

Section 1. Colombian consensus on the diagnosis and follow-up of invasive aspergillosis and *Aspergillus* disease in adult and pediatric patients*

*From the Colombian Association of Infectious Diseases (ACIN) Mycosis Group, for the Development of the Colombian Consensus on the Management of Invasive Fungal Disease

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Abstract

For a long time, the diagnostic approach to IA/*Aspergillus* disease has been a challenge. The definitive diagnosis is made by correct microbiological and/or histopathological documentation, undoubtedly the fundamental cornerstone for therapeutic decision-making. However, it is considered limited by lack of sensitivity and speed, often being counterproductive, as in many cases invasive procedures are needed (e.g., fibrobronchoscopy [FBC] or tissue biopsy), which delays treatment and undermines survival of at-risk patients. This highlights the need for faster and more accurate diagnostic tools. Although novel serological and molecular methods have been developed that have demonstrated their potential to replace conventional diagnostic tests, inconsistencies in interpretation and validation among the different approaches limit their reproducibility and large-scale clinical application. However, whenever possible, an immunological and/or molecular diagnosis should be made, as it has proven useful in different detection and diagnostic strategies, which with an understanding of its strengths and limitations, and the standardization of the different techniques available, can be incorporated into care protocols and diagnostic algorithms, as an aid in administering and monitoring the different antifungal treatments and predicting possible clinical outcomes. Due to the limited sensitivity (SE) of many of the tests, and variations in the specificity (SP) of some of them, the combined use of several diagnostic tools during the high-risk period for invasive infection and/or during the early period in the course of IA/*Aspergillus* disease would allow an adequate diagnostic approach, provided that their results are interpreted together with the existing clinical and imaging information.

Keywords: aspergillosis; *Aspergillus*; guidelines; diagnosis; *Aspergillus* resistance; azole resistance; antifungal resistance; galactomannan; *Aspergillus* PCR

Sección 1. Consenso colombiano para el diagnóstico y seguimiento de la aspergilosis invasora y de la enfermedad aspergilar en pacientes adultos y pediátricos*

*Del Grupo de Micosis de la ACIN, para el Desarrollo del Consenso Colombiano para el Manejo de la Enfermedad Fúngica Invasora

Resumen

Durante mucho tiempo la aproximación diagnóstica de una AI/enfermedad aspergilar ha sido un desafío. El diagnóstico definitivo, se lleva a cabo mediante la correcta documentación microbiológica y/o histopatológica, indudablemente la piedra angular que resulta fundamental para la toma de decisiones terapéuticas, sin embargo, se considera limitada por la falta de sensibilidad y rapidez, siendo a menudo contraproducente, ya que se requiere en muchos casos de procedimientos invasivos (ej., fibrobroncoscopia [FBC] o biopsia de tejido), lo que retrasa el tratamiento y socava la supervivencia de los pacientes en situación de riesgo. Esto pone de relieve la necesidad de herramientas diagnósticas más rápidas y precisas, y aunque se han desarrollado métodos serológicos y moleculares novedosos, que han demostrado su potencial para reemplazar las pruebas de diagnóstico convencionales, las inconsistencias en interpretación y validación entre los diferentes enfoques limitan su reproducibilidad y su aplicación clínica a gran escala. Sin embargo, un diagnóstico inmunológico y/o molecular debería realizarse siempre que sea posible, ya que ha demostrado su utilidad en diferentes estrategias de detección y diagnóstico, que con la comprensión de sus fortalezas y limitaciones, y la estandarización de las diferentes técnicas disponibles, pueden ser incorporados a protocolos de atención y algoritmos de diagnóstico, como una ayuda para administrar y vigilar los diferentes tratamientos antifúngicos y predecir los posibles resultados clínicos. Debido a la limitada sensibilidad (S) de muchas de las pruebas, y las variaciones en la especificidad (E) de algunas de ellas, el uso de varias herramientas diagnósticas disponibles en combinación, durante el período de alto riesgo para una enfermedad invasora y/o durante el período temprano en el curso de una AI/enfermedad aspergilar, permitirían una adecuada aproximación diagnóstica, siempre que sus resultados se interpreten de manera conjunta con la información clínica e imagenológica existente.

Palabras claves: aspergilosis; *Aspergillus*; guías de práctica clínica; diagnóstico; *Aspergillus* resistencia; resistencia a azoles; galactomannan; *Aspergillus* PCR

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Introduction

Aspergillus species cause a wide spectrum of diseases in humans whose clinical manifestation depend almost exclusively on the immune status of the patients. These diseases can be classified into three groups with different pathogenic mechanisms and clinical manifestations, although they have overlapping characteristics¹⁻³: (a) *Invasive aspergillosis (IA)*, which most frequently occurs in severely immunocompromised patients; it includes patients with acute leukemia (AL), chronic lymphoproliferative disorders, allogeneic hematopoietic stem cell transplant (HSCT) recipients and solid organ transplant recipients (SOTRs) (2-5), with an estimated annual incidence of >300,000 cases^{1,6-8}; (b) *Chronic pulmonary aspergillosis (CPA)*, which occurs in immunocompetent or mildly immunocompromised patients; it can manifest itself in different and sometimes overlapping clinical forms, from a fungus ball (aspergilloma) to an inflammatory and/or fibrotic process^{1,9}, with an estimated annual incidence of 3,000,000 cases⁹⁻¹¹; and (c) *Allergic bronchopulmonary aspergillosis (ABPA)*, which occurs almost exclusively in patients with asthma or cystic fibrosis (CF), where fungal sensitization elicits a robust hypersensitivity response, with about 4,800,000 cases diagnosed annually in asthmatic patients⁹⁻¹¹.

In Colombia there are different clinical therapeutic options for the management of patients with IA/*Aspergillus* disease, but there are no national guidelines to consult; little is known about the local epidemiological profile and the costs associated with the clinical-diagnostic approach to the disease.

The following are recommendations for the diagnosis, management, follow-up and prevention of IA/*Aspergillus* disease in adult and pediatric patients in a hospital or community setting, in hemato-oncological units and/or transplant units, in patients with human immunodeficiency virus (HIV/AIDS) infection and in critically ill intensive care unit (ICU) patients, including pediatric or neonatal intensive care unit (PICU/NICU) patients. The process for developing these recommendations included a systematic approach to rating the quality of evidence and the strength of each recommendation (Table 1)^{12,13}, and they contain a detailed description of the methods, background, and evidence summaries supporting each recommendation. This consensus has been reviewed and endorsed by the Colombian Association of Infectious

Table 1. Scale for measuring the quality of evidence and strength of recommendations.

Quality of evidence	
High (i)	The probability that the results will change is minimal.
Moderate (ii)	Results may change over time, but will not change dramatically.
Low (i)	The results can definitely change over time.
Strength of recommendation	
Strong	It is recommended to implement this recommendation in daily clinical practice.
Weak	It is recommended that before implementing this recommendation, the risks and benefits to the patient, as well as the costs or utilization of health resources, be evaluated.

Adapted from: Andrews JC *et al.*¹²

Diseases (ACIN). It is not intended to replace the clinical approach for individual patient management, but rather to be a guide to orient the diagnosis, treatment and prevention of IA/*Aspergillus* disease.

The convened experts were assigned in 4 working groups according to their experience, each one in charge of evaluating and preparing an independent document on a particular topic: I Diagnosis and Follow-Up Invasive Aspergillosis/*Aspergillus* Disease in Adult and Pediatric Patients; II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients; III Diagnosis and Treatment of Extrapulmonary Aspergillosis in Adult Patients; IV Diagnosis and Treatment of Chronic, Saprophytic and/or Allergic Syndromes Associated with *Aspergillus* spp. in Adult and Pediatric Patients. Leaders were selected for each working group, who served as executive secretaries. After completing the first round of assignments within the working groups, they presented the initial recommendations along with supporting evidence. The final draft of the 4 consensus texts was sent to all members for final approval.

Summarized below are the recommendations for the diagnosis and follow-up of invasive aspergillosis and *Aspergillus* disease. The whole documents "Colombian Consensus on the Diagnosis and Follow-Up of Invasive Aspergillosis and *Aspergillus* Disease in Adult and Pediatric Patients", "Colombian Consensus for Prophylaxis, Treatment and Prevention of Invasive Aspergillosis in Adult and Pediatric Patients", "Colombian Consensus on the Diagnosis and Treatment of Extrapulmonary Aspergillosis in Adult Patients" and "Colombian Consensus on the Diagnosis and Treatment of Chronic, Saprophytic and/or Allergic Syndromes Associated with *Aspergillus* Spp. in Adult and Pediatric Patients", which includes the methods, background and evidence summaries that support each recommendation can be found in the full text available in the online version.

For a long time, the diagnostic approach to IA/*Aspergillus* disease has been a challenge. The definitive diagnosis is made by correct microbiological and/or histopathological documentation, undoubtedly the fundamental cornerstone for therapeutic decision-making. However, it is considered limited by lack of sensitivity and speed, often being counterproductive, as in many cases invasive procedures are needed (e.g., fibrobronchoscopy [FBC] or tissue biopsy), which delays treatment and undermines survival of at-risk patients^{4,21,32,34,72,77-82}. This highlights the need for faster and more accurate diagnostic tools. Although novel serological and molecular methods have been developed that have demonstrated their potential to replace conventional diagnostic tests, inconsistencies in interpretation and validation among the different approaches limit their reproducibility and large-scale clinical application^{34,72,78-80}. However, whenever possible, an immunological and/or molecular diagnosis should be made, as it has proven useful in different detection and diagnostic strategies, which with an understanding of its strengths and limitations, and the standardization of

the different techniques available, can be incorporated into care protocols and diagnostic algorithms, as an aid in administering and monitoring the different antifungal treatments and predicting possible clinical outcomes^{4,21,80,81}. Due to the limited sensitivity (SE) of many of the tests, and variations in the specificity (SP) of some of them, the combined use of several diagnostic tools during the high-risk period for invasive infection and/or during the early period in the course of IA/*Aspergillus* disease would allow an adequate diagnostic approach, provided that their results are interpreted together with the existing clinical and imaging information⁷⁸⁻⁸⁰.

It is difficult to make a definitive diagnosis of IA/*Aspergillus* disease, due to the risk of fungal colonization and/or environmental contamination, and the low predictive value of cultures of the different clinical samples from the involved site (mainly respiratory tract samples). Currently, based on the evaluation of clinical signs and symptoms of the disease, and despite the large number of diagnostic tools available (direct microscopy, cultures, antibody [Ab] and/or antigen [Ag] detection, fungal DNA detection [PCR] and/or imaging studies), or the implementation of complementary tests such as *in vitro* antifungal susceptibility testing (AFST) and therapeutic drug monitoring (TDM) of antifungal agents to identify and manage the possible etiologic agent responsible for the infectious disease, uncertainty about their diagnostic performance persists in many cases^{4,21,34,81}. The EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study Group) developed a series of recommendations applicable in clinical, diagnostic and epidemiological research in a wide range of patients at high risk of developing an IFI/IA, which have recently been revised^{77,82}. These recommendations establish three diagnostic criteria: (a) proven infection, (b) probable infection, or (c) possible infection, where the proven IFI category can be applied to any patient, regardless of whether or not he or she is immunocompromised, and the probable and possible categories are proposed only for immunocompromised patients (with the exception of mycoses due to primary pathogens [endemic mycoses]). These three categories are established after analyzing three patient characteristics: (a) host factors (baseline condition), (b) clinical features (including imaging) and (c) mycological evidence (including microbiological/histological and/or serological/molecular documentation)^{4,21,32,34,72,77-82}.

Background

Epidemiology

Invasive aspergillosis in the immunocompromised patient

The reported overall incidence of patients with IA is >300,000 cases annually, with associated high mortality rates, although, due to the lack of global data, there is a wide range between the reported rates (30-95%)^{1-3,6-8}. The estimated number of cases reported in Colombia for the different clinical forms of aspergillosis is approximately 130,000 cases annually, with a higher disease burden in patients with respiratory diseases (mainly asthma or chronic obstructive pulmonary disease

[COPD]]¹⁰. In Colombia, the calculated number of IA cases is around 2,820 cases per year (5.7/100,000 inhabitants), of which 361 were found to be related to transplant recipients (especially HSCT), and 2,549 were found to be associated with patients hospitalized in the ICU. However, the numbers of IA and CPA cases may be underestimated, due to, among other factors: (a) absence of a mandatory registry of such disease, (b) low availability of diagnostic methods, and (c) lack of knowledge and/or low clinical suspicion or awareness¹⁰.

Since the late 1990s, invasive pulmonary aspergillosis (IPA) has surpassed IA as the most commonly diagnosed invasive fungal infection (IFI) from autopsies, with approximately 15-20% of patients with AL who die of fungal pneumonia caused by *Aspergillus* spp.¹⁴⁻¹⁶. Furthermore, due to the prevalence of the disease and its associated treatment, it has become the most expensive fungal disease in the hospital setting^{3,17}.

Several studies suggest that in HSCT patients, mortality rates associated with the development of IA are decreasing significantly, with a reported mortality of 35-57%^{3,6,11,18}. Although no major epidemiological studies have been carried out in recent years, all the data collected show that the situation has not changed much, so the reported morbidity and mortality rates are still valid today^{3,19}. Several surveillance studies have demonstrated in allogeneic HSCT patients a 12-month cumulative incidence for IA of 1.6-3.0%, and a ratio of the risk of invasive disease due to the type of transplant, with higher infection rates after: (a) transplant with HLA disparity, (b) unrelated donors, and (c) umbilical cord blood transplants^{6,11}. The onset of invasive disease has a bimodal distribution. Some cases manifest pre-graft, but most are associated with a second post-graft peak (> 40 days post-transplant), and strongly associated with the development of moderate to severe stage graft-versus-host disease (GVHD), which requires high doses of corticosteroids and/or administration of other immunosuppressive drugs^{11,20}. Numerous demographic and/or clinical factors have been identified as independent predictors of mortality in HSCT patients, such as: (a) male sex, (b) thrombocytopenia, (c) elevated bilirubin, (d) presence of pleural effusion at diagnosis, (e) prolonged corticosteroid use, (f) neutropenia, (g) uncontrolled GVHD, and (h) disseminated or central nervous system (CNS) disease¹¹.

Among SOTR patients, the average incidence for the development of an IA ranges from 0.1-11.6%, with a higher risk in small bowel (11.6%) and lung (8.6%) transplant recipients, followed by liver (4.7%), heart (4.0%), pancreas (3.4%) and kidney (1.3%) transplant recipients^{3,7,21}. In these patients, the incidence of cumulative invasive infection at 12 months is 0.7%, being more common in lung transplant recipients^{8,20}. In SOTR patients, the onset of IFI is variable, with a median onset of 6 months. However, all patients may develop IA after 3 years post-transplant^{7,11}. Early IA (half of the reported cases) occurs in the first 3 months post-transplant and in patients with post-surgical risk factors; late IA is more common in elderly patients and in very immunocompromised patients

due to rejection, post-transplant neoplasia and/or chronically impaired graft function²¹. Risk factors common to all SOTR patients independently associated with mortality include: (a) liver failure, (b) renal failure, (c) malnutrition, (d) thrombocytopenia, (e) cytomegalovirus (CMV) infection, (f) alveolar involvement, (g) disseminated or CNS disease, and (h) proven aspergillosis¹¹.

