization of techniques for effective integration in routine laboratories.

TREATMENT

1.- What is the treatment for IA in hematological patients?

a) Which drug has been associated with better outcomes?

Searched key words: Aspergillosis, *Aspergillus*, Treatment, Therapy, Guidelines

Executive summary

1.- Voriconazole and Isavuconazole should be considered drugs of choice for primary treatment of IA in hematological patients (***AI***).

3.- Liposomal amphotericin B is an alternative for primary or salvage treatment for patients who are intolerant, had hepatitis or are refractory to voriconazole or isavuconazole. Also for patients with suspected or confirmed triazole resistance, or when triazole use is not desirable due to drug interactions (***AII***).

4.- Echinocandins and posaconazole are not recommended as primary treatment of IA in oncohematological patients (***AII***), but they are an alternative as salvage therapy when other azoles and liposomal amphotericin B cannot be used (***BII***).

Evidence summary

Invasive aspergillosis is a complex disease, difficult to study in prospective clinical trials due to heterogeneity of affected population, diagnostic difficulties and the influence of multiple factors apart from therapy in the outcome.

However, the efficacy of voriconazole was assessed in a large randomized trial[70] demonstrating superior efficacy and better survival than amphotericin B deoxycholate (d-AmB) for primary therapy of this infection. Voriconazole (loading dose of 6 mg/kg IV every 12 h for two doses, followed by 4 mg/kg every 12 hours) was compared to d-AmB (1-1.5 mg/kg/day IV), both of which were followed by other licensed agents in the case of failure/intolerance. Voriconazole improved survival at 12 week (71% vs. 58%) and had a significantly higher rate of favorable response (55% vs. 38%) with less side effects. In particular, better results were obtained in neutropenic and allogeneic hematopoietic stem cell transplantation recipients. One criticism for this study is that median duration of the control drug (d-AmB) was 10 days compared to 77 days for voriconazole arm. The high discontinuation rate in the d-AmB group was mainly due to intolerance, and could have been reduced by using premedication and/or supplementation with fluid (which is not specified in the article) or using liposomal amphotericin B (L-AmB) instead of d-AmB[71]. Moreover, 80% of patients in d-AmB group and 36% in voriconazole group received a salvage therapy with another licensed

drug, which makes comparisons problematic. However, the impact of switching to other therapies in the outcome of patents with IA was analyzed in a further study[72], showing better outcomes with initial voriconazole, compared to initial d-AmB in patients intolerant or refractory of initial therapy. Other cohort studies have confirmed the efficacy of voriconazole for the treatment of IA in oncohematological patients[73-75].

Recently, a new broad-spectrum triazole, isavuconazole, has been approved. Isavuconazole has shown non-inferiority when compared with voriconazole for the primary treatment of suspected IA disease in a multicenter, double-blind, randomized clinical trial enrolling 532 patients. For the pre-protocol analysis, all-cause mortality to day was 15% for isavuconazole and 18% for voriconazole. Isavuconazole was better tolerated than the comparator, with fewer study-drug related adverse events, specially those related with hepatobiliary disorders, laboratory investigations, eye disorders, and psychiatric disorders[76]. Permanent drug discontinuation due to drug-realted adverse events was lower for isavuconazole than for voriconazole (8% vs 14%). Isavuconazol has a favorable pharmacokinetic profile and therapeutic drug monitoring might not be necessary[77].

The iv cyclodextrin-free formulation eliminates concerns of the use of this drug in patients with nephrotoxicity.

There are no randomized trials comparing the efficacy and safety of L-AmB with voriconazole for primary therapy of IA. However, several trials have evaluated the efficacy and safety of different formulations of amphotericin B on this indication (reported rate of complete and partial response from 40-70%)[78]. Amphotericin B Lipid Complex (ABLC) has not been studied in randomized trials as primary treatment of IA, although efficacy at a dose of 5 mg/kg has been shown in observational studies with better tolerance than d-AmB[79,80]. Amphotericin B Colloidal Dispersion (ABCD) has similar response rates to those of d-AmB but frequent infusion-related adverse effects[81] have limited its indication. Two large trials evaluated the efficacy of L-AmB for the treatment of IA using different dosages. The first one compared 1 mg versus 4 mg/kg/d, reporting a 55% versus 37% survival rate without advantages for higher doses[82]. Another study, the AmBiLoad study[83], was a double-blind trial that compared conventional doses of 3 mg/kg/day with high doses of 10 mg/kg/day in patients with IA, confirming that efficacy was similar in both arms (50 vs. 46%) with higher rates of adverse effects for the high-dose arm. This study also showed a similar response and 12-week survival rates (50% and 72% respectively) like those reported for voriconazole in the Herbrect et al trial[84].

Comparing d-AmB and its lipid formulations to voriconazole, both have fungicidal activity against most fungal strains, albeit amphotericin B has lower *in vitro* activity against *Aspergillus nidulans,*

Aspergillus lentulus and *Aspergillus terreus* than voriconazole[85,86]. On the other hand, emerging *A. fumigatus* resistance to voriconazole is a growing concern in recent years.

Although all three echinocandins have fungistatic *in vitro* activity against *Aspergillus* spp., there is limited data supporting the use of echinocandins for primary treatment of IA in patients with hematological malignancies[84,87,88]. Other studies have evaluated the efficacy and safety of caspofungin and micafungin in patients who were refractory or intolerant to other therapies with a response rate ranging from 40.9-56.5%[89-92], although response rates may be lower in hematopoietic stem cell transplant (HSCT) recipients[93]. Anidulafungin has been not evaluated in monotherapy for primary or salvage treatment of IA in oncohematological patients. However, this echinocandin has better lung tissue penetration and antimould activity compared with the others.

Posaconazole salvage therapy demonstrated greater efficacy and safety than amphotericin in IA in hematological malignancy[94].

b) When should we use combination therapy and what are the best regimens?

Searched terms: Aspergillosis , *Aspergillus*, Combination therapy, Treatment

Executive summary

1. Antifungal combination therapy should not be generally recommended for primary treatment of IA, but it could be consider in selected hematological patients with documented IA (**BI**).

2. Regarding class of antifungal compounds to be combined, combinations including triazole and echinocandin are the most commonly recommended and specifically voriconazole with anidulafungin would be the best regimen (**BI**).

3. For salvage treatment of refractory IA, the addition to another agent to initial therapy is in general not recommended, although combination therapy may be consider in individual patients (**CIII**).

Evidence summary

The use of antifungal combination therapy (ACT) administering drugs with different mechanisms of action has been proposed in the last years. The combination potentiates the antifungal activity by targeting multiple cellular sites, extends the spectrum of action, compensates pharmacokinetic issues, and avoids resistance development. In contrast, the use of two or more antifungal drugs may also result in increased toxicity, drug interactions, potential attenuation of activity or antagonism and significant higher costs.

First studies that evaluated the impact of ACT on the outcome of IA have a retrospective and monocentric design. Those studies included patients with both primary and salvage indications and had unbalanced study arms regarding sample size or patient characteristics. The reports of the studies either show a significant selection bias by including patients during a long period[95-99], or were not primary designed to analyze the efficacy of the ACT[94].

Regarding primary therapy for IA, most of the non-randomized studies included a very low number of patients and did not find benefits for ACT[89,96,99]. The largest, most important randomized trial that evaluated the efficacy and safety of ACT assessed the combination of voriconazole with anidulafungin in comparison to voriconazole with placebo for primary therapy of IA[100]. The results of this study are difficult to evaluate. Although there was a trend in a higher 6-week survival (primary end-point) for patients under ACT, the global clinical response was lower due to high clinical failure. Unevaluable for missing data was the primary reason for most patients with ACT to be included in the clinical failure group. In contrast, death within the first 6 weeks was the primary reason for most patients with monotherapy to be included in the clinical failure group. In a post-hoc analysis, the subgroup of patients with hematological malignancies or stem cell transplant recipients with probable IA diagnosed by CT scan and positive GM had significanty better outcomes when treated with ACT than with monotherapy. Other randomized trial[101] compared the combination of caspofungin and L-AmB (standard doses) *vs.* the monotherapy with high dose L-AmB. In this study, monotherapy arm was not optimal due to its higher toxicity.

There is a lack of well-designed controlled clinical trials to evaluate the impact of ACT as a salvage treatment of IA. Some clinical studies, with conflicting results, report data on this scenario. However, factors such as the heterogenicity of population or the lack of standard definitions of outcomes complicate the interpretation of results, particularly when using retrospective data[89,92,95,97]. In the real world, the severity of clinical situation and the limitation of other options make ACT a suitable option in most cases.

To conclude, the main concern when using ACT is the potential increased toxicity. Only a few studies analyzed the impact of ACT on toxicity[92,94,96,97,99,100]. Overall, there is no significant impact in hepatic or renal toxicity when ACT includes echinocandins and triazoles, but polyenes, largely at higher doses, may be associated with increased renal toxicity[94,98].

c) Should we monitor treatment response? How?

Searched terms: Aspergillosis, *Aspergillus*, Response

Executive summary

1.- Response assessment of antifungal therapy should be based on a composite of clinical, radiological and mycological criteria in an appropriate period evaluation (***AI***).

2.- A follow-up chest CT scan is recommended to assess the radiological response of invasive aspergillosis to treatment after a minimum of 2 weeks of treatment (***CIII***).

3.- Monitoring of serum galactomannan titers can be used in patients with hematological malignancies and hematopoietic stem cell transplantation recipients to assess therapeutic responses earlier and predict outcomes (***AII***).

Evidence summary

A composite endpoint of clinical, radiological and microbiological outcomes has been used to evaluate the response to therapy of patients with IA[102]. In general, global response requires survival and improvements in fungal disease, including no clinical, radiological or mycological (histological and/or conventional mold isolation) evidence of infection. However, this evaluation of therapeutic response may be difficult, especially in hematological patients, since response criteria are often based on subjective assessments[103,104]. Thus, the symptoms and signs of infection may be absent or diminished in hematological patients, especially in neutropenic patients, and may even worsen during neutropenia recovery. Some of the clinical manifestations appearing during the treatment of IA, as fever, dyspnea or hemoptysis do not necessarily means refractory disease. Also, radiological findings may progress during the first week of treatment, mainly in neutropenic patients, with up to a 4-fold increase in patients with an otherwise favourable evolution; cavitation may appear while recovering from neutropenia[105]. Additionally, the sensitivity of conventional microbiological diagnosis is suboptimal, and serial evaluation of microbiological response is often difficult, since repeating invasive diagnostic test for new samples is not always feasible in clinical practice. Finally, attribution of mortality in patients with hematological malignancies and IA is difficult, since survival is frequently related to the underlying disease, particularly later during the course of IA[106].

In the search for a more specific alternative tool for therapeutic monitoring, several studies have evaluated the utility of serial serum biomarkers like GM index and BDG as predictors of response to treatment and outcome in patients with IA[103,104,107-111]. A review of 27 studies that enrolled patients with hematological cancer; proven or probable IA; and had used sequential GM index testing showed a strong correlation between serum GM index and survival[110]. Several studies are

concordant with the optimal relationship between serum GM values and clinical response at 6 and 12 weeks[24.103,107-109,111].