The overall incidence of IA in lung transplant recipients varies from 4-23%^{8,20}. In a global retrospective study of 900 lung transplant recipients, the rate of first episode development was 29.6/1000 person-years, with a median post-transplant disease onset of 7.7 months^{8,22}, and an incidence of 5% is reported in the pediatric population²³. The associated mortality rate varies according to the clinical presentation of the disease, ranging from 23-29% in patients with *Aspergillus* tracheobronchitis to 67-82% in patients with IPA²⁴. In recent studies, a 78% survival rate has been reported⁸.

In liver transplant recipients the incidence rate ranges from 1-9.2%^{8,25}, and an incidence of 0.5% in the pediatric population²³. In recent cohort studies, the median time to onset of IFI/IA is > 100 days^{8,26}. Liver transplant recipients have the highest rate of disseminated disease with IFI by filamentous fungus (55%), leading to a mortality rate of 64%²⁷. Mortality rates remain high in patients who develop IA after liver retransplantation (82%), particularly in those who undergo retransplantation more than 30 days after the primary transplantation (100%)⁸.

The incidence of IA in heart transplant recipients varies from 1-14%²⁸, and in the pediatric population an incidence of 0.3% is reported²³. A prospective multicenter study that included 15 transplant centers established that the cumulative annual incidence of IFI post-heart transplantation was 3.4%, and IA accounted for 23% of all IFI cases⁸. Overall mortality in heart transplant recipients is 67%. However, a 38% decrease in mortality has been reported in recent years^{6,29}.

Approximately 43-80% of IA cases occur in patients without a diagnosis of hematologic malignancy, where the rate of invasive infection increases when patients are exposed to high concentrations of environmental spores (>25 colony-forming units [CFU]/m³), in specific hospitalization areas (30). The incidence of IA in patients admitted to the ICU varies between 0.3-5.8%, and infection is associated with an overall mortality that can exceed 80%, and an attributable mortality of 20%, where it is difficult to attribute mortality to a specific factor, due to the type of patient involved³¹. IA may affect 0.3% of patients diagnosed with liver cirrhosis, and both acute liver failure and advanced cirrhosis (mainly alcoholic hepatitis treated with corticosteroids) have been recognized as a risk factor for the development of IA, and due to the low level of suspicion, about 53% of cases are recognized *post mortem*. The presence of liver disease is independently associated with increased IA mortality²¹.

The incidence of IA in HIV/AIDS patients has decreased since the advent of highly active antiretroviral therapy (HAART) to 2.2 cases per 10,000/year, although mortality remains high (38%)²¹. The disease usually occurs in patients with low CD4+ cell counts and associated conditions (such as neutropenia, advanced cirrhosis, liver transplantation or corticosteroid therapy). No country has included estimates about the development of IA as a complication in HIV patients. However, an overall estimate of > 45,000 cases/year has been made in these patients^{1,15,21}.

Chronic forms of aspergillosis in immunocompetent and/or mildly immunocompromised patients

The actual incidence of CPA is unknown, as it is considered an under-recognized entity^{1,9}. In most countries, the number of CPA cases correlates with the number of patients diagnosed with pulmonary tuberculosis (TB), as the fungus can colonize and infect the cavities left in the lungs after pulmonary TB⁹. Annually, about 1,170,000 new cases are estimated as a complication of treated pulmonary TB within 12 months after the end of treatment against pulmonary TB^{32,33}, and with at least the same or even a higher number of cases associated with other pulmonary disorders (COPD, sarcoidosis, nontuberculous mycobacteria [NTM] infection, post-pneumothorax, ABPA, and rheumatoid arthritis)⁹. It is estimated that in Colombia, the diagnosis of CPA following pulmonary TB is approximately 2,000 cases/year, with an annual incidence of 458 cases (1/100,000)^{10,34}.

Untreated CPA is associated with high mortality, with about 450,000 deaths per year. There are several factors associated with an increased risk of mortality, such as: (a) NTM infection, (b) COPD, (c) pleural involvement, (d) cavitary lung disease, (e) presence of a fungus ball (aspergilloma), (f) respiratory distress, (g) low physical activity, and (h) low body mass^{9,33}.

Allergic forms of aspergillosis in the atopic patient

It is estimated that about 4,800,000 cases/year of ABPA are diagnosed in adult asthmatic patients (it is rare in children), and about 6,675 cases/year of ABPA are diagnosed in patients with CF (only in adults and in children older than 4 years), with about 6,500,000 cases/year (in adults only and probably rare in children) of patients with severe asthma with fungal sensitization (SAFS), and about 12,000,000 cases/year of patients diagnosed with fungal rhinosinusitis^{1,35}. It is estimated that in Colombia there is a high prevalence of ABPA (52,268 cases, 106/100,000 inhabitants) and SAFS (68,987 cases, 140/100,000 inhabitants), associated with respiratory disease¹⁰.

Risk factors associated with IA/Aspergillus syndrome

The most important determinant for the development of *Aspergillus* infection is the patient's ability to prevent invasion of the etiologic agent, and the invasive process may be favored by the inhalation of large amounts of environmental conidia (e.g., from constructions and/or demolitions), or when the specific clinical characteristics of the patient do not allow adequate clearance of the conidia^{11,36}. The classic risk

factors for the development of IA are: (a) profound and prolonged neutropenia, and (b) administration of high doses of corticosteroids or other drugs that produce severe cellular immunodeficiency^{4,5,21,36}.

A higher incidence of IA occurs in patients with hematologic malignancies, with the following clinical conditions: (a) profound (absolute neutrophil count [ANC]: <100 cells/ μ L) and prolonged (> 7 days) neutropenia, (b) significant deficit of cellular immunity as a consequence of chemotherapy or radiotherapy, (c) CMV infection, (d) diagnosis of GVHD, (e) treatment with corticosteroids, tumor necrosis factor (TNF)- α antagonists or alemtuzumab. Populations at high risk of developing an IFI/IAF are: (a) cord blood allogeneic HSCT patients or patients with HLA disparity, (b) HSCT patients in GVHD phase, and (c) patients with AL (myeloblastic or lymphoblastic) and myelodysplastic syndrome (MDS) in induction, reinduction or salvage (Table 2)^{6,20,36,37}. In recent years, other risk factors for the development of IA have been identified and are related to: (a) comorbidities present, (b) immunosuppressive treatment, (c) degree of environmental contamination, and (d) genetic predisposition, where the importance of the presence of different genetic polymorphisms (e.g., Mannose-binding lectin [MBL], Toll-like receptor [TLR4-2], dectin-1, plasminogen, interleukin-10, pulmonary surfactant) has been recognized³⁶.

The RESITRA (Spanish Network of Infection in Transplantation) study³⁸, investigated the development of early-onset and late-onset IA in SOTR patients with different risk factors present, from 11 Spanish transplant centers (156 cases of

Table 2. Risk factors and patients at risk for an IA.

Risk factors
<ul style="list-style-type: none"> • Neutropenia. • Alteration of phagocytic capacity. • Decreased cellular immunity. • Use of corticosteroids and other immunosuppressive drugs. • Breaches of mucocutaneous barriers. • Environmental exposure (high concentration of conidia).
Population at risk
<ul style="list-style-type: none"> • Patient with neutropenia (ANC < 500 cells/μL, > 10 days): AML, MDS, allogeneic HSCT. • Patients on immunosuppressive therapy for GVHD. • SOTR, mainly lung and heart. • HIV-infected patients without antiretroviral therapy and CD4 < 100 cells/mm³ • Patients with CGD. • Patients treated with adalimumab, alemtuzumab, infliximab or etanercept. • Non-hematology critically ill patients. • COPD patients on chronic corticosteroid therapy. • Patients with liver cirrhosis or advanced liver disease. • Patients with complex major surgery.

AML: Acute myeloid leukemia; ANC: absolute neutrophil count; CGD: Chronic granulomatous disease; COPD: Chronic obstructive pulmonary disease; GVHD, graft versus host disease; HIV: Human immunodeficiency virus; HSCT: Hematopoietic stem-cell transplantation; IA: invasive aspergillosis; MDS: Myelodysplastic syndrome; SOTR: Solid Organ Transplant Recipient. Adapted from: Pemán J et al.³⁷

proven or probable IA were included). The authors established that the risk factors present that favored the development of IA within 3 months post-transplantation (early onset) were: (a) use of vasoactive drugs, (b) prolonged ICU stay, (c) post-transplant renal failure requiring hemodialysis, (d) CMV infection, and (e) an episode of bacterial infection; and the independent risk factors, which favor the development of IA after 3 months post-transplantation (late onset), were: (a) advanced age, (b) renal failure, (c) CMV infection, (d) diagnosed bacterial infection, (e) chronic graft rejection and (f) neoplasia related to immunosuppressant treatment (Table 3)⁸.

In lung transplant recipients, airway colonization by *Aspergillus* spp. during the first 6 months post-transplant increases the risk of developing IA by 11-fold. The risk factors that have traditionally conferred an increased risk in this patient population are: (a) ischemia of the bronchial anastomosis, (b) single lung transplantation, (c) acquired hypogammaglobulinemia, (d)

bronchial prosthesis placement, and (e) CMV infection (Table 3)^{8,20,36}. The diagnosis of CF increases the risk of *Aspergillus* colonization before transplantation. However, the risk of IA in lung transplant recipients with CF has not been clearly established, and the association of respiratory viral infections with the development of invasive disease in this population has not yet been documented⁸.

A number of specific factors have been characterized in liver transplant recipients that significantly increase the risk of IA, including:^{8,20,21,36}: (a) CMV infection, and (b) need for dialysis and retransplantation³⁸. Liver retransplantation has been shown to increase the risk 30-fold, while the presence of renal failure is associated with a 25-fold increase in risk, and are considered risk factors in the early post-transplant period^{18,39}. Fortún et al.⁴⁰, established that CMV infection increased the risk of invasive infection 6 times after 100 days post-transplantation and Saliba et al.³⁹, established that a MELD (Model for End-stage Liver Disease) score greater than 30 was associated with an increased risk of developing IFI. Other associated risk factors in living donor liver transplant recipients are: (a) transplantation for fulminant liver failure, (b) CMV infection, and (c) prolonged ICU stay (Table 3)^{6,41}.

Risk factors for the development of IA in heart transplant recipients include: (a) bronchoalveolar lavage (BAL) isolation of *Aspergillus fumigatus*, (b) reoperation, (c) CMV infection, (d) post-transplant hemodialysis, (e) an episode of IA in any patient in the transplant program 2 months before or after heart transplantation, and (f) identification of *Aspergillus* coinidia in the ICU environment (Table 3)^{8,42}.

Among renal transplant recipients, an IA rate ranging from 0.7-4% has been reported⁴³. The following were identified as independent risk factors for early infection (within 3 months prior to diagnosis of IA/IPA): (a) previous diagnosis of COPD, (b) delayed graft function, (c) bacterial bloodstream infection, and (d) acute graft rejection (Table 3). Previous occurrence of post-transplant complications attributed to over-immunosuppression (e.g., pneumonia, pulmonary TB, CMV) have been identified as an independent risk factor for late IPA. High doses (> 3 g of methylprednisolone) and prolonged duration of corticosteroid therapy, and graft failure requiring hemodialysis, have been shown to be risk factors for post-renal transplant IA^{8,21}.

IA/IPA has also been described in apparently immunocompetent patients as a complication in the event of: (a) acute respiratory distress syndrome (ARDS), (b) COPD, (c) advanced AIDS, (d) liver failure, (e) surgery, (f) burns, (j) Influenza A (H1N1) infection, (h) pneumonia, (i) malnutrition, and (j) critically ill patients requiring admission to the ICU^{11,15,21}. In patients admitted to the ICU, especially in those diagnosed with COPD and/or receiving inhaled or systemic corticosteroid therapy, drug administration is the main associated risk factor, and similarly to cirrhotic or HIV+ patients, a late diagnosis is common. Individuals diagnosed with COPD who require

Table 3. Risk of IA in SOTR patients.

Type of transplant	Risk factor
Liver transplant recipients	
Early (0-3 months)	Re-transplantation Kidney failure, particularly if renal replacement therapy is required. Fulminant liver failure. MELD > 30 Re-operation with thoracic or intra-abdominal cavity.
Late (> 3 months)	CMV infection Creatinine > 3.3 g/dL
Lung transplant recipients	
	Single lung transplantation Early airway ischemia CMV infection Rejection and increased immunosuppression in the last 3 months, particularly in patients with CF. Pre-transplant <i>Aspergillus</i> colonization <i>Aspergillus</i> colonization within one-year post-transplant <i>Aspergillus</i> -positive culture of intraoperative material in CF patients. Acquired hypogammaglobulinemia (IgG <400 mg/dL)
Heart transplant recipients	
	<i>Aspergillus</i> colonization Airborne <i>Aspergillus</i> spores in the ICU Re-operation (thoracic) CMV infection Post-transplant hemodialysis Report of an IA episode in the program, 2 months before or after heart transplantation.
Kidney transplant recipients	
	Pre-transplant diagnosis of COPD Acute rejection episode in the last 3 months Graft failure High-dose and prolonged corticosteroid use

CF: Cystic Fibrosis; CMV: Cytomegalovirus; COPD: Chronic obstructive pulmonary disease; IA: invasive aspergillosis; ICU: Intensive Care Unit; MELD: Model for End-stage Liver Disease; SOTR: Solid Organ Transplant Recipient.

Adapted from: Husain S et al.⁹

corticosteroid treatment represent a group with a particularly high mortality²¹. Other risk factors associated with the manifestation of IA/IPA in patients admitted to the ICU include: (a) chronic heart failure, (b) treatment with broad-spectrum antibiotics, and (c) cumulative doses of corticosteroids^{21,44}.

It has been established that the development of viral pneumonia increases the susceptibility of patients to develop superinfections, both bacterial and fungal⁴⁵⁻⁴⁷. Respiratory viruses cause direct damage to the airway epithelium; this allows invasion of the etiologic agent, where viral infection impairs ciliary clearance and leads to local and systemic immune dysfunction and/or dysregulation. Although the degree of dysregulation associated with the development of ARDS is unknown, in some patients it presents as pronounced immunosuppression, which facilitates fungal superinfection and the subsequent development of IPA or influenza-associated pulmonary aspergillosis (IAPA), which is considered a common complication in many critically ill patients admitted to an ICU^{45,46}. With the current COVID-19 (severe respiratory syndrome caused by SARS-CoV-2 virus) pandemic, there are more and more reports about the development of COVID-19-associated pulmonary aspergillosis (CAPA), which has raised many questions about the possible worsening of the course of COVID-19 disease and as an additional factor contributing to high mortality⁴⁵⁻⁵³. In a prospective cohort⁵², that included 108 critically ill ARDS patients, a higher 30-day mortality was observed in those patients diagnosed with CAPA compared to patients without aspergillosis (44% vs. 19%), and it has been established that patients diagnosed with CAPA would have many baseline prognostic factors with negative effects on survival⁴⁶, and with the latest reports, a greater involvement by CAPA could still be seen if the etiological agent responsible is azole-resistant. (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients: Section: Targeted Antifungal Treatment of IA/IPA [COVID-19-associated pulmonary aspergillosis])⁴⁷.