There is limited data about serial determinations of BDG to predict outcome of IA[103,104,112,113], but it seems that early changes in BDG index do not correlate well with clinical responses since the decline in BDG titers is slower.

On the other hand, as there are many causes of antifungal therapy failure, several issues has to be taken into account when evaluating response to therapy in hematological patients with IA before diagnosing a therapeutic failure (table 5). Discordant clinical, radiological and mycological data may result from an inadequate period of evaluation. The CT scan results are not evaluable before two weeks of antifungal therapy[23] due to clinical and radiological worsening possibly being misleading in hematological patients otherwise responding to treatment as a result of inflammatory immune reconstitution syndrome. Assessing response after 2 weeks of antifungal therapy using both clinical and radiological criteria and the kinetics of serum GM index may allow predicting the ultimate response.

2.- When should we use antifungal empirical treatment for IA in hematological patients?

Searched terms: Preemptive antifungal therapy, Galactomannan screening, Empirical antifungal therapy

1.- Due to the poor diagnostic specificity for IA during the presence of persistent or recurrent fever in spite of broad-spectrum antibiotic therapy, empirical antifungal therapy should not be administered in high-risk patients receiving anti-mold prophylaxis or low-risk patients (***AII***). If indicated, antifungal options include a lipid formulation of amphotericin B (***AI***), caspofungin or micafungin (***AI***), or voriconazole (***AII***). Antifungal treatment different than those used in prophylaxis is recommended (***BII***).

Evidence summary

Empirical (or clinically driven) antifungal therapy refers to that administered only in the presence of persistent or recurrent fever in spite of broad-spectrum antibiotic therapy or other clinical or radiological features suggestive of IA in high-risk patients with prolonged neutropenia. This strategy was originally justified in the early 1980s due to the need to gurantee the prompt

initiation of antifungal treatment immediately in patients suspected of having IA while diagnostic workup was still ongoing, particularly in view of the high incidence of IFD and the low diagnostic yield of conventional microbiological methods at that time. A number of clinical trials using a composite endpoint of efficacy and safety demonstrated the non-inferiority of caspofungin[114.115], micafungin[116] or voriconazole[117] compared to amphotericin B in this indication. In addition, echinocandins performed better in terms of a lower rate of premature study discontinuation because of toxicity or lack of efficacy[114]. However, emphasis should be given to the fact that the stringent implementation of this approach would imply that about 50% of patients with refractory febrile neutropenia be considered candidates for empirical therapy, while the actual incidence of IA in this population does not exceed 10-15%, even in the absence of any other prevention strategy. In the era of new diagnosis approach to IA, this strategy is not recommended.

3.- What is the treatment of IA in patients receiving solid organ transplantation?

Searched terms: Solid organ transplantation, Aspergillosis, Antifungal therapy, Immunosuppression, Voriconazole, Drug-to-drug interaction, Posaconazole, Lung transplantation, Nebulized amphotericin B, Cutaneous squamous cell carcinoma

Executive summary

1.- It is recommended initiate early antifungal therapy in SOT patients with high suspicion of IA. Further diagnostic work-up is mandatory to confirm post-transplant IA (***AII***).

2.- Antifungal treatment should be individualized taking into account the type of transplant, the severity of IA, and the immunosuppressive regimen used (***AII***). The first-line treatment for IA in SOT recipients is voriconazole (***AII***). When the use of voriconazole may be problematic (increased risk of hepatotoxicity, relevant drug-drug interaction, intolerance or allergy to azoles), a lipid formulation of amphotericin B (L-AmB) is recommended, although potential nephrotoxicity should be taken into account (particularly in kidney transplant recipients) (***AIII***).

3.- The overall amount of immunosuppression should be reduced as an adjunct to antifungal therapy, but without threatening graft outcomes (***AII***). Most likely, the preferred approach should be based on reducing steroid doses (***CIII***).

4.- In SOT recipients with severe forms of IA (i.e., central nervous system [CNS] involvement or disseminated disease), initiating treatment with antifungal combination therapy should be

considered, at least until therapeutic concentrations of voriconazole are achieved (***BII***).

5.- Special considerations for lung transplant recipients include prompt treatment of both *Aspergillus* colonization of the lower respiratory tract, and nodular or ulcerative forms of *Aspergillus* tracheobronchitis. Bronchoscopy and high-resolution CT scan should be performed to rule out dissemination (***BII***).

Evidence summary

The role of voriconazole (loading dose of 6 mg/Kg IV q12h or 400 mg PO q12h, then 4 mg/Kg IV q12h or 300 mg PO q12h) as first-line therapy for IA in the specific setting of SOT has not been formally evaluated by means of randomized controlled trials to date. Only 9 SOT recipients were analyzed within the voriconazole arm in the pivotal controlled trial by Herbrecht et al[70], whereas a previous, non-comparative, open-label trial included 6 recipients[75]. However, since this preliminary evidence, an increasing number of observational and comparative studies have reported the use of voriconazole for the treatment of IA in different SOT populations[75,118,119].

If voriconazole is to be used in severely ill patients, the parenteral formulation is preferred to ensure bioavailability. In presence of renal impairment or if the patient is clinically stable, the drug can be administered orally. Therapeutic drug monitoring should be performed to maintain voriconazole plasma concentrations in the range of 1-5.5 µg/mL. Voriconazole use might be associated with a risk of hepatotoxicity and drug-to-drug interactions with immunosuppressive drugs. There is an additional risk of steroid myopathy resulting from drug-to-drug interaction between voriconazole and methylprednisolone at doses >20 mg/day. Long-term voriconazole use may induce the development of cutaneous squamous cell carcinoma (particularly in lung transplant recipients living in areas with high sun exposure)[120,121] and hyperfluorosis-induced periostitis[122]. There lacks specific information regarding isavuconazole use in SOT patients.

Lipid formulations of amphotericin B (3-5 mg/Kg IV q24h) should be considered as a second-line alternative treatment due to its potential for nephrotoxicity (although lower than that associated with the deoxycholate formulation), higher incidence of infusion-related adverse events, and lack of oral bioavailability[123]. On the other hand, the use of any formulation of amphotericin B was identified as a risk factor for graft loss in kidney transplant recipients who remained alive at 12 weeks from the diagnosis of IA[124].

Caspofungin is the only approved echinocandin for the treatment of refractory IA, although the evidence for SOT recipients is mainly limited to non-comparative studies[125,126]. Some case reports have described the successful use of micafungin[127] or posaconazole oral suspension[128,129] as

salvage therapy for IA in SOT recipients, albeit experience so far is limited. No experience with isavuconazole has yet to be published.

The role of antifungal combination therapy in SOT recipients with IA is unclear due to the lack of comparative studies[130]. A prospective multicenter non-randomized study failed to demonstrate significant differences in 90-day survival between 40 SOT recipients treated with voriconazole plus caspofungin as first-line therapy compared with a historic cohort treated with L-AmB monotherapy. However, subgroup analysis revealed better outcomes for combination therapy in patients with renal failure or IA due to *A. fumigatus*[131]. The initial use of antifungal combination therapy for kidney transplant recipients was found to be related to poorer outcomes in a recent multinational retrospective cohort study. However, due to the presence of baseline imbalances between treatment groups, this funding was particularly susceptible to confounding by indication[124].

Therapeutic response monitoring must be regularly performed by clinical follow-up and high-resolution thoracic CT scan. Consider performing a CT scan every 7-10 days. It should be noted that neither the cavitation of pre-existing lesions (denoting necrosis) nor a discrete volume increase necessarily reflects an unfavorable evolution.

Salvage therapy refers to treatment administered for refractory or progressive forms of post-transplant IA (i.e., therapeutic failure) or due to intolerance to first-line drugs. The minimum duration of prior treatment to consider a therapeutic failure is not well defined, but the following findings should be considered as indicators of therapeutic failure: *1)* Clinical evidence of dissemination in the course of therapy. *2)* New or increasing lesions in comparison to the previous examination in a CT scan performed at 7-10 days after the initiation of treatment. *3)* No decrease in the size of lesions in a CT scan performed at 14-21 days after the initiation of treatment. *4)* Intolerance to first-line therapy. If salvage therapy must be given due to therapeutic failure of first-line therapy, the use of antifungal combination therapy is recommended. If salvage therapy must be given due to intolerance to first-line therapy, consider switching from voriconazole to L-AmB in the absence of contraindication. Other agents that have been shown to be effective as salvage therapy include: amphotericin B lipid complex (ABLC) (5 mg/Kg IV q24 h), posaconazole (oral suspension: 400 mg PO q8 h; tablets: 300 mg PO q12 h, then 300 mg q24 h), caspofungin, and micafungin (150-200 mg IV q24 h).

Few special considerations for lung transplant recipients should be done. *Aspergillus* colonization of the lower respiratory tract must be promptly treated to prevent the development of IA. The recommended treatment is nebulized L-AmB (25 mg q24 h for 7 days, then 25 mg q72 h) or nebulized ABLC (50 mg q48 h for 7 days) plus removal of debris by repeated bronchoscopic

procedures. In case of intolerance or difficulties for administering nebulized amphotericin B, consider the use of voriconazole. In presence of nodular or ulcerative forms of *Aspergillus* tracheobronchitis, voriconazole plus nebulized lipid formulations of amphotericin B (at the same doses as those in cases of colonization) are recommended. Bronchoscopy should be performed to evaluate disease extension and clear necrotic debris and fungus balls (repeat every 1-2 weeks). Parenchymal extension must be ruled out by means of high-resolution CT scan.

4.- What antifungal drugs should be used in case of breakthrough aspergillosis (BrA)?

Searched terms: Breakthrough aspergillosis, Breakthrough fungemia, Posaconazole, Micafungin, Caspofungin, Amphotericin B.

Executive summary

1.- In patients with BrA is recommended initiating empirical treatment with an alternative class of antifungal with *Aspergillus* activity until the diagnosis is established and a response to treatment can be documented (***BIII***).

Evidence summary

If IA appears after 3-7 days of an antifungal treatment or prophylaxis, it is considered a BrA. The management of BrA continues being a matter of concern. The optimal approach remains unknown; there are no studies about the clinical efficacy of different strategies of treatment (continuing with the same drug if is susceptible, increase the doses, add another antifungal drug or change to another family of antifungals) nor much is know about what is the best antifungal drug after another one apart from that most frequently occurring with an azole.

Recent guidelines[23,132,133] suggest an individualized approach that takes into consideration the antifungal used in prophylaxis, the rapidity and severity of infection, comorbidities and local epidemiology. BrA on oral suspension posaconazole prophylaxis correlated in most cases with low plasmatic levels[134]. Some data support the use of an alternative triazole (voriconazole or isavuconazole) or the increase of azole dose, but further studies are required to give this recommendation. In clinical practice, most physicians use L-AmB in this situation.