In pediatric population, high risk of developing IA is associated with: (a) HSCT, (b) hematologic malignancies, (c) SOTS, (d) primary or acquired immunodeficiencies, (e) chronic granulomatous disease (CGD), and (f) mannose-binding lectin deficiency (Table 4)^{54,55}.

CPA differs from IA in that it develops when immune dysfunction is present; it differs from ABPA in that the latter occurs in the context of a patient with atopy (asthma) with immune hyperactivity^{34,56,57}. CPA affects apparently immunocompetent individuals, usually with a pre-existing lung condition that may be silent, and approximately 5% of cases without an underlying pulmonary or systemic disorder. Risk factors for developing CPA include: (a) pulmonary TB or NTM infection, (b) previous diagnosis of ABPA, (c) COPD, (d), sarcoidosis, (e) pneumothorax, (f) lung cancer, (g) previous thoracic surgery, (h) diabetes, (i) alcoholism, (j) ankylosing spondylitis, and (k) rheumatoid arthritis⁵⁶.

Table 4. Incidence of IA in pediatric population.

Patient population	Incidence of IA
Neonates and LBWNs	Sporadic (> 5%)
Primary immunodeficiencies	
Chronic granulomatous disease Hyper IgE syndrome	High risk > 10%
Acquired immunodeficiency	
Acute and recurrent leukemia Bone marrow failure syndromes Allogenic HSCT Allogenic HSCT and acute GVHD (2-4) or extensive chronic GVHD	High risk > 10%
Autologous HSCT ALL Non-Hodgkin Lymphoma Solid tumors and brain tumors Hodgkin Lymphoma	Low risk (5%)
SOTR Advanced HIV infection Immunosuppressive therapy Acute illness or trauma Chronic airway disease	Sporadic (<5%)

ALL: Acute Lymphoblastic Leukemia; GVHD: graft-versus-host disease; HIV: human immunodeficiency virus; HSCT: hematopoietic stem cell transplantation; IA: invasive aspergillosis; SOTR: Solid Organ Transplant. Adapted from: Groll AH et al. (54), Tragiannidis A et al.⁵⁵, García-Vidal C et al.⁹¹

Aspergillus species distribution and antifungal resistance

Although more than 300 species of *Aspergillus* are known, only a small number cause opportunistic infections, and the manifestation of the different clinical forms varies depending on the species responsible (Table 5). Some *Aspergillus* species are more likely to cause infection, and some species have a greater predilection for certain hosts. However, the causative agent associated with any specific clinical setting is considered to be a dynamic process that can change by environmental factors and/or clinical practices^{11,58}.

The *A. fumigatus* complex is the species associated with the highest frequency of infectious disease, accounting for up to 90% of infections associated with this genus^{19,59}, both in individual sporadic cases and in those related to outbreaks, as if it were an infection acquired in the community or hospital setting¹¹. This prevalence is not only due to the ubiquity of the species in the environment, but also to the expression of its virulence factors (proteases, phospholipases, superoxide dismutases and toxins). In HSCT patients, the presence of specific genetic polymorphisms also contributes to the inability of the host to stop *A. fumigatus* invasion once it enters the lungs^{36,60}. IA caused by azole-resistant *A. fumigatus* isolates is associated with high mortality (> 50%)⁶¹.

In some environments, certain species are more prominent. The *Aspergillus flavus* complex, in a very distant second place in prevalence, is responsible for a significant number of ENT infections, with a clear tropism for paranasal sinuses. *Aspergillus terreus* complex is not frequently isolated, but is associated with invasive infection in immunocompromised

patients, with a high mortality rate, perhaps due to its innate resistance to amphotericin B (AmB). *Aspergillus niger* complex, which is rarely responsible for invasive infection, is most often found as an airway colonizer in patients with some underlying abnormality, frequently being a causative agent of CPA. *Aspergillus ustus* complex is considered to be an etiologic agent that is infrequently associated with infection; it is responsible for IFI in HSCT patients, and its isolates are usually resistant to the antifungal drugs of choice. The *Aspergillus nidulans* complex is usually a common species in CGD patients, and is more frequently isolated in the pediatric population (Table 5)^{60,62}.

Azole resistance among *Aspergillus* species may reflect an increase in the use of these drugs in prophylaxis or long-term treatment^{60,63}. However, the role of azoles and their environmental use, associated with increased antifungal resistance, has been investigated in global surveillance studies revealing that 3.2% of environmental isolates of *A. fumigatus* are resistant to one or more azoles, and are associated with the use of azole fungicides, which are widely used to protect crops^{8,63}. The variability in resistance reports of these isolates in different regions may reflect the difference in microbiological procedures for *A. fumigatus* isolation and resistance detection (Table 5)⁶⁰. The manifestation of secondary resistance of *A. fumigatus* is also a worldwide concern. Reports indicate that the most commonly implicated mechanism is modification of Cyp51A and its promoter (TR34/Leu98His; TR46/Tyr121Phe/Thr289Ala), and responsible for the manifestation of specific or pan-azole resistance, has been found in both clinical and environmental isolates in 22 countries. The formation of biofilms, due to the presence of extensive exopolysaccharide matrices, is also considered a factor for the development of resistance^{60,62}.

The most common *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger*) are species complexes, with several associated cryptic species. Such "cryptic" species represent a serious threat in clinical practice; taxonomic studies have identified new species that are almost morphologically indistinguishable by standard identification methods. Although the prevalence of cryptic species has been poorly investigated, studies conducted in the United States and Spain report that 10-15% of all species involved, with high minimum inhibitory concentrations (MICs) to antifungal drugs, do not yet have clinical cut-off points for interpretation, and are always associated with a poor prognosis^{60,62}.

Multiple *Aspergillus* species exhibit elevated MICs to different antifungal drugs, although their isolation in a clinical setting is considered uncommon (probably due to under-diagnosis)⁸. Some species of the *A. fumigatus* complex (*Aspergillus lentulus*, *Aspergillus fumigatiaffinis*, *Aspergillus viridinutans* and *Aspergillus pseudofischeri*) exhibit elevated MICs to azoles and/or AmB. The manifestation of resistance of *A. niger* complex species to azoles is isolate-dependent, being more common in *Aspergillus tubingensis*. Several species of the *A.*

Table 5. *Aspergillus* species and antifungal susceptibility.

Species	AmB	VCZ	PCZ	ITZ	CAS
Fumigati					
<i>A. lentulus</i>	R	R	V	R	S/V
<i>A. viridinutans</i>	R	R	S	R	S
<i>A. felis</i>	S	V	V	V	S
<i>A. pseudofischeri</i>	S	R	S	R	S
<i>A. fumigatiaffinis</i>	R	R	S	R	S
<i>A. udagawae</i>	V	V	S	S	S
<i>A. fumisynnematus</i>	S	S	S	S	S
<i>A. hiratsukae</i>	S	S	S	S	S
<i>A. fischerianus</i>	ND	ND	ND	ND	ND
<i>A. novofumigatus</i>	S	R	R	R	S
Flavi					
<i>A. flavus</i>	R	S	S	S	V
<i>A. alliaceus</i>	R	S	S	S	V
<i>A. tamarii</i>	V	S	S	S	S
<i>A. nomius</i>	R	S	S	S	S
Terrei					
<i>A. terreus</i>	R	S	S	S	V
<i>A. alabamensis</i>	R	S	S	S	ND
<i>A. hortai</i>	R	S	S	S	S
Nigri					
<i>A. niger</i>	S	S	S	V	S
<i>A. tubingensis</i>	S	S	S	V	S
<i>A. awamori</i>	S	ND	S	ND	ND
<i>A. brasiliensis</i>	S	S	S	R	ND
Nidulantes					
<i>A. tetrazonus</i>	S	S	S	S	R
<i>A. nidulans</i>	V	S	S	S	V
Versicolores					
<i>A. versicolor</i>	R	S	S	V	S
<i>A. sydowii</i>	R	S	S	V	S
Usti					
<i>A. ustus</i>	V	V	R	R	R
<i>A. calidoustus</i>	V	R	R	R	V
<i>A. insuetus</i>	R	R	R	R	ND
<i>A. pseudodeflectus</i>	V	R	R	R	V
<i>A. keveii</i>	R	R	R	R	ND
Circumdati					
<i>A. persii</i>	R	S	S	S	ND
<i>A. ochraceus</i>	R	S	S	S	S
<i>A. westerdijkiae</i>	R	S	S	S	S

+ For practical reasons, for PCZ, MIC: ≥ 0.25 mg/L is considered resistant; for AmB, ITZ and VCZ, MIC ≥ 2 mg/L is considered resistant. AmB: Amphotericin B; VCZ: Voriconazole; PCZ: Posaconazole; ITZ: Itraconazole; CAS: Caspofungin; S: Susceptible; R: Resistant; V: Variable; ND: No data. Adapted from: Samson RA et al.⁵⁸, Gautier M et al.⁶²

ustus complex exhibit elevated MICs to azoles and other antifungal drugs, and are considered multidrug resistant (62). Certain species of the *A. terreus* complex are typically resistant to polyenes, but are susceptible to voriconazole (VCZ)^{8,61}.

Diseases caused by *Aspergillus*

Depending on the immune status of the patient, *Aspergillus* spp. associated diseases can be roughly classified into three groups with different pathogenic mechanism, clinical manifestations, and overlapping features (Table 6).

Invasive forms of aspergillosis in the immunocompromised patient

IA is a severe and aggressive fungal disease that occurs in severely immunocompromised patients. In these patients, the growth of *Aspergillus* spp. in the lung leads to tissue destruction, angioinvasion, a septic state in its final stage, and sometimes the manifestation of hemoptysis^{1,5,32}. Most IA cases arise sporadically, and a specific point source of infection is never found, consistent with the ubiquitous nature of *Aspergillus* species (Table 6)^{11,32}.

Clinical manifestations of aspergillosis vary from asymptomatic colonization to invasive presentation including sinusitis, tracheobronchitis, IPA and empyema. In most cases of IPA, clinical symptoms are subtle (cough, chest pain, fever), and the presence of secondary acute respiratory failure is rare^{8,32}. Once germination of conidia occurs in the lung, *Aspergillus* hyphae invade pulmonary arterioles and lung parenchyma, leading to ischemic necrosis^{3,14,32}. Hematogenous dissemination associated with thrombosis, hemorrhagic infarction, invasion and organ involvement (mediastinum, paranasal sinuses, CNS, endocardium, musculoskeletal system, organ-specific [eye, kidneys, liver, spleen], and disseminated forms) may result from invasion of arterioles by *Aspergillus* hyphae^{4,5,8}.

In lung transplant recipients, IA develops in a distinct and almost exclusive way, as tracheobronchial aspergillosis (TBA) (affecting 4-6% of patients), characterized by necrotizing infection of the bronchial anastomosis, which can lead to airway obstruction, bronchial ulceration and pseudomembrane formation⁸. Ulcerative tracheobronchitis is the most aggressive form of invasive bronchial aspergillosis (IBA). It is characterized by the presence of endobronchial plaques, nodules or areas of ulceration and necrosis, which may extend into the adjacent lung parenchyma and pulmonary vasculature. (Tables 6 and 7)^{3,32}.

Chronic forms of aspergillosis in the immunocompetent patient

Aspergillus spp. can lead to chronic non-invasive forms with overlapping clinical features, ranging from development of a fungus ball (aspergilloma) to a chronic inflammatory and fibrotic process (Tables 6 and 7)^{3,9,34,57}.

The pathogenesis of a fungus ball (aspergilloma) usually involves fungal colonization and proliferation of the fungus

in a preexisting pulmonary cavity (most commonly due to a history of pulmonary TB), and up to 20% of patients who recover from cavitary tuberculosis develop a fungus ball (aspergilloma) within 3 years. An aspergilloma may also complicate a wide spectrum of cavitary lung diseases (sarcoidosis, other fungal infections, other chronic cavitary lung diseases), and about 50-90% of patients develop mild, self-limited hemoptysis, which occasionally can be massive or even fatal^{3,57}.

Other chronic, inflammatory forms associated with *Aspergillus* have been recognized in patients with chronic cavitary lung disease. These are characterized by an indolent clinical course (months to years) and are considered very complex and heterogeneous diseases, which have been classified into: (a) chronic necrotizing pulmonary aspergillosis (CNPA), (b) chronic cavitary pulmonary aspergillosis (CCPA), and (c) chronic fibrotic pulmonary aspergillosis (CFPA) (Tables 6 and 7)^{32,57}.

Allergic forms of aspergillosis in the atopic patient

The most severe clinical form of aspergillosis among atopic patients is ABPA, which develops following sensitization to *A. fumigatus* allergens in a unique subset of atopic individuals: (a) patients with asthma, (b) patients with CF, or (c) individuals with a genetic predisposition to develop the disease (Table 6)^{3,35}.

Methodology

Panel composition

A multidisciplinary panel of 23 specialists (infectious diseases, critical care, pulmonology, hematology, internal medicine, diagnostic imaging, mycology and epidemiology) from all over Colombia, experts in the care of adult, pediatric and neonatal patients with fungal infection, affiliated to the ACIN and experts from other recognized scientific societies, was convened for this consensus. All panel members were selected based on their expertise in IFI research, diagnosis, treatment and follow-up.

Overview of the process

The consensus work plan was created using the RAND/UCLA method, based on scientific evidence and the collective judgment of a panel of experts⁶⁴. A series of questions was created considering the critical factors that shape decision making in patients with *Aspergillus* spp. associated disease. All panel members were asked to review recent literature on at least one consensus topic to evaluate the evidence, determine the strength of the recommendations, and develop written evidence to support these recommendations. The panel reviewed and discussed all published recommendations, their strength, and the quality of the corresponding evidence. Discrepancies associated with the presentation of evidence were discussed and resolved as a team, and all final recommendations represent the consensus opinion of the entire expert panel. For the final version of the consensus, the expert panel, as a group, reviewed all the individual sections.

Table 6. Diseases caused by *Aspergillus* spp.

Invasive forms of aspergillosis	
IPA	It is considered the most severe infectious form, and usually occurs in severely immunocompromised patients. In the setting of: (a) hematologic patients with prolonged and profound neutropenia, (b) HSCT recipients, or (c) SOTR. The clinical manifestations of an IPA are similar to those of other pathogens that cause pneumonia, although it usually progresses rapidly, evolving over a period of days. Persistent fever despite broad-spectrum antibiotic treatment is often the first symptom that generates a strong suspicion of the disease, although fever may be absent in patients receiving corticosteroids. Other symptoms include cough, which may be productive, dyspnea, hemoptysis and pleuritic pain. Occasionally, in a more diffuse form, the patient presents with hypoxia with rales and pleural friction rub. Manifestations of angioinvasion and tissue infarction, with hemoptysis and pleuritic pain, are late findings.
Sinusitis	<i>Acute invasive sinusitis</i> mainly affects highly immunocompromised patients, and is characterized by rapid progression. In the context of: (a) neutropenic patients or, (b) Allo-HSCT recipients, being rare outside this context. Clinical signs and symptoms include fever, facial pain, nasal congestion, nasal discharge, epistaxis, nasal crusts, nasal ulcers and the presence of a necrotic anesthetic slough in the nose or on the palate. It often spreads to contiguous tissues (similar to rhino-orbital mucormycosis). <i>Chronic invasive sinusitis</i> is more frequent in immunocompetent patients (diabetes, corticosteroid treatment, HIV/AIDS, among others), and is characterized by the presence of a mass within the sinuses which is comprised of friable, necrotic or purulent material. With no specific clinical signs or symptoms, although the orbital apex syndrome is particularly characteristic.
IBA	<i>Pseudomembranous tracheobronchitis</i> occurs in several clinical settings including: (a) lung transplantation, (b) heart and lung transplantation, (c) post-Influenza, (d) hematologic malignancy, (e) HSCT recipients, (f) COPD, and (g) metastatic renal cell carcinoma. It can be clinically silent, with progressive invasion into the airway lumen leading to bronchial obstruction, distal atelectasis or lobar collapse; it manifests clinically as stridor, wheezing, respiratory failure, and finally death. <i>Ulcerative tracheobronchitis</i> usually occurs in the first six months after lung transplantation, where the bronchial anastomosis is the usual site of involvement. It has also been observed in a limited number of other clinical settings such as: (a) HIV/AIDS, (b) solid tumors, and (c) ICU patients with COPD. In these patients, the dyspnea, cough and mucus plug expectoration component predominates.
Extrapulmonary aspergillosis	The different extrapulmonary forms occur in severely immunocompromised patients or patients with different degrees of immunosuppression. Angioinvasion may occur in the context of a disseminated lung infection (e.g. CNS or cutaneous), or as a single organ infection, mainly due to direct inoculation (sinus, tracheobronchial, and less frequently, endocarditis, osteomyelitis, endophthalmitis, peritonitis). Extrapulmonary symptoms may provide clues to the diagnosis of disseminated disease: (a) sinus pressure, facial pain and purulent drainage may be indicative of sinusitis, (b) if it affects the orbit, may be associated with ocular symptoms (blurred vision, proptosis, ecchymosis, oculomotor palsy or blindness due to thrombosis of the central retinal artery), (c) neurological symptoms associated with CNS involvement may involve cognitive impairment, focal deficit or seizures, (d) cutaneous aspergillosis is usually primary, following local inoculation, open trauma, vascular catheter, burns, contaminated dressings, etc., and rarely occurs in the context of disseminated infections, and (e) gastrointestinal aspergillosis may produce local invasion and express itself as typhilitis, colonic ulcers, abdominal pain, and/or intestinal bleeding.
Chronic and/or saprophytic forms associated to <i>Aspergillus</i>	
CNPA (or SAIA)	It occurs in mildly immunocompromised or very weakened patients, with a duration of symptoms of about 1 to 3 months, and clinical and radiological features similar to CCPA, although it usually has no complications, can produce pneumothorax, aspergillomas or even an IPA. Its time course differs from IA, in which the rate of progression depends on the degree of immunosuppression. It occurs in the context of patients with: (a) advanced age with previous pulmonary disease, (b) COPD, (c) diabetes mellitus, (d) malnutrition, (e) alcoholism, (f) prolonged administration of corticosteroids or other immunosuppressive drugs, (g) connective tissue disorders, (h) radiation therapy, (i) NTMB, or (j) HIV.
CCPA	It occurs in subtly immunocompromised patients; symptoms last at least 3 months, in which fungal eradication is poor. It is characterized by the presence of multiple lung cavities, usually in the upper lobes, possibly containing one or more aspergillomas or irregular intraluminal material, which, if they progress, lead to CFPA. They occur in the setting of patients with: (a) TB, (b) ABPA, (c) resolved lung cancer, (d) pneumothorax with bullae formation, (e) COPD, and (f) fibrocavitary sarcoidosis.
CFPA	It is a complication of untreated CCPA, whose main characteristic is a significant loss of lung function due to severe fibrotic destruction.
Simple aspergilloma	Simple aspergilloma is a single immunologically protected fungus ball within a lung cavity; such a cavity may be pre-existing (from TB, sarcoidosis, histoplasmosis or bronchiectasis) or created by <i>Aspergillus</i> colonization; it may also involve the paranasal sinuses (in older persons with some pre-existing sinus abnormality, resulting in headache, rhinorrhea and post-nasal discharge). It is usually asymptomatic or presents mild symptoms that progress slowly for more than 3 months, and without radiological progression during the months of observation. Some may present with hemoptysis, bacterial superinfection or tissue invasion.
<i>Aspergillus</i> bronchitis	It is a rare disease characterized by the persistence of bronchitis symptoms for at least one month, with positive fungal cultures for <i>Aspergillus</i> spp. Respiratory symptoms include dyspnea, cough and expectoration, which may be copious. Although it can occur in immunocompetent patients, it is often associated with underlying pulmonary comorbidity or weakly attenuated immune states.
<i>Aspergillus</i> -associated allergic forms	
ABPA	ABPA is caused by an exaggerated hypersensitivity reaction to antigens produced by <i>Aspergillus</i> species, most commonly <i>A. fumigatus</i> . The pathogenesis of the disease is complex, where several immunological and genetic factors are involved that predispose to the disease. It presents in the context of: (a) immunocompetent patients with healthy lungs, (b) adult patients with underlying steroid-dependent asthma, and (c) patients with CF. The clinical symptoms lead to recurrent episodes of bronchial obstruction in asthmatic patients, with fever, malaise, expectoration of dark mucous plugs, eosinophilia, and occasionally hemoptysis. In chronic cases, pulmonary fibrosis may develop, with gradual loss of lung function.
AFRS	AFRS is not considered a true fungal infection, but rather the result of an inflammatory reaction due to the fungal presence in the sinonasal tract. Fungi colonize the sinonasal tract during the first months of life; however, only a few immunocompetent asthmatic patients develop the disease, with sensitization to <i>Aspergillus</i> or other fungal allergens, in the absence of clinical and radiographic evidence of ABPA. The pathogenesis of the disease is not fully understood.

IA: invasive aspergillosis; IPA: Invasive pulmonary aspergillosis; IBA: Invasive bronchial aspergillosis; HSCT: Hematopoietic stem-cell transplantation; SOTR: Solid organ transplant recipient; HIV: Human Immunodeficiency Virus; COPD: Chronic obstructive pulmonary disease; ICU: Intensive Care Unit; CNPA: Chronic necrotizing pulmonary aspergillosis; SAIA: subacute invasive/chronic necrotizing/semi-invasive aspergillosis; CCPA: Chronic cavitary pulmonary aspergillosis; CFPA: Chronic fibrosing pulmonary aspergillosis; TB: Pulmonary tuberculosis; NTMB: Non-tuberculous mycobacteria infection; CF: Cystic Fibrosis; ABPA: Allergic bronchopulmonary aspergillosis; AFRS: Allergic fungal rhinosinusitis.

Adapted from: Wilopo BAP et al. (9), Gregg KS et al. (11), Hope WW et al. (32), Denning DW et al. (34), García-Vidal C et al. (81), Page ID et al. (86), Muldoon EG et al.¹⁰².

Review of the evidence

To assess the quality of the evidence and the strength of the recommendations, the modified GRADE methodology^{12,13} was used. It assigns each recommendation with separate ratings for the underlying quality of the evidence supporting the recommendation, and for the strength with which the recommendation is made, establishing the following levels of evidence: LOW (III): results may definitely change over time; MODERATE (II): results may change over time, but will not change dramatically; HIGH (I): the likelihood that the results will change is minimal. The strength of the recommendation (STRONG OR WEAK) was evaluated taking into account the balance between benefits and risks, quality of evidence, patient values and preferences, and cost or resource utilization⁶⁵. The quality of the evidence was evaluated using the AGREE II instrument⁶⁶⁻⁶⁹, including as a substrate for consensus the guidelines selected with an average score of the evaluated domains higher than 60%. (Annexes 1 and 2)⁷⁰. With the selected guidelines and consensus, a document was prepared issuing recommendations to the questions asked. The panel met once and held a series of videoconferences over a 10-month period, in which the recommendations were individually scored using the modified Delphi methodology⁷¹, with two rounds of voting (anonymous and open). Consensus was established by an agreement of more than 75% of the expert panel for each recommendation. (Annexes 1 and 2).

Systematic reviews

A literature search for clinical practice guidelines for *Aspergillus* spp. diseases and aspergillosis guidelines integrating recommendations for the different groups of the consensus target population (adult, pediatric and neonatal) was conducted using sources from compiling agencies (NGC, National Guideline Clearinghouse, Guideline International Network), producers of clinical practice guidelines (New Zealand Guidelines Group, National Institute for Clinical Excellence, Scottish Intercollegiate Network), Ibero-American clinical practice guidelines and general databases (Pubmed, Medline, EMBASE). Se utilizaron los siguientes términos MESH: aspergillosis, *Aspergillus*, guidelines, invasive aspergillosis, chronic pulmonary aspergillosis, *Aspergillus* allergic and noninvasive Infectious, ABPA, aspergilloma, *Aspergillus* fungal ball, chronic cavitary pulmonary aspergillosis, subacute invasive aspergillosis, *Aspergillus* surgery, extra pulmonary aspergillosis, *Aspergillus* central nervous system, *Aspergillus* endocarditis, *Aspergillus* sinusitis, *Aspergillus* osteomyelitis, *Aspergillus* endophthalmitis, oncologic patients, haematologic patients, neutropenia, diagnostic, *Aspergillus* diagnostic, *Aspergillus* resistance, TDM, antifungal exposure, azole resistance, antifungal resistance, tests galactomannan, Glucan, BDG, *Aspergillus* LFD, *Aspergillus* PCR, *Aspergillus* breakthrough fungal infection, nonneutropenic, SOT (lung, renal, kidney, liver, intestinal), diagnostic approach for IA, ICU patients, *Aspergillus* antifungal prophylaxis, secondary prophylaxis, antifungal agents, Invasive aspergillosis *Aspergillus* empirical antifungal therapy, *Aspergillus* diagnostic driven antifungal therapy, galactomannan screening, antifungal treatment, *Aspergillus*

therapy, *Aspergillus* adjuvant therapy, *Aspergillus* combination therapy, *Aspergillus* treatment, voriconazole, posaconazole, isavuconazole, caspofungin, micafungin, andiflulafungin, amphotericin B.

Only guidelines published after 2012 and topic-specific reviews from 2010 onwards were taken into account.

Conflicts of interest

The expert panel complied with the international conflict of interest policy, which requires disclosure of any financial or other interest that could be construed as an actual, potential or apparent conflict. All panel members received the Conflict-of-Interest Disclosure Statement and were asked to clearly identify ties to companies developing products that may be affected by the enactment of the consensus. Information on employment, consultancies, stock ownership, fees, research funding, expert testimony and membership on advisory committees of these companies was also requested. Potential conflicts of interest are listed in the Annexes section. (Annexes 3 and 4)

Consensus review and approval process. The panel asked 2 external reviewers, international experts on the specific topic of consent, to review it and give their comments. The guide was reviewed and approved by all panel members before it was released.

Future consensus reviews. Annually, the panel leaders will be asked for their opinion on the need to update the guide based on an evaluation of the current literature; based on this consideration, the need and timing of an update will be determined. If deemed warranted, the full panel of experts or a subset of the panel will be convened to discuss possible changes.

QUESTIONS

1. **In a patient with suspected IA/*Aspergillus* disease, how is the conventional diagnostic approach performed?**

Recommendation

1. *The consensus considers that in the patient with a high suspicion of developing IA/*Aspergillus* disease, a direct microscopic examination of the involved site is a rapid and accessible tool that allows an initial diagnostic approach. It is considered that this makes it possible to establish whether it is an invasive infection, a fungal colonization, or an environmental contamination. (strong recommendation, high-quality evidence)*^{4,21,32,34,72-82}.

Invasive infection

2. *To confirm or rule out invasive *Aspergillus* spp. infection in a patient with a high suspicion of developing an IFI, the consensus recommends that, whenever possible, a histopathological study of biopsy and/or sterile body fluid from*

the involved site be performed. Evidence of angioinvasion and the presence of septate hyphae, associated with necrosis, and/or hemorrhage of the surrounding tissue is considered suggestive of an IF/IA. **(strong recommendation, high-quality evidence)** (Tables 6 and 7)^{4,21,32,79–82}.

3. In a patient with a high suspicion of developing an IF/IA, the consensus recommends performing a diagnostic approach using optical brighteners (Calcofluor white™, Uvitex 2B, Blancophor™), and/or Gomori silver methenamine (GSM) and Periodic Acid Schiff (PAS) stains, for direct microscopic examination from fresh clinical specimens and/or tissue sections from the involved site. **(strong recommendation, high-quality evidence)** (Table 7)^{4,21,32,72–74,79–82}.
4. It is considered that in a patient with a high suspicion of developing an IF/IA, the use of immunohistochemical techniques, through the use of monoclonal antibodies (acs) (WF-AF-1 or EB-A1) and in situ hybridization, allow the identification of possible fungal elements from tissue sections of the involved site. **(weak recommendation, low-quality evidence)**^{21,75,76,83,84}.

Aspergillus disease

5. The consensus considers that in a patient with a high suspicion of developing an Aspergillus disease, a direct microscopic examination from a biopsy of the involved site allows the diagnosis of a chronic and/or allergic form associated with *Aspergillus* spp. **(strong recommendation, moderate-quality evidence)**^{32,34,72,85}.
6. It is considered that in a patient suspected of developing a chronic and/or allergic form associated with *Aspergillus* spp. the presence of septate hyphae within the cavity, without evidence of involvement of the pulmonary parenchyma and/or circulating tissue, is highly suggestive. **(strong recommendation, moderate-quality evidence)** (Table 7)^{32,34,72,85}.
 - a. In a patient with suspected IA/*Aspergillus* disease, how are the involved *Aspergillus* species identified?

Recommendation

7. The consensus considers that in a patient at high risk of developing an IA/*Aspergillus* disease, direct microscopic examination and mycological culture from samples from involved sites and/or sterile body fluids allow an adequate diagnostic approach. **(strong recommendation, high-quality evidence)**^{3,9,32,72–76,83–94}.
8. The consensus is that the recovery of an *Aspergillus* species from a biopsy culture and/or sterile body fluid and/or from the involved site may be suggestive of: (a) an invasive process, (b) a chronic process, (c) an allergic syndrome, or (d) fungal colonization or environmental contamination. **(strong recommendation, high-quality evidence)**^{92–94}.
9. The consensus recommends identifying the *Aspergillus* species involved in an IA/*Aspergillus* disease through gross

examination and microscopic examination from primary cultures. The use of special identification media (malt extract agar 2% and/or Czapek-Dox agar), incubated at 25–30°C, 37°C and 50°C is recommended. **(strong recommendation, moderate-quality evidence)**^{4,21,34,77–80}.