One mandatory key point is to establish a specific diagnosis with celerity carrying on the susceptibility testing of any *Aspergillus* isolates. Breakthrough infections caused by non-*A. fumigatus* species in patients undergoing azole-prophylaxis have been described (e.g. *A. terreus*

and *Aspergillus calidoustus*). All species of *Aspergillus* section *Usti* were found to be resistant to azole drugs, and resistance was also found among *Aspergillus* section *Fumigati* isolates[63]. *A. terreus* has been isolated in patients on prophylaxis. Overall survival was greater for patients who received voriconazole or another triazole as a part of their antifungal regimen than for patients who received other systemic antifungal therapies, such as amphotericin B-containing regimens. Nonetheless, resistance to triazoles has yet to be described[135].

Despite the absence of prospective trials on the optimal treatment, these authors recommend initiating empirical treatment with an alternative class of antifungal with *Aspergillus* activity until the diagnosis is established and a response to treatment can be documented. Table 6 summarizes different options of antifungal therapy in BrA.

5.- What is the treatment for IPA in ICU patients?

Searched terms: Aspergillosis, ICU, Treatment

Executive summary

1.- Voriconazole is the recommended first-line agent for critically ill patients with invasive pulmonary aspergillosis IPA (***BII***). Monitoring of serum levels is recommended, even though this triazole is administered intravenously (***BII***). Isavuconazole iv is the recommended alternative in those patients with severe renal disfunction (***BII***).

2.- Liposomal AmB is the alternative (***BII***). Echinocandins can be used as salvage therapy preferably in combination therapy (***CIII***).

3.- We do not recommend nebulized AmB as adjunctive therapy in patients with API (***CIII***).

Evidence summary

A retrospective study that evaluated 412 ICU patients with IPA has demonstrated that a delay in the initiation of antifungal therapy implicates an increase in hospital length of stay with the corresponding increase in hospital costs[136]. Thus, early initiation of antifungal treatment, often empirically, is recommended[137]. It should be highlighted that antifungal therapy clinical trials carried out to obtain the indication for IPA treatment did not include critically ill patients. Thus, these studies excluded patients requiring mechanical ventilation[70,100]. Therefore, current recommendations are extrapolated from those trials that enrolled mostly onco-hematologic patients in non-critical condition.

Some observational studies carried out in critically ill patients with IPA have shed light on our current knowledge about the best treatment options of these patients. A retrospective study of all hematology patients that required mechanical ventilation for IPA concluded that the use of voriconazole was an independent variable of survival[138].

However, voriconazole has limited aqueous solubility, as the intravenous voriconazole form includes the solvent vehicle sulfobutylether beta cyclodextrin sodium. This excipient elimination is linearly related to creatinine clearance and accumulation has been described in subjects with moderate to severe renal impairment. Renal replacement therapies (either continuous or intermittent) do not remove this compound significantly and toxic accumulation may occur. The other concern with the used of voriconazole is that its plasma concentrations fluctuate widely and serum concentrations outside the therapeutic range are associated with either worse outcome in IPA or increased toxicity. An observational study concluded that in ICU patients, only 45% of them had serum levels within therapeutic range and the majority had low sub-therapeutic concentrations[139].

Isavuconazole is a suitable alternative for voriconazole in critically ill patients with invasive pulmonary aspergillosis IPA, but current information about the use of this drug in this subset of patients is scarce. The better tolerance in comparison to voriconazole and avaiablility of a water-soluble solution for intravenous administration make this agent more easy to use in critically ill patients. The high concentration of isavuconazole achieved in the lung makes potentially this drug of great interest in patients with IPA. No definitive recommendations about the need of TDM of isavuconazole in ICU patients can be formulated given the lack of clinical studies in this scenario.

A debated issue is the use of combination therapy for patients with IPA. Recently published meta-analysis and systematic reviews concluded that cumulative evidence on combination therapy is moderate and controversial. It is worth mentioning that 40-60% of the critically patients included in observational studies received combination therapy for IPA[140]. A recent clinical trial that compared monotherapy with voriconazole with the combination of anidulafungin plus voriconazole also excluded patients on mechanical ventilation[100].

Nebulized antifungal administration is an attractive option for IPA management especially in intubated patients. Up to now, this route of administration has been employed for amphotericin B and its lipid formulations only. Regrettably, no clinical trial has been conducted to determine its efficacy and safety.

6.- What is the treatment for chronic pulmonary aspergillosis?

Searched terms: Chronic pulmonary aspergillosis, Aspergilloma, *Aspergillus* fungal ball, Chronic cavitary pulmonary aspergillosis, Subacute invasive aspergillosis, Treatment, Therapy, Surgery

Executive summary

Treatment for *Aspergillus* fungal ball (Aspergilloma)

1.- Asymptomatic patients with stable single aspergillomas may be kept in observation (***BIII***).

2.- Single aspergillomas should undergo surgical resection if there are no contraindications (***AIII***).

3.- If surgery is not feasible, long-term antifungal therapy is recommended. Instillation of antifungal agents in an aspergilloma cavity could be considered in patients with recurrent hemoptysis (***CIII***).

4.- If there is a moderate risk of surgical spillage of the aspergilloma, antifungal therapy with triazoles or an equinocandin should be given peri-/postoperatively (***CIII***).

Treatment for chronic pulmonary aspergillosis (CPA)

5.- In symptomatic patients or with progressive disease, oral antifungal therapy for a minimum of 6 months is the recommended approach (***BII***).

6.- Oral itraconazole (***BI***) or voriconazole (***BII***) are the first-line agents. Oral posaconazole or isavuconazole are a potential alternative treatment (***BIII***).

7.- In patients who fail therapy, who are intolerant, or develop triazole resistance, intravenous therapy with equinocandins (***BI***) or amphotericin B (***CIII***) are alternatives to triazoles.

8.- Surgical resection may be necessary in patients with localized disease and intractable hemoptysis, destroyed lung, or azole resistance (***BIII***).

Evidence summary

Patients with aspergilloma should undergo surgery only if they are symptomatic, either by conventional lobectomy[26,141-144] or preferably, by a video-assisted thoracic surgical procedure[145-148].

If spillage of aspergilloma in to the pleural space occurs during surgery or residual disease remains, antifungal therapy with triazoles (voriconazole) or an equinocandin (micafungin) should be given peri-/postoperatively[146]. In these patients, long-term therapy is recommended[146,149,150]. Patients with two separate aspergillomas may undergo resection depending on the locations and their respiratory reserve[151].

If surgical resection is not possible, instillation of antifungal agents in an aspergilloma cavity could be considered in patients with recurrent hemoptysis. Several clinical reports have described the

cessation of hemoptysis and/or the resolution of aspergilloma when systemic antifungals are not effective or well tolerated[152-157]. Amphotericin B is the drug of choice, although other antifungals have been used.

Response to antifungal therapy is generally slow. Thus, oral antifungal therapy for a minimum of 6 months is the recommended approach in order to reduce general and respiratory symptoms and minimize hemoptysis and prevent lung destruction and fibrosis[26,149,150,158-162]. Patients who deteriorate in this period should be considered treatment failures and an alternative regimen should be used. In responders, continuing therapy, which may be indefinite long-term suppressive treatment, is usually required and associated with better outcomes[150]. Oral itraconazole or voriconazole are the preferred agents, depending on tolerance and affordability[150,159-161,163-170].

In patients with subacute invasive aspergillosis voriconazole has shown to be superior than in patients with CPA[159,161,168]. A retrospective cohort study supports that posaconazole is a potential alternative treatment[158]. There is no published data on isavuconazole; however, the PK/PD characteristics of this drug might suggest a good treatment option for this infection. In patients with prolonged QTc, isavuconazole may be the treatment of choice.

Micafungin, caspofungin, and liposomal amphotericin B may be necessary in patients who fail therapy, who are intolerant, or develop triazole resistance[162,171-178].

Adjuvant therapy with prednisolone may be considered for symptom control only if patients are adequately treated with antifungals[179]. Mild and moderate hemoptysis usually responds to tranexamic acid[180,181]. Severe hemoptysis should be arrested with bronchial artery embolization[182]. Surgical resection may be necessary in patients with intractable hemoptysis, destroyed lung, with poor quality of life, or azole resistance. The risk of complications and mortality are significantly higher in these patients compared with those with single aspergillomas, and relapse rates are high (up to 25%)[146,148]. These patients require an active follow up.

In despite of nebulized antifungal administration being an attractive option for the management of these patients, no clinical trial has been conducted to determine its efficacy.

7.- What is the treatment for central nervous system (CNS) aspergillosis?

Searched terms: Aspergillosis, *Aspergillus*, Central nervous system

Executive summary

1.- Voriconazole is currently considered the standard of treatment of CNS aspergillosis (AIII) and liposomal amphotericin B is the best alternative in cases of intolerance or those refractory to voriconazole (***AIII***).

2.- Clinical experience with posaconazole is scarce in CNS aspergillosis; experimental studies suggest that posaconazole is equivalent to amphotericin B and superior to itraconazole and caspofungin (***CIII***).

3.- The evidence to recommend a combination therapy is weak; however, voriconazole in combination with liposomal amphotericin B has been superior to other combinations or monotherapy in experimental CNS aspergillosis (***CIII***)

4.- Surgical approach should be proposed for therapy, mainly in located lesions, and for diagnosis if conservative procedures have resulted no-conclusive (***AIII***).

5.- Intrathecal or intralesional antifungal chemotherapy and corticosteroids use is currently not recommended for treatment of CNS aspergillosis (***CIII***).

Evidence summary

Voriconazole is the smallest molecule with activity against *Aspergillus* species. Data from animal models and humans demonstrate sufficient penetration of voriconazole across the blood-brain barrier to attain fungicidal drug concentrations in the CNS, making this antifungal agent an ideal candidate to treat cerebral aspergillosis[183,184]. Weiler reported a median of 3.41 mg/L, for voriconazole brain tissue concentrations among 128 autopsy samples from eight human patients[185]. Various other studies have focused on cerebral voriconazole concentrations and have reported CSF levels from 0.08 to 3.93 mg/L in meningitis patients[186,187].

Most observations of treatment of CNS aspergillosis are based on open-label studies. A recent retrospective review of 192 voriconazole-treated patients with CNS fungal disease, including 120 CNS aspergillosis has been performed[188]. The 47% response rate for the 120 patients with CNS aspergillosis is an improvement over the previously 35% recorded in other series[189]. However, comparison of the database cases with those from the literature revealed highly significant response and survival differences in favor of the published cases, which is likely due to a publication bias. Underlying conditions influenced success (only 14% of responses in HSCT and 72% in other underlying conditions, $p<0.001$)[188]. The PK/PD properties of echinocandins with a poor penetration in CNS makes this treatment not recommended for the management of CNS aspergillosis.

Clinical studies dealing with the role of combination treatment in CNS aspergillosis are scarce. In the study commented above[188], patients treated with antifungal combination therapy had an improved response rate and superior survival.

The duration of medical therapy is controversial and highly variable in the literature: it depends on factors such as host response and residual size, but it must be maintained until clinical and radiological (CT images) have confirmed a satisfactory response. A minimum of 12 months is recommended.