Invasive infection

10. The consensus considers that in a high-risk immunocompromised patient, recovery of an *Aspergillus* species by mycological culture from a biopsy and/or sterile body fluid from the involved site allows the diagnostic approach of a proven/probable IA. **(strong recommendation, high-quality evidence)** (Annex 5)^{4,21,32,34,77–82,93}.
11. The consensus considers that in a high-risk immunocompromised patient, the demonstration of colonization by an *Aspergillus* species increases the risk of developing subsequent invasive disease. **(strong recommendation, moderate-quality evidence)**^{86–95}.
12. It is considered that in an immunocompetent or mildly immunocompromised patient at high risk of an IFI, direct microscopic examination and mycological culture allows a diagnostic approach of a proven/probable IA. **(strong recommendation, moderate-quality evidence)** (Annex 5)^{21,34,77,93}.

Aspergillus disease

13. The consensus considers that in an immunocompetent or mildly immunocompromised patient, the recovery of an *Aspergillus* species by mycological culture from clinical samples of the respiratory tract (induced sputum, tracheal aspirates, BAL, etc.), allows an adequate diagnostic approach of a chronic and/or allergic form associated with *Aspergillus* spp. **(recomendación fuerte, moderada de la evidencia)**^{32,34,93}.
 - i. What is the clinical significance of the isolation of an *Aspergillus* species from a clinical specimen from the respiratory tract?

Recommendation

14. The consensus recommends that in a patient at high risk of developing an IA/*Aspergillus* disease, mycological cultures from clinical samples of the respiratory tract (induced sputum, tracheal aspirates, BAL, etc.) are routinely performed in order to recover the possible etiological agent involved in the infectious process. **(strong recommendation, moderate-quality evidence)**^{8,20,21,32,58–60,62,81,93–109}.
15. The consensus considers that in order to perform an adequate diagnostic approach without a previous positive microscopic examination in a patient with suspicion of an invasive process, a chronic process, or an allergic syndrome associated with *Aspergillus* spp, the patient's risk and predisposing factors must be taken into account, together with the isolated species and its ability to grow at 37°C. **(strong recommendation, high-quality evidence)**^{19,21,36,56,57,81,84,93,110–112}.

Table 7. Pathological and imaging findings in diseases caused by *Aspergillus* spp.

Aspergillosis of the lower respiratory tract		
	Pathological findings	Imaging findings
IPA (angioinvasive)	Evidence of tissue plane disruption and vascular invasion by adhesion of surface components of fungal structures (including vascular wall components, basement membrane, extracellular matrix, and cellular constituents), associated with coagulative necrosis and hemorrhagic infarction. Fungal lesion (or fungal sequestration) and areas of distal wedge-shaped pulmonary infarction are manifestations of angioinvasion.	Imaging findings depend on the patient's characteristics, and a wide variety of nonspecific radiographic patterns may be present. X-ray may show peripheral opacities (ill-defined, 1-3 cm, gradually merging into larger opacities) with or without cavitation. The opacities may increase in size and become necrotic in their central part, which reduces their density and favors air trapping, producing the "air-crescent sign"; such cavitation occurs after neutrophil recovery, which is a sign of good prognosis. An early but non-specific finding on CT is the presence of nodular opacities with a ground-glass border "halo sign" (reflecting hemorrhage and edema surrounding the lesion), also the presence of peripheral opacities by complete alveolar occupation, wedge-shaped with a base towards the pleura which, in the appropriate clinical setting, are highly suggestive of angioinvasive aspergillosis. On multislice CT, a budding tree pattern can be seen. Pleural effusion and mediastinal adenopathies are rare. Invasion of the chest wall or mediastinal pleura may occur.
IPA (non-angioinvasive)	There is no evidence of vascular invasion by the fungal structures, with the presence of a pyogranulomatous inflammatory infiltrate, inflammatory necrosis or cavitation (occasionally a mixed histologic picture may be observed).	Almost any radiologic pattern may be present. Nonspecific abnormalities may be evident, including airspace disease, single or multiple nodular infiltrates (with or without halo sign), segmental or subsegmental consolidation, diffuse ground-glass opacities or cavitation. CT allows a better definition of halo and crescent signs.
Chronic forms of pulmonary aspergillosis (CNPA, CCPA, CFPA)	Presence of hyphal elements within a cavity, without evidence of parenchymal invasion (occasionally, direct hyphal invasion of the tissue is observed, which defines a non-angioinvasive IPA). In <i>CNPA</i> there is colonization of pre-existing spaces by hyphal elements, often with dilated airways, mucosal invasion and necrotizing granulomatous inflammatory reaction. The airway lumen often has a mixture of hyphal elements and necrotic debris. In <i>CCPA</i> , a discrete mass is present, with the presence of intertwined hyphal elements, mucus, fibrin and cellular debris that colonize a cavity. There is no evidence of tissue invasion, although necrosis is common, and multiple cavities form and expand over time.	The most suggestive features of CPA are the presence of a cavitated lesion, with nodular opacities in the upper lobe, progressive enlargement of new cavities and/or adjacent pleural thickening. Associated involvement of bronchial or non-bronchial systemic arteries and, less frequently, the formation of pseudoaneurysms, can lead to hemoptysis, sometimes fatal. In a <i>CNPA</i> there may not be a previous cavitated lesion. Generally, an area of consolidation is found in an upper lobe, progressing over days or weeks. The predominant characteristic is the presence of a thin-walled cavity, which expands during 1-3 months. It may present pleural thickening, presence of aspergillomas, pneumothorax and pleural effusion. The presence of the "air-crescent sign" is a sign of necrosis, indicative of worsening disease. In <i>CCPA</i> , unilateral or bilateral areas of consolidation are typically seen, associated with multiple thick-walled, usually expandable cavities, which may contain one or more aspergillomas, with variable pleural thickening. Thickened pleura is often associated with more evident extrapleural fat than normal, indicating chronicity. These findings are frequently asymmetric and predominantly located in areas with pre-existing abnormalities due to underlying lung disease. Radiologic evolution is usually slower and may take years. <i>CFPA</i> is the terminal fibrosing evolution of <i>CCPA</i> , and occurs when it remains untreated, resulting in extensive pulmonary fibrosis. Fibrosis may be limited to one or both upper lobes, but may also affect the entire hemithorax. There is no distinguishing feature of a CPA-related fibrosis other than the presence of cavitation and aspergillomas. Progression over time may provide a clue to the etiology of the fibrosis.
Aspergilloma	A conglomerate of intertwined hyphal elements is observed, mixed with fibrin, mucus, cellular debris and other blood products. There is no evidence of parenchymal invasion by hyphal elements.	An aspergilloma usually presents as a solid, round or oval upper lobe intracavitary mass, partially surrounded by a crescent of air, the mobile "air-crescent" sign. This finding can be demonstrated by acquiring the images in the supine and prone position (the aspergilloma often moves within the cavity). Calcification can be seen in the aspergilloma either extensively or as dense nodules. Adjacent pleural thickening is often seen, which may be the first radiographic sign, before visible mass-forming changes within a cavity.
ABI	It is an invasive disease that mainly affects the large airways, (bronchoscopically accessible). It is classified as: <i>Aspergillus tracheobronchitis</i> , in which there is tracheobronchial inflammation, with a mucus exudate containing hyphal elements of <i>Aspergillus</i> spp. with no other identifiable pathogen. The inflammation is superficial, the mucosa is intact, without pseudomembrane formation, deep focal ulceration or other focal endobronchial abnormalities. <i>Pseudomembranous tracheobronchitis</i> , in which there is necrosis and detachment of the bronchial epithelium, together with formation of a pseudomembrane containing necrotic debris and hyphal elements. The depth of infection is variable and there is superficial invasion, which does not extend beyond the bronchial cartilage. <i>Ulcerative tracheobronchitis</i> , in which there are single or multiple, discretely abnormal focal areas with endobronchial plaques, nodules or areas of ulceration and necrosis. The depth of the ulcer varies, and may extend into the adjacent lung parenchyma and pulmonary vasculature.	Generally, imaging findings are normal, although X-ray and CT scan may show airway wall thickening, presence of patchy opacities or centrolobular nodules, atelectasis and/or lobar collapse.

Aspergillosis of the upper respiratory tract		
	Pathological findings	Imaging findings
Acute invasive sinusitis	There is evidence of an acute and relatively sparse inflammatory infiltrate, with tissue infiltration by hyphal elements, angioinvasion and coagulative necrosis. It is locally more invasive, causes bone destruction, spreads to adjacent soft tissues and invades the pterygopalatine fossa, cavernous sinus and intracranial cavity. With vascular invasion and hematogenous dissemination.	Initially, noncontrast CT shows low-density mucosal thickening or soft tissue attenuation within the paranasal cavity, with a predilection for unilateral involvement of the ethmoid and the sphenoid cell. Aggressive bone destruction of the sinus walls can be rapid, and is associated with intracranial and intra-orbital extension. Bone erosion and mucosal thickening is sometimes subtle, as these fungi tend to extend along the vessels; extension beyond the sinuses may occur with intact bone walls. Intracranial extension of the disease from the sphenoid sinus leads to thrombosis of the cavernous sinus and even invasion. Severe unilateral thickening of the soft tissues of the nasal cavity is the earliest finding and the most consistent, although nonspecific, sign seen on CT. More extensive changes, such as inflammation of the periantral fat, erosion, and orbital or intracranial invasion are more specific but late and infrequent features. <i>MRI</i> is the imaging of choice to evaluate intracranial and intra-orbital extension, as it better characterizes inflammatory changes in the orbital fat, extraocular muscles and proptosis resulting from intra-orbital invasion. Obliteration of periantral fat is a subtle sign of such extension, and should be looked for in patients at risk. Leptomeningeal enhancement with intracranial invasion may be seen, which is subtle in the early stages of infection. With the progression of infection there may be adjacent cerebritis, granulomas and brain abscess formation. Intracranial granulomas are hypointense in T1 and T2, with minimal enhancement in the contrasted T1 sequence.
Chronic invasive sinusitis	Presence of a mass composed of friable, necrotic or purulent material, associated with a mixed cellular infiltrate, inflammatory necrosis and invasion of contiguous structures, such as the skull base, orbit and brain.	Noncontrast <i>CT</i> shows a pseudomass with hyper-attenuated (dense) soft tissue in one or more of the paranasal sinuses, with an infiltrative appearance (mimicking cancer) and destruction of the sinus walls, with extension beyond the paranasal cavities. <i>MRI</i> is preferred to evaluate the associated intracranial extension, which behaves isointense on T1 and hypointense on T2 and FLAIR, without diffusion restriction, with mottled appearance in the bone tissue due to irregular bone destruction.
Sinus aspergilloma	Presence of a fungus ball composed of cheesy and friable material and a conglomerate of hyphal elements in concentric circles. The antral mucosa is well preserved, with chronic non-granulomatous inflammatory response, and no evidence of bone erosion.	There is evidence of sinus opacification with one or more oval or rounded soft tissue dense images, called foreign bodies, concretions or antroliths, found centrally or towards the orifice of the antrum, without evidence of tissue invasion; there may be thickening or sclerosis of the sinus wall due to pressure, which may lead to necrosis.
Cardiovascular aspergillosis		
	Pathological findings	Imaging findings
Native valve endocarditis	Presence of large friable vegetations, mural vegetations, extension to paravalvular structure and development of pancarditis. Embolic events in the main vessels, such as the aorta, iliac and femoral arteries, are characteristic.	Endocarditis may be occult or manifest as valvular vegetations on images. Right-sided vegetations on the tricuspid or pulmonary valves may indicate a venous source of the infection, which can cause pulmonary septic embolism. Vegetations on the mitral and aortic valves are symptomatic and can cause downstream arterial embolic effects such as stroke or visceral infarction. Despite their larger size (compared to that of bacterial origin), these vegetations are difficult to visualize at CT and MR imaging, unless gated cardiac examinations are performed, which improves the anatomical resolution of the images displayed in 4 Ch, 3 Ch and 2 Ch cine sequences. Approximately 78% of patients with <i>Aspergillus</i> endocarditis have demonstrable vegetations on transthoracic echocardiography.
Prosthetic valve endocarditis	Probably associated with direct inoculation of fungal elements during surgery, or by seeding of the endocardium from the lungs in the perioperative period. There is evidence of vegetation causing prosthetic malfunction and valvular dehiscence.	Echocardiographic findings are non-specific, and include compromised mechanical valve function and seating, paravalvular leak and paravalvular abscess formation.
Pericardial aspergillosis	It may arise by contiguous dissemination from the pleura or myocardium. There is evidence of fibrinous or exudative pericarditis, or contiguous IPA, or myocardial aspergillosis. <i>Postmortem</i> findings demonstrate patchy, diffuse pericarditis, or the presence of multiple raised nodules or plaques on one or both pericardial surfaces.	Pericardial aspergillosis is not a frequent <i>ante mortem</i> diagnosis, but should be suspected when a patient with IPA develops pericardial effusion (suspected clinically or seen on imaging), or there is other evidence of pericardial disease. There is echocardiographic evidence of pericardial disease, including pericardial effusion and signs of pericardial tamponade, with a widened cardiac silhouette on chest X-ray.
CNS Aspergillosis		
	Pathological findings	Imaging findings
Cerebrovascular aspergillosis	Evidence of vessel invasion by scarce hyphal elements, cerebral infarction in vascular territory, necrosis and hemorrhagic transformation with little inflammatory response, without evidence of abscess or granuloma formation, and with contiguous involvement of the meninges.	The radiologic appearance is non-specific, and may be normal or near normal in the early stages of infection. CT and MRI features in a specific clinical setting may support the diagnosis. Imaging features include intraparenchymal and extraparenchymal (skull base) lesions with perilesional edema, basal meningeal enhancement, hydrocephalus and infarcts, with or without hemorrhage. In addition, imaging may provide evidence of concomitant pulmonary, sinus, orbital or mastoid involvement. Massive lesions may have a homogeneous enhancement (granuloma) or a peripheral ring (abscess). The presence of lesions at the base of the skull, with homogeneous enhancement associated with intrasinus, orbital or mastoid lesions, suggest the diagnostic possibility of <i>Aspergillus</i> infection.