Neurosurgical procedure is recommended whenever possible. In the mentioned voriconazole series patients receiving neurosurgical interventions showed superior responses (p=0.017) and survival (*p*=0.039)[188]. In other series of CNS aspergillosis, of the 49 patients who underwent a neurosurgical procedure, six had an unknown outcome (12.2%) and 14 patients died (28.6%), but in 74 patients who did not undergo neurosurgery, 50 died (67.5%)[190]. Although this could reflect a selection bias in both studies because surgery was applied more frequently in located lesions and immunocompetent patients, until more efficacious treatment becomes available, the combination of effective antifungal therapy with neurosurgery must be strongly considered. Resection might be effective in particular in patients with a focal CNS aspergillosis lesion; one study stated that mortality can be reduced from 64% to 39% under such circumstances[191].

Intrathecal or intralesional antifungal chemotherapy has the potential for AmB-induced chemical meningitis, arachnoiditis, seizures, headache, or altered mental status in absence of a clear clinical benefit[23]. Progressive neurologic deficits have led to the use of corticosteroid therapy in patients with evolving CNS disease; however, this practice is deleterious and should be avoided[23].

8.- What is the treatment for other forms of extra-pulmonary IA (intravascular infections, osteomyelitis, septic arthritis, ocular infections and others)?

Searched terms: Extra-pulmonary aspergillosis, *Aspergillus* endocarditis, *Aspergillus* sinusitis, *Aspergillus* osteomyelitis, *Aspergillus* endophthalmitis

Executive summary

1.- The treatment of extra-pulmonary forms of IA must include antifungal therapy plus adjunctive surgery (Table 7) (***AIII***). The preferred regimens are the same as those previously discussed for IPA.

Evidence summary

The diagnosis of extra-pulmonary aspergillosis is often difficult and/or made postmortem. Treatment recommendations are quite similar to those discussed for other forms of aspergillosis. No randomized trials focused in the efficacy of treatment of extrapulmonary aspergillosis had been conducted. In our view, thee major points should be taken into account when treating patients with extrapulmory aspergillosis: 1) understanding the pathogenesis of the infections, 2) knowing the antifungal PK/PD parameters to treat particularly infections, and 3) performing antifungal therapy plus surgery whenever possible. Table 7 shows the indications for adjunctive surgery in extrapulmonary IA.

9.- When and how often should we use therapeutic drug monitoring (TDM) for antifungal drugs in aspergillosis? Which levels of antifungals have been related with better outcomes in IA?

Searched terms: TDM, Antifungal exposure, Drug concentration, Amphoterycin B, Voriconazole, Itraconazole, Posaconazole, Isavuconazole, Caspofungin, Micafungin Andifulafungin.

Executive summary

1.- TDM of antifungal agents is generally recommended (***AII***), especially where non-compliance, non-linear pharmacokinetics, inadequate absorption, a narrow therapeutic window, suspected drug interaction or unexpected toxicity are encountered (***AI***).

2.- First sample (trough sample) for TDM must be obtained once the steady state has been reached (3-7 days depending on the antifungal) (***AI***) and then repeated at least once per week after dose stability is achieved (***CIII***).

3.- A therapeutic range to treat IA between 1 mg/L and 6 mg/L has been defined for voriconazole (AII). Trough levels > 0.7 mg/L for prophylaxis and > 1.0–1.25 mg/L for treatment may be predictive of efficacy for posaconazole (***AII***). A new target needs to be defined for new posaconazole formulations (***BIII***). Regarding itraconazole, a trough concentration of 0.5–1 mg/L (measured by HPLC) is recommended (***AII***). TDM for isavuconazole is not currently recommended (***BIII***).

4.- When trough concentration does not reach or exceed the target established, drug dosage should be increased or decreased consequently (***AIII***).

Evidence summary

Most of the studies exploring the impact of antifungals TDM on efficacy and safety are observational and prospective; most of them have found TDM to be beneficial, especially for triazoles (itraconazole, voriconazole and posacoanzole) commonly used in aspergillosis management. A randomized, assessor-blinded, controlled trial involving 110 patients with IFD demonstrated that TDM significantly reduced drug discontinuation and better response in patients treated with voriconazole[192].

The optimal frequency of TDM has yet to be well defined. In a randomized trial conducted by Park et al[192], voriconazole serum levels were repeated when dosage regimen or administration route was altered for any reason, if an interacting drug was introduced or stopped or if there was any change in patient clinical conditions.

Therapeutic concentration targets to optimize the antifungal effect have been derived exclusively within the context of prevention or treatment of IFD. For voriconazole TDM, literature has suggested variable therapeutic range, primarily spanning between 1 and 6 mg/L. A voriconazole level higher than 1 mg/L should be considered the lowest threshold associated with efficacy. This value has been considered as the most predictive of successful outcomes in a recently published meta-analysis[193]. Noteworthily, other meta-analysis published by Jin H and coworkers[194], including fewer studies, considered 0.5 mg/L the lowest threshold associated with efficacy. Additionally, emphasis has been placed on the importance of considering MIC of the isolate, when it is available, together with trough concentration, when predicting therapeutic response for patients receiving voriconazole. Therefore, MIC may also be considered in targets for TDM, which suggests a tenable trough concentration: MIC ratio of 2–5 (when the MIC is estimated using CLSI methodology) may be useful for better outcome[195]. A supratherapeutic threshold of 6.0 mg/L was most predictive of toxicity (193). A trough concentration>4.0 mg/L is associated with increased neurotoxicity and >3.0 mg/L is associated with increased hepatotoxicity, particularly for the Asian population[194].

Additionally, drug dosage should be based on the results of TDM. Park et al established a strategy on voriconazole TDM group of patients: a dose increase of 100% if level was less than 1 mg/L, and a reduction of 50% if drug level was higher than 5.5 mg/L. Also, they proposed to skip dosage if level was >10 mg/L or if any adverse effect appeared, followed by dosage reductions of 50% until therapeutic levels were reached again. What is worthy to mention are the strategies of voriconazole dosage based on CYP2C19 genotyping. A meta-analysis published recently did not find any significant association between CYP2C19 variants and daily maintenance dose or adverse outcomes of voriconazole[196]. However, *CYP2C19* genotyping appears useful in guiding voriconazole

initial dosing when coupled with TDM in order to characterize voriconazole disposition[197].

In the case of posaconazole, clinical studies suggest a trough level of >0.7 mg/L, a through level of >0.35 mg/L after 2 days of treatment for prophylaxis and trough level of >1.0–1.25 mg/L as predictive of efficacy after 1 week of treatment with the oral suspension[198]. Additionally, an upper boundary of 3.75 mg/L has been suggested for average posaconazole plasma concentrations by the European Medicines Agency (European Medicines Agency 2016), albeit no evidences of toxicity has been described yet. Dekkers and coworkers recommended a new target of posaconazole trough of 0.9 mg/L for prophylaxis and a trough level of 1.8 mg/L for treatment when tablet formulation is used. Also, Park et al established new startegies for reaching the therapeutic level with posaconazole syrup by increasing the frequency of dose administration, 200 mg four times daily[199].

For itraconazole, a trough concentration of 0.5–1 mg/L (measured using High Preasure Liquid Cromatography) was established for successful outcome. Itraconazole concentrations measured by bioassay are typically 2 -10 times higher than those estimated using HPLC (due to the active metabolite). When measured by bioassay, a reasonable lower limit for TDM is approximately 5 mg/L[200].

TDM for isavuconazole is not currently recommended due to their predictable kinetic.

10.- What is the best treatment for *Aspergillus* infections caused by azole-resistant isolates?

Searched terms: Azole resistance, Antifungal treatment, Manage azole resistance, *Aspergillus lentulus*.

Executive summary

1.- Therapy of *Aspergillus* infections caused by cryptic or resistant species should be selected per *in vitro* susceptibility data, site of infection, and patient characteristics (***AIII***).

2.- Isolates resistant to voriconazole (MIC >2 mg/l) are recommended to be treated with amphotericin B (***AIII***) or the combination of voriconazole with an echinocandin (***CIII***).

3.- In areas with a rate of azole resistance ⩾10%, azole monotherapy should be avoided in empirical primary treatment of severe cases of IA (***BIII***).

Evidence summary

Some studies suggest that azole resistance is related to treatment failure and high mortality[201-204]. No guidelines are available and clinical evidence is lacking, but a recent international consensus

document, based on case reports, preclinical studies and expert opinion, has dealed with the treatment of azole-resistant aspergillosis[205].

Therapy of *Aspergillus* infections caused by cryptic or resistant species should be selected per *in vitro* susceptibility data, site of infection, and patient characteristics (drug-drug interactions, toxicity, etc). Isolates may be resistant only to itraconazole or voriconazole; some to itraconazole and posaconazole, and others to all azole drugs. Resistance to AmB is rare, apart from *A. terreus*, *A. nidulans*, and *A. lentulus*.

Isolates resistant to voriconazole (MIC >2 mg/l) should not be treated with voriconazole monotherapy. Liposomal-AMB is the preferred option. Another alternative is the combination of voriconazole with an echinocandin, although evidence is scarce[206,207]. It is not clear if voriconazole monotherapy could be used for intermediate strains (MIC =2 mg/l)[205]. Recent preliminary data suggest, that, contrary to expectation, lower voriconazole exposures may be required for strains with higher MICs[208]. In any case, treatment may need to be prolonged and surgery can be considered in selected cases.

In azole-resistant chronic pulmonary aspergillosis, micafungin or liposomal AmB can be considered[205]. Inhaled AmB is also an option for azole-resistant airways aspergillosis.

Experts recommend that in areas or departments with a rate of azole resistance ⩾10%, azole monotherapy should be avoided in empirical primary treatment of severe cases of invasive aspergillosis[205]. However, most countries remain far below this threshold[60,209-213].

11.- What is the role of adjunctive therapy in IA (including surgical resection and granulocyte transfusion therapy?

Searched terms: Aspergillosis, Adjuvant therapy, Surgery, Granulocyte Transfusion therapy, Interferon-γ

Executive summary

1.- Doses of immunosuppressive agents should be reduced as much as possible as an adjunct to antifungal therapy (***AII***).

2.- Granulocyte transfusion therapy may be considered for neutropenic patients with refractory forms of IA and an anticipated duration of neutropenia >7 days. There is no indication for this type of adjunctive therapy in other populations (***BII***).

3.- The administration of recombinant interferon (IFN)-γ may be considered in patients with refractory forms of IA, although its benefit as adjunctive therapy is unclear and must be weighed against the potential consequences of enhancing alloimmune responses (***CIII***)

4.- Adjunctive surgery is recommended in patients with massive hemoptysis, endocarditis, pericardial involvement, invasive sinusitis, or infection of large vessels, bone, subcutaneous tissue, or central nervous system during treatment (***BII***).

Evidence summary

The role of granulocyte transfusion therapy is still a matter of debate. A recently randomized controlled trial that recruited 114 neutropenic patients with infection (including 8 cases of IA) failed to demonstrate a favorable effect of transfusions from donors stimulated with granulocyte colony-stimulating factor (G-CSF), nonetheless,subjects who received a higher average dose per transfusion (≥0.6 x 10^9 granulocytes per Kg) tended to have better outcomes than those receiving lower doses[214]. Acute lung injury is the most common adverse event associated to granulocyte transfusions, and this risk may be higher with the concurrent use of amphotericin B[215].