Brain abscess	It may be the result of hematogenous seeding in the context of significant immunologic impairment. There is evidence of abscess formation, consisting of an area of central liquefactive necrosis in which hyphal elements are scarce or absent, and surrounded by an area of hemorrhagic necrosis with an acute inflammatory infiltrate interspersed with multiple hyphal elements.	CT or MRI shows single or multiple space-occupying lesions showing ring enhancement, mass effect or cerebral edema.
Brain granuloma	There is evidence of a firm, rubbery or gritty mass, without suppuration, with florid granulomatous inflammation and fibrinous tissue interspersed with hyphal elements.	CT shows a space-occupying lesion. The MRI characteristic of <i>Aspergillus</i> granulomas, shows to be extremely hypointense on T2, and hypointense or isointense on T1.
Fungal cerebral aneurysm	There is evidence of aneurysm formation in the intracranial artery, and invasion of the vessel wall by hyphal elements. The aneurysm may rupture or leak, which usually manifests as hemorrhage into the subarachnoid space.	In catheter angiographies and CT and MRI angiographies, aneurysms are usually fusiform, most often involving the proximal vasculature, such as internal carotid arteries and vessels forming the polygon of Willis. Intracranial hemorrhage may be a complication of vasculitis or fungal aneurysm formation, which appears hyperdense on non-contrast CT.
Meningitis	Presence of a thick grayish-white membrane covering the brain, in cases of dissemination from sinuses; or of focal and contiguous meningitis in cases of cerebrovascular aspergillosis, with the formation of a cerebral abscess, meninges infiltrated by hyphal elements and inflammatory infiltrate. CSF shows neutrophil pleocytosis, elevated protein and low to normal glucose.	Radiologic abnormalities within the meninges are usually absent. MRI shows thick meningeal enhancement on T1-weighted images, particularly at the skull base. The presence of leptomeningeal enhancement is nonspecific, but nodularity may be more specific of a fungal infection.
Ocular aspergillosis		
	Pathological findings	Imaging findings
Endogenous endophthalmitis	There is evidence of hyphal elements within the retinal and subretinal structures, with chorioretinal abscess formation, secondary vitritis, thrombosis and retinal vessel rupture, with retinal detachment. A fundus examination shows white, spongy preretinal lesions with deep creamy-white retinal lesions and intraretinal hemorrhages, with large wedge-shaped quadrants of pigmented and scarred chorioretinal atrophy.	<i>A fluorescein angiogram</i> shows hyperfluorescence of the preretinal granuloma and surrounding deep retinal lesions, with staining of the chorioretinal scars, focal vasculitis and leakage of the involved vessels. <i>Ocular ultrasound</i> shows dense opacities in the vitreous chamber for vitritis, thickening of the retinochoroidal layer due to subretinal exudative lesions, pre and intraretinal hemorrhages, preretinal layering of exudates that lead to epiretinal membranes and retinal detachment.
Exogenous endophthalmitis	It is due to direct inoculation of viable organisms, which may be accidental or iatrogenic, after penetrating ocular wounds or cataract surgery. Ocular abnormalities are not necessarily limited to the posterior globe.	Ocular ultrasound may reveal intraocular abnormalities.
Scleritis	Infiltration of the sclera by hyphal elements is more common in the setting of recent surgery, accidental or traumatic injury, or may be due to hematogenous seeding.	Diagnostic imaging is not usually performed.
Keratitis	Situations in which the integrity of the cornea is altered, such as surgery, trauma or use of contact lenses. It presents with stromal infiltrate, stromal abscess formation, invasion by hyphal elements and coagulative necrosis.	Diagnostic imaging is not usually performed.
Cutaneous aspergillosis		
	Pathological findings	Imaging findings
Primary cutaneous aspergillosis	There is evidence of significant local tissue destruction, spread to contiguous structures and disseminated infection, where it may be difficult to determine whether hyphal elements are found on the surface, or involve deeper structures.	Diagnostic imaging is not usually performed. Diagnosis is made by histopathology and culture.
Secondary cutaneous aspergillosis	There is evidence that cutaneous involvement is due to hematogenous seeding.	Diagnostic imaging is not usually performed. Diagnosis is made by histopathology and culture.
Osteoarticular aspergillosis		
	Pathological findings	Imaging findings
Osteomyelitis	It can occur by direct traumatic or iatrogenic inoculation, dissemination from contiguous structures or hematogenous seeding.	Fungal lesions can be seen on simple X-ray, CT and MRI, and may be helpful in stratifying and guiding needle biopsy of the lesion. The imaging features of infections are often nonspecific and difficult to differentiate from findings due to pyogenic infections or other causes of inflammatory arthropathy. CT findings include bone destruction, mixed lytic and sclerotic foci, cortical thickening and joint space narrowing.
Septic arthritis	It may be the result of hematogenous seeding, or direct inoculation after joint instrumentation.	CT and MRI findings are nonspecific. The most common findings are bone erosion, joint space narrowing, synovitis and joint effusions.

Renal aspergillosis		
	Pathological findings	Imaging findings
Renal aspergillosis	This entity is the result of hematogenous seeding, and is usually an incidental finding in the context of disseminated disease. Several different pathological processes are observed, ranging from vascular involvement with tissue infarction to renal abscess formation. Involvement of the renal vasculature results in multiple areas of renal infarction and ischemic and papillary necrosis. The renal vein may also be involved with complications including renal vein thrombosis and renal infarction.	CT reveals typically heterogeneous focal abscesses; these appear as hypoattenuating collections, which replace the renal parenchyma, with or without associated hydronephrosis. Advanced infection can lead to renal infarction and necrosis, which appears on contrast-enhanced MRI and on CT as a hypointense image, rarely progressing to emphysematous pyelonephritis.
Renal aspergilloma	Similar to formation of aspergillomas of pulmonary or sinus origin; the renal pelvis is the most common site of involvement. Aspergilloma is composed of gray or brown cheesy material that completely fills and occludes the pelvis and other components of the collecting system. The renal parenchyma is not involved, although there may be an ascending infection.	The presence of an aspergilloma occupying the renal collecting system, appearing as a heterogeneous hypoechoic mass within the dilated calyces, can be seen by ultrasound. On CT they appear as soft tissue dense masses within the collecting system. MRI often shows isointense masses on T1 and hyperintense on T2. They may cause local vascular inflammation resulting in thrombosis. More indolent chronic infections may cause parenchymal calcifications.
Gastrointestinal aspergillosis		
	Pathological findings	Imaging findings
Intestinal aspergillosis	An upper and lower intestinal aspergillosis arises from hematogenous seeding, and occurs as part of a disseminated infection. Focal areas of ulceration with variable depth and abscess formation are observed. Infiltration of hyphal elements in the intestinal wall, arteritis and thrombus formation within intramural vessels are seen.	Imaging findings of abdominal infections are variable and include nonspecific lesions, organomegaly and lymphadenopathy. Intestinal aspergillosis can manifest itself in a variety of ways, with intestinal obstruction, perforation or toxic megacolon, as a result of ischemia or tissue infarction.
Various gastrointestinal syndromes	Several rare syndromes are related to <i>Aspergillus</i> infection: stomatitis (with purplish discoloration of the gingiva, necrotic ulceration with an overlying gray membrane, and subsequent invasion of the alveolar bone), hepatosplenic disease (with formation of a hepatosplenic abscess) and peritonitis secondary to chronic ambulatory peritonitis.	CT shows a wide range of findings; the angioinvasive characteristic of <i>Aspergillus</i> can help predict its diagnosis. Fungal vascular invasion can cause vascular thrombosis, occlusion or pseudoaneurysm with or without rupture, ischemic or hemorrhagic tissue necrosis.
Aspergillus-associated allergic forms		
	Pathological findings	Imaging findings
ABPA	Macroscopically, lung specimens usually show airways filled with thick, tough sputum, with fibrous material consisting of scattered, typically fragmented, hyphal elements. <i>Charcot-Leyden</i> crystals (a by-product of eosinophil breakdown), Curschmann spirals (desquamated epithelium associated with eosinophilic infiltration) and inflammatory cells (macrophages, eosinophils and lymphocytes) are often seen.	Imaging findings consist of recurrent pneumonic consolidation (80%), mucoid airway impaction (30%), and atelectasis (20%). Chronic (permanent) findings consist of increased lung volume, tubular or annular shadows and lobar contraction. Multislice CT findings include bronchiectasis (cylindrical or cystic), mucus plugging, atelectasis, peripheral consolidation or ground-glass opacity, mosaic attenuation due to air trapping evident on the expiration sequence.
RAF	This entity occurs in patients with a history of chronic allergic rhinitis, often with hyperplastic nasal mucosa, which forms nasal polyps. Sinus contents evidence a thick, clayey, green, brown or grayish mucus. Microscopically there is evidence of eosinophilic ("allergic") mucin, containing mucin mixed with sloughed epithelial cells, eosinophils, <i>Charcot-Leyden</i> crystals, eosinophilic debris and other inflammatory cells arranged in a lamellar pattern, and associated with sparse and scattered hyphal elements.	In contrast to ABPA, there are no radiographic criteria or characteristic findings in an AFRS. On the CT scan, almost complete opacification of some cavities can be seen, with heterogeneous density resembling soft tissues within the affected sinus, and thinning of the bony walls of the affected sinus.

IPA: Invasive pulmonary aspergillosis; IBA: Invasive bronchial aspergillosis; CT: Computed tomography; MRI: Magnetic resonance imaging; HSCT: Hematopoietic stem-cell transplantation; GVHD: Graft-versus-host disease; HIV: human immunodeficiency virus; CGD: Chronic granulomatous disease; SOT: Solid organ transplant; CPA: Chronic pulmonary aspergillosis; CNPA: Chronic necrotizing pulmonary aspergillosis; CFPA: Chronic fibrosing pulmonary aspergillosis; CCPA: Chronic cavitary pulmonary aspergillosis; CNS: Central nervous system; CSF: Cerebrospinal fluid; ABPA: Allergic bronchopulmonary aspergillosis; AFRS: Allergic fungal rhinosinusitis.

Adapted from: Gregg KS et al.¹¹, Hope WW et al.³², Riscili BP et al.⁹², uldoon EG et al.¹⁰², Orlowski HL et al.²¹¹, Chong S et al.²¹⁵, Payne SJ et al.²²², Spadea L et al.²²⁴, Murthy JM et al.²²⁵, Bariteau JT et al.²²⁶, Yeom SK et al.²²⁷, Aribandi M et al.²³⁸, Hage CA et al.²³⁹.

Invasive infection

16. It is recommended that in a patient at high risk of developing an IF/IA, *Aspergillus* species recovered from biopsy of the involved site and/or respiratory tract specimens (induced sputum, tracheal aspirates, BAL, etc.) are identified at the complex level. It is considered that all isolates from the Fumigati section/complex should be identified at the species level. **(strong recommendation, high-quality evidence)** (Table 5)^{4,60,79,97,108,109}.
17. The consensus considers that in a high-risk patient with a hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, the isolation of an *Aspergillus* species from biopsy of the involved site and/or respiratory tract samples (induced sputum, tracheal aspirates, BAL, etc.) is highly suggestive of proven/probable IA/IPA, provides useful information to guide antifungal treatment and allows monitoring of antifungal resistance development. **(strong recommendation, high-quality evidence)**^{4,20,21,32,58-60,62,75,77,79,81,94,96-103,109}.
18. The consensus considers that in a SOTR patient, especially with lung transplantation, the isolation of an *Aspergillus* species by mycological culture from biopsy of the involved site and/or respiratory tract samples (induced sputum, tracheal aspirates, BAL, etc.), is highly suggestive of proven/probable IA/IPA. **(strong recommendation, moderate-quality evidence)**^{4,21,77,79,81}.
19. The consensus considers that in a non-hematologic patient in the ICU, and according to his/her specific clinical condition, based on biopsy of the involved site and/or respiratory tract samples (induced sputum, tracheal aspirates, BAL, etc.), it is possible to make a diagnostic approximation of a proven/probable IA/IPA. **(strong recommendation, moderate-quality evidence)**^{4,8,21,77,79,81,104-107}.

2. In a patient with suspected IA/*Aspergillus* disease, what is the value of antibody (Ab) detection tests?

Recommendation**Invasive infection**

20. In a high-risk immunocompromised patient, the consensus does not recommend the use of tests based on the detection of *Aspergillus*-specific antibodies (Abs) and/or *Aspergillus* precipitins for the diagnostic approach of proven/probable IA, due to his/her immunocompromised status and poor humoral response. **(strong recommendation, moderate-quality evidence)**^{4,21,32,34,35,77-82,86,95,113-123}.

Aspergillus disease

21. The consensus considers that in an immunocompetent or mildly immunocompromised patient, the use of tests based on the detection of *Aspergillus*-specific Abs and/or *Aspergillus* precipitins allows a diagnostic approach of a chronic or allergic form associated with *Aspergillus* spp. **(strong recommendation, moderate-quality evidence)** (Annex 6)^{19,32,34,35,86,95,110,113-126}.
22. The consensus considers that in an immunocompetent or mildly immunocompromised patient, high titers of

Aspergillus-specific Abs are a diagnostic marker of non-invasive infection, with limited utility in a severely immunocompromised patient. **(strong recommendation, moderate-quality evidence)**^{34,86,113}.

- a. In a patient with a chronic and/or allergic form associated with *Aspergillus*, what commercial tests are available for the diagnosis and therapeutic management?

Recommendation

23. The consensus considers that in a patient with a high suspicion of developing a chronic and/or allergic form associated with *Aspergillus*, the use of commercial tests based on the detection of *Aspergillus*-specific Abs titers are a useful tool for the diagnostic approach and therapeutic management. **(strong recommendation, moderate-quality evidence)** (Annex 6)^{34,85,86,95,113}.
24. The consensus considers that in a patient with high suspicion of developing a chronic and/or allergic form associated with *Aspergillus*, the measurement of IgG and IgE Abs has a higher sensitivity, compared to the measurement of IgM or IgA Abs, for the diagnostic approach and therapeutic management. **(strong recommendation, moderate-quality evidence)** (Annex 6)^{34,86,95,117}.
25. The consensus considers that for a diagnostic approach of a chronic and/or allergic form associated with *Aspergillus* spp. the measurement of IgG-*Aspergillus*-specific Abs and IgE-*Aspergillus*-specific Abs based on commercial enzyme immunoassay (EIA)-based tests is more sensitive than the *Aspergillus* precipitin test. **(strong recommendation, moderate-quality evidence)** (Annex 6)^{9,34,86,95,113,117}.

- i. What is the usefulness of antibody (Ab) detection tests for the therapeutic management of the disease?

Recommendation**Invasive infection**

26. The consensus does not recommend that in an immunocompromised patient with a diagnosis of a proven/probable IA, measurement of *Aspergillus*-specific Abs and/or *Aspergillus* precipitins be used as a diagnostic tool to monitor response to treatment. **(strong recommendation, moderate-quality evidence)**^{16,34,86,95,96,125-127}.
27. It is considered that in an immunocompromised patient who is colonized by *Aspergillus* species, the measurement of *Aspergillus*-specific Abs and/or *Aspergillus* precipitins could be a useful diagnostic tool for the initiation of prophylaxis or empirical antifungal treatment. **(weak recommendation, moderate-quality evidence)**^{16,32,81,86,125-127}.

Aspergillus disease

28. The consensus considers that in an immunocompetent or mildly immunocompromised patient, a high titer of IgG-*Aspergillus*-specific Abs allows a diagnostic approach of

a chronic *Aspergillus*-associated form, and a high titer of IgE-*Aspergillus*-specific Abs allows a diagnostic approach of an allergic *Aspergillus*-associated form. It is recommended to consider the degree of overlap of these clinical conditions with similar clinical and/or serological features. **(strong recommendation, moderate-quality evidence)**^{9,34,86,92,95}.

29. The consensus considers that in a patient diagnosed with a chronic form associated with *Aspergillus* spp. the measurement of *Aspergillus*-specific Abs and/or *Aspergillus* precipitins is the best tool to monitor the response to antifungal therapy. Decreased *Aspergillus*-specific IgG titers indicate a good response to treatment. **(strong recommendation, moderate-quality evidence)**^{9,34,86}.
 30. It is considered that in a patient with an allergic form associated with *Aspergillus* spp. the measurement of IgE-*Aspergillus*-specific Abs and total IgE Abs is the best diagnostic tool to monitor the response to treatment. Limitations of the tests should always be considered. **(strong recommendation, moderate-quality evidence)**^{32,34,35,86,92}.
- 3. In a patient with suspected IA/*Aspergillus* disease, what is the value of antigen (Ag) and/or biomarker testing?**

Recommendation

31. The consensus considers that the use of tests based on the detection of Ags and/or biomarkers in a patient with high suspicion, and according to his/her specific clinical condition, allows a diagnostic approach of a proven/probable IA/*Aspergillus* disease. **(strong recommendation, high-quality evidence)** (Annex 7)^{4,16,21,32,34,75–83,126–152}.