Various case series have reported the use of recombinant IFN-γ (usually at doses of 100-200 µg SC thrice a week) as adjunctive therapy for refractory forms of IA in hematological patients[216], SOT recipients[217,218] and patients with chronic granulomatous diseases (CGD)[219,220]. The rationale for such approach stems from the instrumental role played by IFN-γ in promoting Th_1 differentiation and skewing adaptive immune response towards a protective phenotype. In addition, IFN-γ enhances *ex vivo* antifungal activity of macrophages and neutrophils[221]. Recombinant IFN-γ has been FDA-approved for prophylaxis of IA and other types of infection in patients with CGD[222]. Concerns have arisen regarding the potential of exogenous IFN-γ in triggering alloimmune responses, with the subsequent risk of inducing graft versus host disease (GVHD) and graft rejection in HSCT and SOT recipients, respectively. Nevertheless, reported experience to date suggests safety for such therapy[216].

Surgical excision remains an integral component of the therapeutic approach to IA, particularly for localized forms of disease that are accessible to debridement (i.e., invasive sinusitis or subcutaneous tissue involvement) or in the presence of life-threatening complications (i.e., massive hemoptysis)[223]. A recent single-center retrospective study analyzed the role of emergency

and elective pulmonary surgical resection in 50 hematological patients with invasive fungal disease (including 29 cases of IA). At the time of surgery, 30% of patients were still neutropenic and 54% required platelet transfusions. Lobectomy was the procedure most commonly performed (80% of cases), with survival rates at 30 and 90 days after surgery of 94% and 78%, respectively. No outcome differences were found between emergency and elective surgery[224]. Various non-comparative studies have reported a survival benefit in patients with patients with CNS involvement undergoing different types of neurosurgical approaches (craniotomy/abscess resection, abscess drainage or ventricular shunt)[188,189]. The role of image-guided stereotactic resection has also been proposed[225]. In patients with suspected *Aspergillus* endocarditis, resection of vegetations and mural lesions with prosthetic replacement of the infected valves should be considered in confirming the diagnosis of this uncommon form of IA and improving outcomes, as survival has rarely been reported in the absence of surgical intervention[226-229].

12.- What drugs interact with IA treatment?

Searched terms: Aspergillosis, Drug interaction

Executive summary

1.- Antifungal agents may be associated with significant drug-to-drug interactions, leading to sub-therapeutic antifungal drug concentrations and poorer clinical outcomes (***AI***).

Evidence summary

The knowledge of interactions between antifungals and other drugs is crucial in the daily clinical practice to improve antifungal activity and avoid potential undesirable side effects. Main interactions between antifungal agents and other drugs are shown in table 8.

Drug–to-drug interactions are higher for azoles. Among different azoles, metabolic pathways may be different; therefore, a 'class effect' cannot always be assumed. In general, azoles are metabolized by the cytochrome P450 (*CYP450*) system, mainly *CYP3A4*, although posaconazole primarily undergoes glucuronidation[230,231] and fluconazole is largely excreted renally[232].

Itraconazole and voriconazole have higher number of drug-to drug interactions, as these are metabolized to a greater extent by cytochrome isoenzymes than fluconazole and posaconazole (230). Variability in CYP enzyme activity may be observed between patients due to genetic polymorphisms. Isavuconazole is a moderate inhibitor of CYP3A4, but it not affect other substrates,

resulting in fewer drug–to-drug interactions compared with other azoles.

Some or more relevant azole interactions are the following: astemizole, cisapride, terfenadine, and quinidine. The aforementioned should not be co-administered with azole antifungal agents due to the risk of QT interval prolongation[233]. Other medications that may prolong the QT interval (e.g. ciprofloxacin, cotrimoxazole, macrolide antibiotics and conventional antipsychotics) should be used with caution. Unlike the other members of the triazole antifungal class, isavuconazole does not induce QT-interval prolongation. In fact, this drug might produce QT-interval shortening. Ergot alkaloids are contraindicated with azoles because of the risk of ergotism[230]. If azoles are coadministered with coumarins or phenytoin, dose reduction of these drugs may be required and close monitoring is recommended. The University of Liverpool human immunodeficiency virus drug interaction chart is available for assessment of potential azole drug interactions in patients with HIV infection[234]. Drug to drug interactions should be evaluated very carefully in SOT, if voriconazole is administered; the calcineurin inhibitor dose should be reduced by 50-60%[235,236]; co-administration of voriconazole and sirolimus is formally contraindicated, although some authors have applied this combination by reducing the dose of sirolimus by 75-90%[237]. If patient is receiving posaconazole, then dosage of tacrolimus or cyclosporine A should be reduced by 60-75% and 14-29% respectively[238]. Posaconazole is contraindicated in patients cotreated with sirolimus. Conversely, Isavuconazole is not contraindicated in sirolimus treated patients.

The echinocandin class of drugs is not significantly metabolized by the *CYP450* system. Anidulafungin is not metabolized by these enzymes (239), caspofungin is a poor substrate for *CYP450* enzymes[240] and hydrolysis by *CYP3A* plays only a minor role in the metabolism of micafungin[239].

Concommitant administration of *CYP450* inducers (e.g. efavirenz, nevirapina, fenitoin, rifampin, dexametasone and carbamacepin) with some echinocandins (e.g. caspofungin) may reduce serum antifungal drug concentration and dose should be increased[241]. Combination therapy with caspofungin and cyclosporin may lead to transient elevations in transaminases. Caspofungin may also reduce plasma concentrations of tacrolimus[242]. Anidulafungin is not expected to alter the plasma concentrations of either cyclosporin or tacrolimus[243]. Rifampicin does not appear to alter the clearance of anidulafungin[244]. Micafungin, however, may have varied effects on the pharmacokinetics of cyclosporine.

AmB-D and its lipid-based formulations are excreted renally and may be associated with nephrotoxicity, hypokalemia and hypomagnesemia. The nephrotoxic potential of amphotericin preparations is enhanced when used with other nephrotoxic medications[245] Associated

hypokalemia may be exacerbated by non-potassium sparing diuretics. AmB-D-associated nephrotoxicity is typically less severe in infants and children[246]. **Table 8.** Potential drug-to-drug interactions between antifungal agents recommended for IA and other drugs.

13.- When should we stop treatment for invasive aspergillosis?

Searched terms: Aspergillosis, *Aspergillus*, Neutropenia, Solid organ trasplant immunosuppression, Treatment, Therapy

Executive summary

1.- Treatment for IA should be continued for a minimum of 6-12 weeks. The duration of the antifungal therapy should be individualized, depending on the degree and duration of neutropenia and other immunosuppressive conditions, the site of the disease, and evidence of disease improvement (***BIII***).

Evidence summary

Duration of antifungal therapy for invasive aspergillosis is not well defined. There are no studies specifically addressing this issue. Therefore, recommendations emerge mainly from randomized clinical trials that focus on the safety and efficacy of several antifungal agents for the treatment of IA[70,75,76,83,100]. Several factors influence the decision of determining when to stop antifungal therapy, such as the persistence of the immunosuppressed status, especially neutropenia; the localization of the disease, and the response to therapy. Thus, the decision should be individualized.

There is no evidence to recommend that the persistence of neutropenia itself is a definitive criterion in maintaining antifungal therapy. However, persistent neutropenia might lead to diminished response. In order to stop treatment, a rather good partial or complete remission is needed, which is measured by radiographic imaging and/or microbiological absence of disease.

Patients should undergo treatment response, which includes a clinical assessment of all symptoms and signs, as well as a periodical radiological evaluation, usually with CT. The frequency of radiological assessments will be determined by the rapidity of evolution of the pulmonary infiltrates and the acuity of illness in the individual patient. It is important to be aware that the volume of pulmonary infiltrates may increase for the first 7-10 days of therapy, especially within the context of granulocyte recovery, without this meaning a worse evolution[105].

The progression over time of *Aspergillus* GM assay has been associated with poor prognosis[103,247]. The use of serial serum GM assays could be useful as a therapeutic monitoring tool[104]. However, resolution of GM antigenemia to a normal level is not sufficient as a sole criterion for discontinuation of antifungal therapy. Close monitoring (e.g. radiographic imaging) is suggested once antifungal treatment is discontinued.

The duration of antifungal therapy in patients without neutropenia and invasive aspergillosis should be guided by the same considerations as in patients with neutropenia. However, in some of these patients physicians may have the possibility of improving patient's immune system. Thus, immunosuppressive agents should be tapered or removed whenever possible.

Hematopoietic stem cell transplant recipients without neutropenia have been frequently included in randomized clinical trials, and experience may be derived from these studies[70,75,76,83,100]. However, there are no comparative studies of antifungal therapy in non-hematological patients, and as such, recommendations in these patients arise mainly from cohort and observational studies[119,124,126,131,248-251].

Therapeutic monitoring should be similar as that in patients with neutropenia. In non-neutropenic patients (e.g. HIV infected patients and solid organ recipients), immune reconstitution-syndrome like may occur after therapy initiation[130].

14.- What are the specific recommendations for IA in pediatric population (diagnostic approach, therapy and prophylaxis)?

Searched terms: IA, Children, Diagnosis, Treatment, Prophylaxis

Executive summary

1.- The authors recommendation diagnostic approach for pediatric population is the same a that for adults (***BII***).

2.- Voriconazole (***AI***), and liposomal amphotericin B (***BI***) are the preferred options for IA treatment. Primary antifungal combined therapy is not routinely recommended in children (***CIII***). For salvage treatment, voriconazole (***AI***), liposomal amphotericin B (***BI***) and caspofungin (***AII***) are the drugs of choice.

3.- High-risk patients (expected IFD incidence >10%) should receive mold active prophylaxis (***AII***). The drug of choice depends on the studied population.

Evidence Summary

1) Diagnostic approach

Standard procedures for IA diagnosis include cultures and microscopic examination of appropriate specimens (including tissues), imaging studies and biomarkers detection.

Radiological data on imaging findings in children with IA are scarce. In contrast to adults, it is uncommon to see specific images in high-resolution chest CT (halo sign, air crescent sign), particularly in younger children[252,253]. Nodules or fluffy masses and mass-like lesions are the two main types of abnormalities254-258. It remains unclear why there are differences between children and adults.

There is a paucity of data on GM test in children[259-270]. A recent consensus from the ECIL group has re-analyzed[254] patients pooled from five studies[264,267,269-271] providing adequate information about individual patients and using EORTC/MSG criteria. They found similar operating characteristics to adult patients[23,254,272]. The sensitivity of the test is very low in non-neutropenic patients and patients with chronic granulomatous disease[23]. Pediatric information regarding the value of the GM test in bronchoalveolar lavage[273] is scarce.

Published data are very limited in pediatrics on BDG and amplification of nucleic acids and no specific recommendations can be made.