Invasive infection

32. The consensus recommends that in order to make a diagnostic approach of a proven/probable IA in a patient at high risk of developing IFI, and in view of the variability in the prevalence of the disease and the diagnostic performance of Ag and/or biomarker detection tests, the results should always be interpreted in conjunction with clinical, imaging and microbiological criteria. **(strong recommendation, high-quality evidence)** (Annex 7)^{4,77,80,82,128–130}.
33. It is considered that in a patient at high risk of developing an IFI where the prevalence of invasive disease is greater than 15%, when making a diagnostic approach of a proven/probable IA, a negative fungal Ag/biomarker result excludes the disease, and a positive fungal Ag/biomarker result allows its diagnosis. **(strong recommendation, moderate-quality evidence)**^{76–78,83,128–130}.
 - a. In a patient with proven/probable IA, what commercial tests are available for the diagnosis and therapeutic management?

Recommendation

34. The consensus considers that in a high-risk patient, and according to the patient population involved, the use of commercial tests based on the detection of *Aspergillus*

galactomannan Ag (AGA) and (1,3)- β -D-glucan (BDG), is a useful tool for the diagnosis and therapeutic management of a proven/probable IA. **(strong recommendation, high-quality evidence)** (Annex 7)^{4,76–78,105,113,128–130}.

35. The consensus considers that in a patient with a high suspicion of developing an IFI/IA, the diagnostic yield of AGA detection is variable, and will depend on: (a) the patient population studied, (b) the type of sample analyzed, (c) the number of tests performed, and (d) the optimal density index (ODI) used to interpret the result. **(strong recommendation, moderate-quality evidence)** (Annex 7)^{4,21,76,78,81,83,105,113,128–130}.
36. In a patient at high risk of developing an IFI/IA, AGA testing from serum and/or BAL is considered to have an excellent diagnostic yield in: (a) high-risk onco-hematologic patient, (b) patient with profound and prolonged neutropenia after chemotherapy for leukemia, and (c) allogeneic HSCT patient. **(strong recommendation, high-quality evidence)**^{4,21,76–78,81,83,105,113,128–130}.
37. In a patient at high risk of developing an IFI/IA, AGA testing from serum and/or BAL is considered to have an acceptable diagnostic yield in: (a) immunocompromised critically ill patient, (b) lung transplant recipient patient, (c) non-hematologic patient with aspergillosis, and (d) cortico-dependent critically ill patient. **(strong recommendation, high-quality evidence)**^{4,21,76,78,81,83,105,113,128–130}.
38. In a SOTR patient other than a lung transplant patient, or in patients with chronic granulomatous disease (CGD), the consensus does not recommend measurement of serum AGA for a diagnostic approach of proven/probable IA/IPA. **(strong recommendation, low-quality of evidence)**^{4,21,34,77–83,105,110,113,128–132,137,152}.
39. It is considered that in a patient at high risk of developing an IFI/IA, the serum BDG test allows a diagnostic approach of a non-specific invasive disease for *Aspergillus* spp. in: (a) patient with hematologic malignancy and/or HSCT, (b) immunocompromised critically ill patient. **(strong recommendation, moderate-quality evidence)**^{4,21,76,78,81,83,105,113,128–130}.
40. The consensus considers that in a patient at high risk of developing an IFI/IA, the *Aspergillus* lateral-flow device (LFD) is a rapid and easy to manipulate and perform test that allows the diagnosis of a proven/probable IA from serum and/or BAL. **(strong recommendation, moderate-quality evidence)**^{75,77,78,129,133}.
 - i. What is the usefulness of AGA and BDG for the diagnosis of invasive disease?

Recommendation

41. In a patient at high risk of developing an IFI/IA, the consensus recommends performing the AGA test from serum, within a serial measurement protocol (x3/wk), as a test for early detection and/or for diagnosis of a proven/probable IA. **(strong recommendation, high-quality evidence)**^{4,21,77,79–82,128,134–137}.
42. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutro-

penia, the consensus recommends measurement of AGA from serum and/or BAL for the diagnostic approach of proven/probable IA. **(strong recommendation, moderate-quality evidence)**^{4,21,77,79-82,128,134-137}.

43. The consensus considers that in a pediatric patient at high risk of developing an IFI/IA, the diagnostic performance of AGA from serum is similar to that of an adult patient, but with a lower specificity. **(strong recommendation, moderate-quality evidence)**^{21,78,81,105,110,128-132,137,152}.
44. In a high-risk non-hematologic patient in the ICU, the consensus does not recommend measurement of AGA from serum for the diagnostic approach of proven/probable IA. Measurement of AGA from BAL is considered for the diagnostic approach of proven/probable IA/IPA. **(strong recommendation, moderate-quality evidence)**^{21,77,78,81,83,105,110,128-132,137,152}.
45. In a patient at high risk of developing an IFI/IA, the consensus considers the measurement of AGA from CSF to make the diagnostic approach of cerebral aspergillosis. **(strong recommendation, moderate-quality evidence)**^{21,34,77,128,134}.
46. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, the consensus recommends measurement of serum BDG as an early detection and/or diagnostic test for a proven/probable IFI/IA. **(strong recommendation, moderate-quality evidence)**^{21,77,79,128,138,139}.
47. In a high-risk immunocompromised adult patient in the ICU (hematologic, transplant recipient, immunosuppressive therapy, liver failure, and/or HIV), the consensus recommends measurement of serum BDG as an early detection and/or diagnostic test for a proven/probable IFI/IA. **(strong recommendation, moderate-quality evidence)**^{21,77,78,81,83,105,110,128-132,137,152}.
48. The consensus recommends that in a high-risk patient with hematologic malignancy and/or HSCT or SOTR, measurement of Aspergillus Ag by DFL-Aspergillus as a detection and diagnostic test for proven/probable IA is made from serum and/or BAL. **(strong recommendation, moderate-quality evidence)**^{21,77,129,133,140,141}.

ii. What is the usefulness of AGA and BDG for the therapeutic management of invasive disease?

Recommendation

49. The consensus considers that in a patient at high risk of developing an IFI/IA, whether adult or pediatric, measurement of serum AGA (ODI: ≥ 0.5 [x2]; ≥ 1.0 [x1]), BAL (ODI: ≥ 1.0) and/or CSF (ODI: ≥ 1.0) is a useful tool as an accurate diagnostic marker of proven/probable IA, allowing monitoring of response to antifungal therapy. **(strong recommendation, high-quality evidence)** (Annex 7)^{4,21,77,79,81,82,129}.
50. The consensus considers that in a patient at high risk of developing an IFI/IA, whether adult or pediatric, measurement of serum BDG (ODI: $>60-80$ pg/mL) is a useful tool as a diagnostic marker of proven/probable IFI/IA, allowing monitoring of response to antifungal therapy. **(strong recommendation, high-quality evidence)** (Annex 7)^{4,21,77,79,81,82,129,138,139}.
51. The consensus considers that in a high-risk patient with

hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, who is not on antifungal prophylaxis or treatment, the use of the serum AGA test with serial monitoring (x3/wk), is a useful tool to initiate diagnostic-driven antifungal treatment. **(strong recommendation, high-quality evidence)**^{4,21,81,94,113,134-137,144,146}.

52. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, who is on antifungal prophylaxis or treatment, the consensus does not recommend the use of the serum AGA test as an early detection test for proven/probable IA. Measurement of AGA from BAL is considered for the diagnostic approach of proven/probable IA/IPA. **(strong recommendation, high-quality evidence)**^{4,21,77,81,94,113,134-137,144,146}.

iii. What is the usefulness of AGA and BDG in assessing response to antifungal treatment?

Recommendation

53. The consensus considers that in a patient diagnosed with a proven/probable IA, the evaluation of the response to antifungal therapy should be based on the study of clinical, imaging and mycological criteria over an adequate period of time. **(strong recommendation, moderate-quality evidence)**^{77,81,134-137,144,146}.
54. The consensus considers that in a high-risk patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, the use of serum AGA test with serial monitoring (x3/wk) is useful for monitoring disease progression, therapeutic response and prediction of clinical outcome. **(strong recommendation, high-quality evidence)**^{4,78,81,94,113,128,134,145-151}.
55. The consensus considers that in a patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, diagnosed with a proven/probable IA, the serum AGA test result correlates with survival and with the result of the response to antifungal therapy. **(strong recommendation, high-quality evidence)**^{4,21,77,78,94,134-137,144,146}.
56. The consensus considers that in a patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, diagnosed with a proven/probable IA, the result of the serum BDG test has not been fully validated to predict the result of the response to antifungal therapy. **(weak recommendation, moderate-quality evidence)**^{4,21,77,78,113,129,138,139}.

iv. What is the usefulness of AGA and BDG for the prognosis of invasive disease?

Recommendation

57. The consensus considers that in a patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, diagnosed with a proven/probable IA, a decrease in serum AGA titers correlates with a de-

crease in associated mortality. Persistently positive results are a sign of a poor prognosis, and a clinical-therapeutic re-evaluation should be considered. **(strong recommendation, high-quality evidence)**^{4,21,77,79,81,130-132,134}.

58. It is considered that in a patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, diagnosed with a proven/probable IA, the results of serial monitoring (x3/wk) of serum AGA often precede positive culture and/or imaging findings from 6 to 10 days. **(strong recommendation, moderate-quality evidence)**^{4,77,94,113,134,148,149}.
59. The consensus considers that in a patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, diagnosed with a proven/probable IA, a decrease in serum BDG titers correlates with a decrease in associated mortality. It is considered that results often precede positive culture and/or imaging findings from 6 to 10 days. **(strong recommendation, high-quality evidence)**^{4,21,77,79,81,130-132,138,139}.
4. In a patient with suspected IA/*Aspergillus* disease, what is the value of nucleic acid testing (PCR) and mass spectrometry (MALDI-TOF MS system)?

Recommendation

60. The consensus considers that in a patient at high risk of developing an IFI, the identification of the *Aspergillus* species involved from fresh and/or paraffin-embedded biopsies, by a universal PCR test, with/without prior observation of septate hyphae in a direct microscopic examination, allows the diagnostic approach of a proven/probable IA/*Aspergillus* disease. **(strong recommendation, moderate-quality evidence)**^{21,32,58,59,76,77,112,144,153-161}.
61. It is considered that in a high-risk patient diagnosed with proven/probable IA/*Aspergillus* disease, the use of the MALDI-TOF MS system allows routine identification of the *Aspergillus* spp. involved. It is recommended to implement in-house databases, according to local epidemiology. **(strong recommendation, moderate-quality evidence)**^{76,77,108,144,162-174}.

Invasive infection

62. In a high-risk patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, the consensus recommends detecting fungal DNA by PCR-*Aspergillus* test from blood, serum, BAL and/or biopsy of the involved site, to make the diagnostic approach of proven/probable IA. **(strong recommendation, moderate-quality evidence)** (Annex 7)^{21,32,58,59,77,81,144,153-161,169}.
63. In a high-risk non-hematologic patient in the ICU, the consensus recommends detecting fungal DNA by PCR-*Aspergillus* test from blood, serum, BAL and/or biopsy of the involved site to make the diagnostic approach of proven/probable IA. **(strong recommendation, moderate-quality evidence)**^{21,32,77,81,153,154,160,161}.
64. In a patient with a high suspicion of developing an IFI, the consensus recommends using the MALDI-TOF MS system for routine identification of *Aspergillus* spp. complex/sec-

tion isolates from biopsy of the involved site and/or clinical samples from the respiratory tract (induced sputum, tracheal aspirates, BAL, etc.). **(strong recommendation, moderate-quality evidence)**^{21,76,77,108,170,173}.

Aspergillus disease

65. In a patient with a high suspicion of developing an *Aspergillus* disease, the consensus recommends detecting fungal DNA by PCR-*Aspergillus* test from blood, serum, BAL and/or biopsy of the involved site, to make the diagnostic approach of a chronic and/or allergic form associated with *Aspergillus* spp. (weak recommendation, moderate-quality evidence)^{21,32,34,58,59,129}.
66. The consensus recommends using the MALDI-TOF MS system for routine identification of *Aspergillus* spp. complex/section isolates from biopsy of the involved site and/or clinical samples from the respiratory tract (induced sputum, tracheal aspirates, BAL, etc.), from a patient diagnosed with a chronic and/or allergic form associated with *Aspergillus* spp. (strong recommendation, moderate-quality evidence)^{21,76,170,173}.
- a. In a patient with an IA/*Aspergillus* disease, what is the usefulness of PCR and MALDI-TOF MS testing in diagnosing the disease?

Recommendation

Invasive infection

67. The consensus considers that in a patient at high risk of developing an IFI/IA, the use of PCR-based diagnostic tools through sequential testing allows detecting and/or ruling out a probable/possible IA. **(strong recommendation, moderate-quality evidence)** (Annex 7)^{4,21,32,76,77,81,94,129,135,144}.
68. In a high-risk patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, and with evidence of pulmonary opacities, the consensus recommends detecting fungal DNA by PCR as an early detecting and/or diagnostic test for a proven/probable IA. **(strong recommendation, moderate-quality evidence)**^{4,21,32,76,77,81,94,129,135,144}.
69. In a high-risk patient with hematologic malignancy and/or HSCT with/without profound and prolonged neutropenia, who is not on antifungal prophylaxis or treatment, the consensus recommends detecting fungal DNA by PCR from blood, serum and/or BAL, as an early detection test for proven/probable IA. **(strong recommendation, moderate-quality evidence)**^{21,77,79,81,94,129,135,136,153,154,160,161}.
70. In a patient diagnosed with proven/probable IA, with clinical isolates of the relevant *Aspergillus* spp. complex/section, the consensus recommends direct identification by PCR test and sequencing of ITS, β -tubulin and calmodulin genes. **(strong recommendation, high-quality evidence)**^{21,58,59,62,77,101}.
71. In a patient with diagnosed with proven/probable IA, the consensus recommends using MALDI-TOF MS for routine identification of implicated *Aspergillus* spp. complex/section isolates from primary cultures of biopsies from the involved site and/or sterile body fluids. **(strong recommendation, moderate-quality evidence)**^{21,77,108,156,162,163,173,174}.

Aspergillus disease

72. The consensus considers that in a patient diagnosed with a chronic and/or allergic form associated with *Aspergillus* spp. with clinical isolates of the relevant *Aspergillus* spp. complex/section, direct identification should be made by PCR testing and by sequencing of ITS, β -tubulin and calmodulin genes, the interpretation of which will depend on the clinical context of the patient. **(strong recommendation, moderate-quality evidence)**^{9,32,34}.

b. In a patient with IA/Aspergillus disease, what is the usefulness of PCR and MALDI-TOF MS testing for the therapeutic management of the disease?