2) Targeted therapy

The recommendations and ratings for initial antifungal treatment are mostly based on adult trials and are summarized in table 9. It is important to understand the paucity of systematic data available on the efficacy of both, voriconazole and L-AmB in paediatric patients with IA. As the dosage of voriconazole in <2 years of age has yet to be well defined, L-AmB is the only approved first line option in this age group[272].

The duration of treatment remains uncertain. Treatment for 6-12 weeks or until resolution of clinical /radiological evidence of disease and recovery of the underlying deficiency in host defences is recommended[132,274].

3*) Primary and secondary chemoprofilaxis*

Table 10 summarizes pedriatic population as divided by risk of suffering IA.

Specific pediatric guidelines have been established for prevention and treatment of IFD in children[254,278,279]. However, most information regarding this topic comes from randomized controlled trials conducted predominantly in adults (280). As a summary, the current recommendations for primary antifungal chemoprophylaxis with antimold antifungal agents in children are shown in table 11. Secondary prophylaxis against previous fungal pathogen is recommended based on data mainly from adults, as long as patient is granulocytic or immunosuppressed[254]. The role of surgical resection is debated but patients should have had at least a partial response before continuing with planned anticancer treatment. Patients must continue receiving effective antifungal therapy[272]. Pediatric data are scarce[281,282].

Recommended dosages for antifungal prophylaxis in children, beyond the neonatal period, are:

1) Voriconazole**:** 2 to >12 years or 12 to 14 years and < 50 Kg, 16 mg/kg/day iv in two divided doses (day 1: 18 mg/Kg/day iv or po in two divided doses); >15 years or 12 to 14 years and >50 Kg, 8 mg /kg/day iv in two divided doses (day 1: 12 mg/kg/day iv in two divided doses or 400 mg/ day po in two divided doses).
2) Posaconazole: 600 mg/day po in three divided doses.
3) Micafungin: 1 mg/Kg/day iv in one single dose (≥50 Kg: 50 mg/ day).
4) Caspofungin: 3 months to 17 years: 50 mg/m^2/day iv (day 1: 70 mg/m^2/day in one single dose; maximum 70 mg/day).
5) Liposomal amphotericin B: 1 mg/Kg/day every other day or 2.5 mg/ Kg twice weekly in one single dose.

Prophylaxis

1.- Does anti-mold prophylaxis reduce the incidence of IA in high-risk populations, and what are the best drugs?

Searched terms: IA, Prophylaxis, Hematologic malignances, Hematopoietic stem-cell transplantation, Organ solid transplantation, Kidney transplantation, Pancreas transplantation, Heart transplantation, Lung transplantation, and Small bowel transplantation

a) Prophylaxis in patients with hematological malignancy and hematopoietic stem-cell transplantation.

Executive summary

1.- Prophylaxis with an anti-mold agent is recommended for IA prevention in patients with acute leukemia and prolonged and profound neutropenia; allogeneic HSCT recipients during the neutropenic phase; and those with moderate to severe graft versus host disease (GVHD) and/or intensified immunosuppression (AI).

2.- Several antifungal drugs can be used to reduce the incidence of IA in high-risk patients, including posaconazole (AI), voriconazole (AI), itraconazole (BII), micafungin (BIII), caspofungin (CIII), aerosolized L-AmB (BI), and intravenous lipidic formulations of AmB (CII).

Evidence summary

Anti-mold prophylaxis has been shown to reduce the incidence of IA in high-risk populations, such as patients with acute myelogenous leukemia (AML) or myelodisplastic syndrome (MDS) with profound and prolonged neutropenia, those with allogeneic HSCT during the neutropenic phase, and those with cases of moderate to severe GVHD and/or intensified immunosuppression.

Nowadays, azoles are the main antifungals used in fungal prophylaxis in hematological patients.

Posaconazole showed to be superior to fluconazole or itraconazole in IA prevention among neutropenic patients with AML and SMD in a large randomized clinical trial of oral posaconazole solution. Remarkably, antifungal prophylaxis was associated with higher overall survival. Conversely, patients receiving posaconazole presented greater toxicity, mainly due to gastrointestinal disturbances[45]. In another randomized clinical trial involving HSCT recipients with moderate to severe GVHD, the use of posaconazole solution also significantly reduced the incidence of IA compared to fluconazole, which has no mold activity, with similar toxicity profiles[284]. Since these studies, posaconazole extended-release tablets and an intravenous form are now available, which, in turn, may improve serum posaconazole levels.

A large randomized, double-blind clinical trial showed less *Aspergillus* infections in HSCT recipients with and without GVHD receiving voriconazole prophylaxis, in comparison with fluconazole[277]. Similar results were obtained in a retrospective study of patients receiving glucocorticoid therapy for GVHD[285]. In a large randomized, open-label study involving allo-HSCT recipients, voriconazole was superior to itraconazole in the primary composite objective, which included tolerability, but was equally effective in preventing IA[286]. Voriconazole prophylaxis was also retrospectively assessed in patients with leukemia during a construction risk period, showing a reduction in the

incidence of IA[287]. Itraconazole has been widely assessed in several clinial trials, showing controversial results when compared to other antifungals in hematological patients with neutropenia[288-292]. In HSCT recipients, the potential benefit appears to be more evident[293-296]. Nevertheless, due to the erratic bioavailability and high risk of toxicity of the drug, the use of itraconazole in the clinical practice has been increasingly replaced.

In a randomized, placebo-controlled trial, involving 271 patients with chemotherapy-induced prolonged neutropenia, aerosolized liposomal amphotericin B (L-AmB) combined with oral fluconazole significantly reduced the incidence of IA[275]. A subsequent analysis of data from that trial showed that short-term prophylactic nebulization of L-AmB was well tolerated and not associated with decline in pulmonary function or systemic adverse events[297]. However, the beneficial protective effect of L-AmB against IA was not found in a prospective randomized multicenter trial using aerosolized conventional AmB[298]. Intravenous amphotericin B lipid complex (ABLC) given 3 times weekly (2.5 mg/Kg), has been evaluated for prophylaxis of IFD in patients with AML and MDS undergoing induction chemotherapy, and it appeared to have similar efficacy than that of intravenous L-AmB (3 mg/Kg) in a historical control group[299]. In a randomized, open-label trial, comparing intravenous low-dose L-AmB (50 mg every other day) with no systemic antifungal profilaxis in patients with hematologic malignancies and prolonged neutropenia to reduce the incidence of IFD, IA occurred less frequently in the intervention arm[283]. An open-label, prospective phase II study of only 48 patients found that a single 15 mg/Kg L-AmB dose was as safe as antifungal prophylaxis in AML patients undergoing induction therapy[300].

In a randomized, double-blind trial, involving 882 adult and pediatric patients undergoing HSCT, 50 mg of micafungin (1 mg/Kg for patients weighing <50 Kg) was compared with 400 mg of fluconazole (8 mg/Kg for patients weighing <50 Kg) administered once per day, for prophylaxis against IFD during neutropenia[301]. The overall efficacy as antifungal prophylaxis of micafungin was superior to that of fluconazole during the neutropenic phase after HSCT (80% in the micafungin arm vs. 73.5% in the fluconazole arm; P= .03). There was a trend toward reduced breakthrough aspergillosis (0.2% vs 1.5%; P=.07). The efficacy and safety of caspofungin has been found to be similar to that of other antifungal prophylactic regimens in acute leukemia patients undergoing induction therapy and in HSCT recipients, per studies with several limitations[302,303].

b) Prophylaxis in solid organ transplantation.

Executive summary

1.- Prophylaxis with an anti-mold agent is recommended for prevention of IA only in high risk patients with organ solid transplantation. Our recommendations and evidence level are summarized in table 12.

Evidence summary

The correct identification of SOT recipients with increased susceptibility to IA is critical in selecting the optimal prevention strategy. In addition, the effectiveness, safety profile, costs and potential for drug-to-drug interactions of the intervention must be considered[130]. The paucity of randomized controlled trials and the heterogeneity in the incidence of IA across different transplant programs or the optimization of surgical procedures, the accurate tapering of immunosuppression, and the environmental control of filamentous fungi make it difficult to provide definitive evidence-based recommendations for IA prevention after SOT[236,304].

Some specific conditions (i.e., retransplantation, fulminant hepatic failure or post-operative requirement for renal replacement therapy, among others) identify a high-risk subgroup of liver transplant recipients that share predisposing factors for IA. Within this category, the incidence of invasive fungal disease in the absence of any prevention strategy may be historically over 30%[305,306]. Therefore, these patients must receive antifungal prophylaxis with an agent active against both *Candida* spp. and *Aspergillus* spp (table 12). The duration of prophylaxis has not been clearly established, although most previous trials were comprised of a 21-day course or until discharge of patient[307,308]. The drug of choice remains controversial. Amphotericin B, either deoxycholate or lipid formulations, has been used in some studies[305,306,309-311]. However, since renal failure is one of the risk factors for IA, the nephrotoxicity associated to this agent represents a limitation for its use, as well as the relatively common occurrence of infusion-related adverse events[311]. Both randomized trials and non-comparative studies have demonstrated the role of caspofungin[312,313], micafungin[307,314]. and anidulafungin[308] in preventing the occurrence of IA and other invasive fungal diseases in high-risk liver transplant recipients. Although some liver transplant groups use universal prophylaxis with fluconazole, there are several doubts about this strategy since this agent lacks activity against *Aspergillus* spp. and the incidence of invasive fungal disease in the absence of risk factors is usually below 4%[315]. In addition, universal prophylaxis is associated to hepatotoxicity and drug-to-drug interactions, and may lead to an increase in the rates of antifungal resistance[316].

Among heart transplant recipients, IA occurs in 1% to 14% of patients, depending on the series[317-320]. Given this wide variation in incidence and the absence of randomized controlled trials, there is

no clear agreement between transplant groups for recommending universal antifungal prophylaxis in this population. (Table 12).

Universal prophylaxis against IA is commonly accepted in lung transplant recipients, although the applied strategy widely varies across centers[321]. The efficacy and safety of nebulized lipid formulations of amphotericin B have been demonstrated for lung transplant recipients[322-325]. The duration of prophylaxis is usually limited to the first 3-6 months after transplantation, although some groups recommend long-term maintenance in case of persistence of risk factors[324]. Alternatively, antifungal prophylaxis may be performed with broad-spectrum azoles. One single-center study demonstrated that the use of voriconazole as universal prophylaxis was associated with a significant reduction in the overall incidence of IA compared to the group managed with targeted prophylaxis (1.5% versus 23.5%, respectively), although the incidence of hepatotoxicity and drug discontinuation due to adverse events was higher in the former group[321]. The occurrence of hepatotoxicity seems to increase when prophylaxis is initiated in the perioperative period (within the first 30 days from transplantation)[326]. By the same token, the long-term risk of voriconazole-induced phototoxicity should be also considered[120,121].

The incidence of IA after kidney transplantation is lower compared to those observed for other SOT populations (usually below 0.5%)[320,327,328]. Nevertheless, kidney transplant recipients suffer from the highest burden of post-transplant IA in absolute terms since this transplant procedure is, by far, the most frequently performed worldwide. Although recent studies have identified a number of specific risk factors (i.e., pre-transplant diagnosis of chronic obstructive pulmonary disease, delayed graft function, acute graft rejection or prior CMV disease)[329,330], the low prevalence of IA among kidney transplant recipients precludes a formal recommendation for using antifungal prophylaxis in this setting.

Finally, there is no agreement on an optimal prevention strategy for late-onset post-transplant IA (i.e., beyond the first 90-180 days after transplantation), even when this condition poses a significant problem given that half of the episodes in some centers fall within this category. Risk factors for late IA include chronic graft rejection, renal failure and over-immunosuppression[328].

Specific subgroups of small bowel and pancreas recipients may also benefit from antifungal prophylaxis for IA in the presence of certain risk factors (i.e., renal replacement therapy, surgical re-exploration or anastomotic complications)[331,332] or when institution's annual incidence of post-transplant IA exceeds 5% (Table 12).

2.- Is there indication for secondary prophylaxis to prevent IA relapse?

Search terms: Secondary prophylaxis, antifungal agents, Invasive aspergillosis

Executive summary

1.- Secondary prophylaxis aimed at preventing relapse of a previous IA is recommended in immunosuppressed patients, such as allogeneic HSCT in the early phase and with acute or extensive chronic GVHD; with severe and prolonged neutropenia; or undergoing T-cell suppressing therapy, and should be based on treatment response to initial antifungal therapy (***AII***).

Evidence summary

Secondary prophylaxis aims at preventing relapse of a previous IFD, or the onset of another IFD during a new at-risk period, defined as either a prolonged neutropenic phase or a phase of immunosuppression, mainly after allo HSCT. For patients with AML or allo-HSCT with severe neutropenia, the relapse rate of previous IFDs is high; therefore, the use of secondary prophylaxis is recommended[333,334]. In this regard, a single-arm, multicenter trial of a cohort of 45 recipients of allo-HSCT with a previous history of IFD (of whom 69% had prior probable/proven IA) evaluated secondary prophylaxis with voriconazole and found that only 6.7% (3 of 45 patients) patients developed an IFD within the first year after transplant[282]. A prospective study evaluated the efficacy and safety of secondary prophylaxis for allo-HSCT patients with a history of IA[335]. The prophylactic agents were chosen based on treatment response to initial antifungal therapy and included itraconazole, voriconazole, caspofungin and L-AmB. The 1-year cumulative incidence of IFD and IA was 27% and 25%, respectively. Other studies with a small number of patients have found that secondary prophylaxis with either L-AmB or caspofungin appear to be feasible and safe in HSCT[281,336,337].

Conflicts of interest

Carolina Garcia-Vidal has received honoraria for talks on behalf of Gilead Science, Merck Sharp and Dohme, Pfizer, Jannsen and a grant support from Gilead Science. She is a recipient of a INTENSIFICACIÓ Grant from the "Strategic plan for research and innovation in health-PERIS 2016-2020", a research grant from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III [FIS PI15/00744] and belong to FungiCLINIC Research group (AGAUR); Ana Alastruey-Izquierdo has received honoraria for talks on behalf of Gilead Science and grant support from Gilead Science, F2G Ltd. and Scynexis; Jordi Carratalà has

received honoraria for lectures from Gilead and Merck Sharp and Dohme; Mario Fernández-Ruiz has received honoraria for talks on behalf of Gilead Science, Astellas and Pfizer; JM Aguado has received honoraria for speaking at symposia organised on behalf of Pfizer, Astellas, Merck Sharp & Dohme (MSD), Angelini, and Gilead Science and has sat on advisory boards for antifungal agents on behalf of Pfizer, Astellas, MSD, Angelini, and Gilead Science; Jesús Fortún has received honoraria for speaking at symposia organised MSD and Astellas and a grant support from MSD; José Garnacho-Montero has participated in conferences sponsored by MSD and Astellas and a grant support from Astellas; Jesus Guinea has received funds for educational activities organized on behalf of Astellas, Gilead, MSD, Scynexis, and United Medical. He has also received funds for research from Fondo de Investigación Sanitaria, Gilead, Scynexis, and Cidara; Carlota Gudiol has received honoraria as a speaker from Gilead Science and Merck Sharp; Patricia Muñoz has received honoraria as a speaker from Gilead Science, Merck Sharp and Dohme, Astellas, and Pfizer; Javier Pemán has received honoraria as a speaker from Gilead Science, Merck Sharp and Dohme, Astellas, and Pfizer; Montserrat Rovira has received honoraria for talks on behalf of Gilead Science, MSD-Merck and Pfizer; Isabel Ruiz-Camps has received honoraria for talks on behalf of Gilead Science, MSD-Merck, Astellas, Celgene and Pfizer and has sat on advisory boards for Pfizer and Celgene; Manuel Cuenca-Estrella has received grant support from Astellas, bioMerieux, Gilead Sciences, Merck Sharp & Dohme, Pfizer, Schering Plough, Soria Melguizo, Ferrer International, CIDARA, F2G, Basilea, Amplyx and Scynexis

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Table 1. Definitions of strength of recommendation and quality of evidence

Strength of recommendation	
A	Strongly support a recommendation for use
B	Moderately support a recommendation for use
C	Marginally support a recommendation for use
Quality of evidence	
I	Evidence from at least one randomized, controlled trial supporting the recommendation being made
II	Evidence from at least one well-designed clinical trial, without randomization, cohort study or case-controlled study
III	Evidence from expert opinions based on clinical experience or descriptive cases.

Table 2. Diseases caused by *Aspergillus*

Acute forms of invasive aspergillosis	
Invasive pulmonary aspergillosis	It is the most severe form of disease, usually occurs in severely immunocompromised patients. *Aspergillus* spores germinate in deficient macrophages, and hyphae produce angioinvasion and invasion in tissue. As a result, vascular thrombosis, and pulmonary necrosis appear with the characterisc "halo" sign in computed tomagraphy scan. Usually, *Aspergillus* antigen is positive in serum of hematological patients.
Extrapulmonary forms	Occurs in the context of disseminated infection from the lung in severely immunocomppromissed patients (mainly central nervous system, cutaneous,…), or as a single-organ infection mainly due to direct inoculation (sinonasal, tracheobronchitis, and less frequently endocarditis, osteomyelitis, endophthalmitis, peritonitis,…) in patients with different degrees of immunosupression.
Chronic forms of aspergillosis	
Chronic necrotizing pulmonary aspergillosis or subacute invasive pulmonary aspergillosis	Mildy immunocompromised patients, neutrophils fight *Aspergillus* spores, a few germinate and might produce hyphae, but angioinvasion and invasion in tissue is low. Pulmonary inflammation is high. Radiological features include marked pleiotropic findings. Serum *Aspergillus* antigen is usually negative. Bronchoscopy is necessary to microbiological diagnosis.
Chronic fibrosing pulmonary aspergillosis	Similar to previous condition but with severe fibrotic destruction. Main characteristic is a major loss of lung function.

Chronic cavitary pulmonary aspergillosis	One or more pulmonary cavities that may or may not contain a fungal ball, with serological or microbiological evidence involving *Aspergillus* spp. in a non-immunocompromised patient.
Aspergilloma	Fungal balls that can develop in preexisting lung cavities without tissue or angioinvasion.
Non-invasive aspergillosis	Onycomycosis, keratitis, otomycosis or fungal sinusitis in an immunocompetent host.
Allergic forms of aspergillosis	The most common is allergic bronchopulmonary aspergillosis, followed by allergic sinusitis and severe asthma with fungal sensitization.

Table 3. Putative invasive pulmonary aspergillosis (all four criteria must be present) (adapted from reference 38)

1. *Aspergillus*-positive lower respiratory tract specimen culture.
2. Compatible signs and symptoms (one of the following). - 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 - 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 or 4b 4a. Host risk factors (one of the following conditions) - Neutropenia (absolute neutrophil count <500/mm3) preceding or at the time of ICU admission - Underlying hematological or oncological malignancy treated with cytotoxic agents - Glucocorticoid treatment (prednisone equivalent, ≥ 20 mg/day) - Congenital or acquired immunodeficiency 4b. Semiquantitative *Aspergillus*-positive culture of BAL fluid (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae.

Table 4. Characteristics of commercialized and standardized methods for *in vitro Aspergillus* antifungal susceptibility testing.

	Reading Results	Price	Time-consuming	Recommended use
Four-well plate*	Growth inhibition	$	No	Clinical screening
SYO	Colorimetric (MIC)	$$	No	Clinical practice
Etest®	Visual (MIC)	$$$	No	Clinical practice
EUCAST	Visual (MIC/MEC)	$$	Yes	Research
CLSI	Visual (MIC/MEC)	$$	Yes	Research

SYO, Sensititre YeastOne®; MIC: Minimum Inhibitory Concentration, MEC: Minimum Effective Concentration, EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical Laboratory Standard Institute

* Four well plates containing three azoles (itraconazole, voriconazole and posaconazole) and one control to screen for azole resistance. (Vipcheck, Netherlands).

Table 5: Causes of antifungal therapy failure in hematological patients with invasive aspergillosis

Causes of antifungal therapy failure	Management
Host factors	
• **Uncontrolled disease** • **Persistence of immunosuppression (prolonged neutropenia or steroids)**	• Adequate management of underlying disease • Reduce immunosuppression (specially steroids)
Non-accurate diagnosis	
• **Wrong diagnosis** • **Bacterial or viral coinfection or superinfection**	• Confirm the diagnosis of invasive aspergillosis if not done prior • Rule out coinfections or superinfections
Drug resistance	
• **Primary** • **Acquired (secondary)**	• Identification to species level • Azole susceptibility testing
Pharmacokinetic/pharmacodynamics issues	
• **Inadequate drug levels (azoles)** • **Drug interactions** • **Poor penetration at site of infection (CNS, sinus)**	• Confirm adequate doses of antifungal drug • Check potential interactions • TDM (azoles) • Need for surgical treatment
Immune reconstitution syndrome	
• **Clinical and radiological worsening coinciding with neutrophil recovery**	• Assess temporal relationship with neutrophil recovery • Exclude new extrapulmonary lesions of aspergillosis • Confirm serum GM titers decrease

CNS: central nervous system; TDM; therapeutic drug monitoring; GM: galactomannan.

Table 6. Different options of antifungal therapy in breakthrough invasive aspergillosis

Previous treatment or prophylaxis	First line	Alternative	Observations
Posaconazole	Liposomal amphotericin B	Liposomal amphotericin B + echinocandin Voriconazole + anidulafungin Isavuconazole	TDM before starting treatment Treat according to fungigram if possible
Voriconazole	Liposomal amphotericin B Voriconazole + anidulafungin	Liposomal amphotericin B + echinocandin Isavuconazole	TDM before starting treatment Treat according to fungigram if possible, deescalate to voriconazole if possible
Echinocandin	Voriconazole Voriconazole +anidulafungin	Liposomal amphotericin B Liposomal amphotericin B + echinocandin Isavuconazole	Treat according to fungigram if possible
Lipidic amphotericin B	Voriconazole Voriconazole +anidulafungin	Liposomal amphotericin B + echinocandin Isavuconazole Posaconazole	Treat according to fungigrama if possible

TDM: Therapeutic drug monitoring.

Table 7. Indications for adjunctive surgery in extra-pulmonary IA.

Organ involvement	***Recommended approach***
Large vessels and/or pericardium	Resection of the lesion
Pericardium	Pericardiectomy
Chest wall invasion associated with lung involvement	Chest wall resection (with later reconstruction if possible)
Empyema	Chest tube drainage, consider surgical drainage and thoracotomy (in case of fibrinopurulent o organized empyema)
Hemoptysis secondary to lung lesion	Resection of the lesion or embolization
Skin and soft tissue involvement	Debridement and resection with wide margins
Endocarditis	Device removal, excision of vegetation and resection of infected valves
Osteomyelitis	Debridement and cleaning of the affected tissue, with subsequent reconstruction (musculoskeletal grafts or bone grafts) if possible
Sinusitis	Cleaning, curettage and resection of affected tissues
Endophthalmitis or panophthalmitis	Vitrectomy, evisceration or enucleation. Consider intravitreal administration of antifungal agents

Table 8. Interactions between antifungal agents and other drugs

Antifungal agent	*Other drug*	*Resulting interaction*	*Suggested action*
Azoles			
Posaconazole	Cyclosporine, tacrolimus	Increased levels of the immunosuppressive drug[a]	**↓ tacrolimus dose by 2/3, ↓ Cyclosporine levels by 1/4, TDM of immunosuppressive drugs,**
	Other: mTOR inhibitors (sirolimus), rifabutina, midazolam; fenitoine, cimetidine, cumarins	Decresing Posaconazole levels	**Avoid combination when possible or use posaconazole TDM**
Voriconazole	Cyclosporine, tacrolimus,mTOR inhibitors.	Increased levels of the immunosuppressive drug[a]	**↓ tacrolimus dose by 2/3, ↓ Cyclosporine dose by 1/2, TDM of immunosuppressive drugs, avoid combination with mTOR inhibitors or sirolimus.**
	Fenitoine, carbamacepin, rifampin, rifabutin, fenobarbital and ritonavir	Decreseased levels of voriconazol	**Avoid combination when possible or use voriconazole TDM**
	Omeprazol, fenitoine, astemizol, cisapride, ergotamine, quinidine, terfenadine, cumarinic anticoagulants, statins, benzodiazepine, prednisone	Increased levels of this drugs	**Avoid combination when possible or use these drugs with caution and lower dose**
	Astemizole, cisapride, terfenadine, and quinidine. Should be used with caution: ciprofloxacin, cotrimoxazole, macrolide, antibiotics and conventional antipsychotics	Prolonged QT, risk of *torsades de pointes*	**Avoid combination**
Isavuconazole	Cyclosporine, Tacrolimus, Sirolimus		**TDM-based tacrolimus and cyclosporine dose. No empiric reduction while waiting for TDM required. Consider early dose reduction with sirolimus**
Echinocandins			
Caspofungin	Cyclosporine	Increased levels of caspofungin	**No adjustment. Monitor liver function**
	Tacrolimus	Decreased levels of tacrolimus (20%)	**No adjustment**

	Other: Efavirenz, nevirapina,fenitoin, rifampin, dexametasone and carbamacepin	Decreased level of caspofungin	
Micafungin	mTOR inhibitors	Increased levels of the immunosuppressive drug[a]	**No adjustment**
Anidulafungin	Cyclosporine	Increased levels of anidulafungin	**No adjustment**
Polyenes			
Amphotericin B (deoxycholate formulation)	Cyclosporine, tacrolimus	Markedly increased risk of nephrotoxicity	**Avoid other concomitant nephrotoxic drugs, TDM of immunosuppressive drugs, monitor renal function**
Amphotericin B (lipid formulations)	Cyclosporine, tacrolimus	Increased risk of nephrotoxicity	**Avoid other concomitant nephrotoxic drugs, TDM of immunosuppressive drugs, monitor renal function**

IA: invasive aspergillosis; mTOR: mammalian target of rapamycin; TDM: therapeutic drug monitoring.

[a] It takes approximately 1 week for the full magnitude of the interaction to be appreciated.

Table 9: Recommendations and rating for targeted therapy of IA in children

Drug, doses and comments	Rating
First line	
Voriconazole	**AI**
2 to >12 y/ 12-14 y< 50Kg	
16 mg/Kg/day iv in two divided doses	
day 1: 18 mg/kg/day iv in two divided doses	
18 mg/Kg/day po in two divided doses	
>15 y and 12-14 y >50Kg	
8 mg /Kg/day iv in two divided doses	
day 1: 12 mg/Kg/day iv in two divided doses	
400 mg/ day po in two divided doses	
Not approved in children <2y	
Trough concentrations should be 1- 5 mg/L	
TDM recommended	
Liposomal Amphotericin B	**BI**
3 mg/Kg/day iv in one dose	
Amphotericin B lipid complex	**BII**
5 mg/ Kg/ day in one dose	
Combined therapy	
Polyene or triazole plus echinocandin	**CIII**
Primary not routinely recommended	
Anidulafungin is not approved in children	
Posaconazole approved in >13 years of age	
Second line	
Voriconazole	**AI**
2nd line option for voriconazole naive patients	
Doses and TDM recommendation as above	
Liposomal amphotericin B	**BI**

2nd line option for patients not responding to or intolerant to voriconazole	
Caspofungin **3 months- 17 y: 50 mg/ m^2/day iv** **day 1: 70 mg/m^2/day** **in one single dose** **(maximum 70 mg/day)**	**AII**

Based on (23, 254, 272). Modified from (254) .

TDM: Therapeutic drug monitoring

Table 10: Pediatric populations and invasive aspergillosis incidence.

Patient population	Incidence of IA
Low birth infants and neonates	Sporadic occurrence (>5%)
Primary immunodeficiencies	
- Chronic granulomatous disease	
- Hyper IgE syndrome	High risk >10%
Acquired immunodeficiency	
- Acute and recurrent leukemia	High risk >10%
- Bone marrow failure syndromes	
- Allogeneic hematopoietic stem cell transplantation	
- Allogeneic hematopoietic stem cell transplantation and acute GVHD (2-4) or chronic extensive GVHD	
- Autologous stem cell transplantation	Low risk (5%)
- Acute lymphoblastic leukemia	
- Non Hodgkin´s lymphoma	
- Solid tumors and brain tumors	
- Hodgkin´s lymphoma	
- Solid organ transplantation	Sporadic (<5%)
- Advanced HIV infection	
- Immunosuppressive therapy	
- Acute illness or trauma	
- Chronic airway disease	

GVHD: graft versus host disease

HIV: Human immunodeficiency virus

Modified from several sources (272, 275-277)

Table 11: Recommendations and rating for various antimold compounds for the prophylactic setting in children.

Allogeneic SCT (during and immediately following conditioning until engraftment), absence of GVHD; prophylaxis may be continued after engraftment, until discontinuation of immune suppression and immune recovery	
Voriconazole	B I
Micafungin	C I
Caspofungin	CIII
Liposomal Amphotericin B	CIII
GVHD (acute II-IV or chronic extensive) treated with augmented immunosuppression	
Posaconazole + TDM	B I
> 13y	
Voriconazole +TDM	B I
De novo or recurrent acute myeloid Leukemia	
Posaconazole + TDM	B I
> 13y	
Itraconazole + TDM	B I
Liposomal amphotericin B	B II

Based on (254, 283). Modified from (45)

STC: Stem cell transplantation

GVHD: Graft-Versus-Host-Disease

TDM: therapeutic drug monitoring

Table 12. Indications for antifungal prophylaxis in SOT recipients.

Type of transplant	*Target population*	*Elective antifungal drug*	*Alternative antifungal drug*	*Duration*
Kidney	Prophylaxis not recommended (CIII)			
Liver	If one major or two minor criteria: • *Major criteria:* retransplantation, fulminant hepatic failure, requirement for renal replacement therapy • *Minor criteria:* high transfusion requirement (≥40 units of cellular blood products), renal failure not requiring replacement therapy (eGFR: <50 mL/min), choledochojejunostomy, early reintervention, multifocal colonization or infection by *Candida* spp.	Micafungin (AI) Anidulafungin (AI) Caspofungin (AII)	L-AmB (BII) ABLC (BII)	**For 2-4 weeks or until resolution of risk factors**
Pancreas, pancreas-kidney	All recipients	Fluconazole (AII)		**For 1-2 weeks**
	If one of the following criteria: ▪ Enteric drainage ▪ Requirement for renal replacement therapy ▪ Acute graft rejection ▪ Delayed graft function ▪ Surgical re-exploration ▪ Vascular graft thrombosis ▪ Postperfusion pancreatitis ▪ Anastomotic problems	Micafungin (AII) Caspofungin (AII) Anidulafungin (AII)	L-AmB (AIII)	**Until resolution of risk factors**
Heart	If one of the following criteria: ▪ Requirement for renal replacement therapy ▪ Acute graft rejection ▪ Surgical re-exploration ▪ CMV disease ▪ High levels of airborne *Aspergillus* conidia or another case of IA in the program within the 2 months before or after the procedure	Itraconazole (AII) Caspofungin (AII)	Voriconazole (BIII) Posaconazole (CIII)	**At least for 3 months or until resolution of risk factors**
Lung, lung-heart	All recipients	Nebulized L-AmB 25 mg: until resolution of bronchial suture: 3 times a week; 2 to 6 months: once a week; >6 month: once every 2 weeks (AII)	Nebulized ABLC: 50 mg every 2 days for 2 weeks, then 50 mg once a week (BII) Voriconazole (CII)	**Indefinite or at least for 12 months**
	If one of the following criteria (targeted prophylaxis): ▪ Induction with alemtuzumab or ATG ▪ Acute graft rejection ▪ Single-lung transplant ▪ Colonization with *Aspergillus* spp. prior to transplantation or during the first 12 months	Nebulized L-AmB: 25 mg 3 times a week for 2 weeks, then once a week (BII)	Voriconazole [initiate beyond the first month after transplantation] (BII)	**Until resolution of risk factors**

	▪ Severe IgG hypogammaglobulinemia (<400 mg/dL)			
Small bowel, multivisceral	All recipients	Fluconazole (AII)		**For 3-4 weeks or until healing of anastomoses**
	If one of the following criteria: ▪ Requirement for renal replacement therapy ▪ Acute graft rejection ▪ Delayed graft function ▪ Surgical re-exploration ▪ Anastomotic problems	L-AmB (AII) Caspofungin (AII) Micafungin (AII) Anidulafungin (AII)	ABLC (AIII)	**Until resolution of risk factors**

ABLC: amphotericin B lipid complex; ATG: anti-thymocyte globulin; CMV: cytomegalovirus; eGFR: estimated glomerular filtration rate; IgG: immunoglobulin G, L-AmB: liposomal amphotericin B; SOT: solid organ transplantation