Recommendation**Invasive infection**

73. The consensus considers that in certain adult or pediatric immunocompromised patient populations, detection of fungal DNA by PCR testing is an accurate diagnostic marker to monitor the response to antifungal treatment of a proven/probable IA, with: (a) two or more consecutive positive tests from plasma, serum or whole blood, (b) two or more duplicate positive tests from BAL, and (c) at least one positive test from plasma, serum or whole blood, together with a positive test from BAL. **(strong recommendation, high-quality evidence)**^{4,21,76,77,81,94,129,135,144}.

74. The consensus considers that in certain adult or pediatric populations of immunocompromised patients, the use of diagnostic methods based on PCR and/or biomarkers (AGA and/or BDG), used in combination and performed at regular intervals and during the period of risk, allows therapeutic follow-up of proven/probable IA. **(strong recommendation, moderate-quality evidence)** (Annex 7)^{77,79,94,129,135,136,153-155,160,161}.

75. In a patient diagnosed with a proven/probable IA, the consensus does not recommend using MALDI-TOF MS testing for therapeutic follow-up. **(strong recommendation, low-quality of evidence)**^{77,156,162,163,170,173,174}.

Aspergillus disease

76. In a patient diagnosed with a chronic and/or allergic form associated with *Aspergillus* spp. the consensus does not recommend using PCR-based diagnostic methods for therapeutic follow-up. **(strong recommendation, low-quality of evidence)**^{9,32,34}.

77. In a patient diagnosed with a chronic and/or allergic form associated with *Aspergillus* spp. the consensus does not recommend using MALDI-TOF MS testing for therapeutic follow-up. **(strong recommendation, low-quality of evidence)**^{156,162,163,170,173,174}.

5. In a patient diagnosed with IA/Aspergillus disease, what is the value of using in vitro antifungal susceptibility testing (AFST)?

Recommendation

78. In a patient diagnosed with an IFI/IA, the consensus does not formally recommend using AFST, which is routinely

used to evaluate yeast isolates, to evaluate all clinical isolates of *Aspergillus* spp. AFST is recommended for clinical isolates suspected of being azole resistant and/or coming from a patient without an adequate response to the antifungal treatment of choice. **(strong recommendation, moderate-quality evidence)**^{21,109,130,175,176}.

79. The consensus considers that in a patient diagnosed with an IFI/IA, performing AFST of relevant clinical isolates of *Aspergillus* spp. allows establishing an epidemiological surveillance program, since there is variability in the sensitivity profiles of the different clinical isolates of the same species. **(strong recommendation, moderate-quality evidence)**^{21,109,130,175,176}.

80. The consensus is that in a patient diagnosed with an IA/Aspergillus disease, with clinical isolates of *Aspergillus* species from different clinical samples, the interpretation of the AFST result varies according to: (a) the clinical context of the patient, (b) individual risk factors, (c) the response to antifungal treatment, and (d) the high probability of resistance of a specific clinical isolate. **(strong recommendation, moderate-quality evidence)**¹⁷⁵⁻¹⁸⁹.

a. In a patient diagnosed with IA/Aspergillus disease, what methods are recommended to assess susceptibility to antifungal agents?

Recommendation

81. In a patient diagnosed with an IA/Aspergillus disease, the consensus recommends performing AFST on all clinical isolates of *Aspergillus* spp. that are considered clinically relevant by a reference method (CLSI or EUCAST). **(strong recommendation, moderate-quality evidence)**^{21,60,81,109,130,176,182,183,188,189}.

82. The consensus considers that in a patient diagnosed with an IA/Aspergillus disease, commercially available AFST tests, standardized in multicenter studies, can be used for in vitro detection of antifungal resistance. Reference methods (CLSI or EUCAST) should be used to confirm such antifungal resistance. **(strong recommendation, high-quality evidence)** (Table 5)^{21,60,81,109,176,184-189}.

i. When should Aspergillus resistance to antifungal agents be suspected? Does an AFST result change the choice of antifungal treatment?

Recommendation

83. The consensus considers that in a patient diagnosed with an IA/Aspergillus disease, the use of AFST, if available and/or accessible, provides valuable information to help guide antifungal therapy and allows for pharmacological surveillance studies and identification of changes in antifungal sensitivity patterns. **(strong recommendation, moderate-quality evidence)**^{60,109,130,182,183}.

84. In certain populations of patients diagnosed with IA/Aspergillus disease from geographic areas with a high prevalence of antifungal resistance, and/or in patients who do not respond to primary targeted antifungal therapy, the consensus recommends performing AFST of relevant clinical

isolates to identify intrinsic and/or acquired resistance to azoles and amphotericin B (AmB). **(strong recommendation, moderate-quality evidence)** (Table 5)^{4,21,60,81,109,130,178}.

85. In a patient diagnosed with an IA/*Aspergillus* disease, in case of therapeutic failure, and with a relevant clinical isolation of *Aspergillus* spp. and/or if a cryptic species involved in the infectious process is identified, the consensus recommends considering the manifestation of antifungal resistance. **(strong recommendation, moderate-quality evidence)** (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients [approach to therapeutic management of refractory/progressive aspergillosis]) (Table 5)^{4,21,34,60,81,130}.
86. In a patient diagnosed with an IA/CPA, associated with a clinically relevant isolate of *Aspergillus* spp. and an AFST result with a MIC to AmB > 1 mg/L, the consensus recommends replacing antifungal treatment with AmB with an azole, provided that the isolate is sensitive to the azole drug. **(strong recommendation, moderate-quality evidence)** (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients [clinical situations for initiating primary targeted antifungal therapy]) (Table 5)^{4,21,34,60,181}.
87. In a patient diagnosed with an IA/CPA associated with a clinically relevant isolation of *Aspergillus* spp. and an AFST with a MIC to VCZ > 2 mg/L, the consensus recommends initiating antifungal treatment with AmB (L-AmB or LC-AmB) in monotherapy, or with VCZ + echinocandin or L-AmB in combination. **(strong recommendation, moderate-quality evidence)** (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients [clinical situations for initiating primary targeted antifungal therapy]) (Table 5)^{4,21,34,60,181}.
88. In a patient diagnosed with an IA/CPA associated with a clinically relevant isolation of *A. terreus*, the consensus recommends initiating antifungal therapy with standard-dose voriconazole (VCZ), isavuconazole (ISZ), posaconazole (PCZ) or itraconazole (ITZ). **(strong recommendation, moderate-quality evidence)** (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients [clinical situations for initiating primary targeted antifungal therapy]) (Table 5)^{4,21,34,60,181}.
89. In a patient diagnosed with an IA/CPA associated with a clinically relevant isolation of *A. calidoustus* (part of the *A. ustus* complex), the consensus recommends initiating antifungal therapy with standard-dose AmB, (L-AmB or LC-AmB). **(strong recommendation, moderate-quality evidence)** (II Prophylaxis, Treatment and Prevention of IA in Adult and Pediatric Patients [clinical situations for initiating primary targeted antifungal therapy]) (Table 5)^{4,21,34,60,181}.

6. In a patient diagnosed with IA/*Aspergillus* disease, what is the value of using therapeutic drug monitoring (TDM) of antifungal agents?

Recommendation

90. The consensus considers that in a patient diagnosed with an IA/CPA and according to the clinical scenario, TDM is a useful complementary tool to guide a safe and effective

clinical-therapeutic approach. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{4,21,81,190-197}.

91. The consensus considers that if, when performing TDM in a patient diagnosed with an IA/CPA, the results of the minimum allowed concentration are not achieved, or they exceed the plasma levels of efficacy, the dose of the antifungal agent should be modified. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{4,21,81,190-197}.

Invasive infection

92. The consensus recommends performing TDM in a patient diagnosed with proven/probable IA who is on prophylaxis/treatment with antifungal agents (VCZ, ITZ or PCZ), in the event of: (a) non-linear pharmacokinetics, (b) inadequate absorption, (c) narrow therapeutic window, or (d) suspicion of possible drug interactions and/or unspecified toxicity. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{4,21,77,81,190-197}.

Aspergillus disease

93. In a patient diagnosed with a chronic form associated with *Aspergillus* spp. especially in progressive disease and/or requiring prolonged or lifelong suppressive treatment with antifungal agents (VCZ, ITZ or PCZ), the consensus recommends TDM in the event of: (a) non-linear pharmacokinetics, (b) inadequate absorption, (c) a narrow therapeutic window, or (d) suspicion of possible drug interactions and/or unspecified toxicity. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{4,21,81,190-197}.

a. In a patient diagnosed with IA/*Aspergillus* disease, what is the usefulness of TDM for the therapeutic management of the disease?

Recommendation

94. To improve pharmacological efficacy in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/treatment with VCZ, the consensus recommends performing TDM from day 4-7 after initiating treatment (or on day 4 after dose adjustment), and repeating it at least once a week (after achieving dose stability). A plasma concentration in prophylaxis > 1mg/L (by high-performance liquid chromatography [HPLC]), or 1-5.5 mg/L (by HPLC) in treatment, is considered to be associated with pharmacological efficacy. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.
95. To decrease drug toxicity in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/treatment with VCZ, the consensus recommends performing TDM from day 4-7 after initiating treatment (or on day 4 after dose adjustment), and repeating it at least once a week (after achieving dose stability). A plasma concentration of 4.5-5.5 mg/L (by HPLC) is considered to be associated with pharmacological toxicity. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.

Table 8. Recommendations for TDM.

Drug	Indications	Time to TDM after treatment initiation	Effective plasma concentration	Toxicity plasma concentration
ITZ	To improve efficacy in patients (immunocompromised or not) receiving ITZ, in prophylaxis or for treatment of an IFD or an allergic fungal disease: <ul style="list-style-type: none"> When there are drug interactions, when starting or stopping therapy (either by inhibiting absorption or affecting its metabolism) In co-medications (with Cytochrome P450 inducers). In case of suspicion of non-adherence to oral therapy. In the absence of pharmacological response. Concern about gastrointestinal absorption, especially over prolonged periods. Possible clinical or laboratory manifestations of toxicity. 	Measure from day 4-7, after the start of treatment.	In prophylaxis: 0.5 mg/L, (HPLC), or; > 3 mg/L (bioassay) For treatment: > 1-4 mg/, (HPLC)	Toxicity is associated with serum levels of ITZ > 17.1 mg/L (bioassay), or ~4 mg/L, (HPLC).
VCZ	To improve efficacy in patients (immunocompromised or not) receiving VCZ, in prophylaxis or for treatment of an IFD: <ul style="list-style-type: none"> When drug interactions are present, when starting or stopping therapy. In case of suspicion of non-adherence to oral therapy. Concern about gastrointestinal absorption, especially over prolonged periods. In the absence of pharmacological response. In interactions with drugs administered simultaneously. When changing from oral to intravenous administration or vice versa. In case of hepatic insufficiency. In its administration in pediatric patients. 	Measure from day 4-7, after initiation of treatment, or on day 4 after dose adjustment.	In prophylaxis: > 1 mg/L. For treatment: 1-5.5 mg/L Repeat TDM during week 2 of treatment.	< 4.5-5.5 mg/L, (HPLC)
PCZ	To improve efficacy in patients (immunocompromised or not) receiving PCZ, in prophylaxis or for salvage treatment of an IFD: <ul style="list-style-type: none"> When drug interactions are present, when starting or stopping therapy. In case of suspicion of non-adherence to oral therapy. Concern about gastrointestinal absorption, especially over prolonged periods. In the absence of pharmacological response. In co-medications, including H2 antagonists and proton pump inhibitors. In mucositis and other types of gastrointestinal disorders. 	Measure from day 4-7, after the start of treatment.	In prophylaxis: > 0.7 mg/L at steady state, or, 0.35 mg/L after 48 hours from the start of treatment. For treatment: > 1 mg/L.	Serum PCZ levels of, 0.5-3.75 mg/L are considered safe and effective in all three formulations. Serum PCZ levels above this exposure range may be associated with toxicity.
ISZ	To improve efficacy, safety and treatment adherence in patients receiving ISZ	Measure serum concentration on day 5, after initiation of treatment, and then regularly thereafter.	Data are limited to support routine TDM, but may be indicated in case of treatment failure, drug interactions or if toxicity is suspected.	

TDM: Therapeutic drug monitoring of antifungal agents; ITZ: Itraconazole; VCZ: Voriconazole; PCZ: Posaconazole; ISZ: Isavuconazole; IFD: Invasive fungal disease; HPLC: High-performance liquid chromatography.

Adapted from: Ullmann AJ et al. (21), Fortún J et al.²⁰, Ashbee HR et al.¹⁹³, Cendejas-Bueno E. et al.²⁴⁰.

96. To improve pharmacological efficacy in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/treatment with ITZ, the consensus recommends performing TDM from day 4-7 after starting treatment (or on day 4 after dose adjustment) and repeating it at least once a week (after achieving dose stability). A plasma concentration in prophylaxis of 0.5 mg/L (by HPLC), or in treatment of >1-4 mg/L (by HPLC), is considered to be associated with pharmacological efficacy. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.
97. To decrease drug toxicity in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/treatment with ITZ, the consensus recommends performing TDM from day 4-7 after initiating treatment (or on day 4 after dose adjustment) and repeating it at least once a week (after achieving dose stability). A plasma concentration of ~4 mg/L (by HPLC) is considered to be associated with pharmacological toxicity. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.
98. To improve pharmacological efficacy in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/

treatment with PCZ suspension, the consensus recommends performing TDM from day 4-7 after initiating treatment (or on day 4 after dose adjustment) and repeating it at least once a week (after achieving dose stability). A plasma concentration in prophylaxis of > 0.7 mg/L at steady state, or 0.35 mg/L after 48 hours (by HPLC), or in treatment of > 1 mg/L (by HPLC), is considered to be associated with pharmacological efficacy. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.

99. To decrease drug toxicity in a patient diagnosed with an IA/CPA who is on antifungal prophylaxis/treatment with PCZ suspension, the consensus recommends performing TDM from day 4-7 after initiating treatment (or on day 4 after dose adjustment), and repeating it at least once a week (after achieving dose stability). A plasma concentration of 0.5-3.75 mg/L (by HPLC) is considered safe and effective (in all three formulations), and a serum level above this range is associated with pharmacological toxicity. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,191-193}.
100. The consensus does not recommend performing TDM to assess efficacy and/or drug toxicity in a patient diagnosed with an IA/CPA who is on antifungal therapy with ISZ. **(strong recommendation, moderate-quality evidence)** (Table 8, Annexes 8 and 9)^{21,81,192,193}.
7. **In a patient with suspected IFI/IA, what is the diagnostic utility of using the D-index (area over the neutrophil curve)?**

Recommendation

101. The consensus considers that in a patient with a high suspicion of developing an IFI, using the D-index to stratify the risk of a proven/probable IA can be a useful tool within a targeted primary antifungal treatment strategy. **(strong recommendation, low-quality evidence)**^{77,198-200}.
102. The consensus considers that in a febrile neutropenic patient with a high suspicion of developing an IFI, using the D-index can help define different degrees of risk for a proven/probable IA. It is considered that in a low-risk patient, it reduces the use of unnecessary empirical antifungal therapy, and in a high-risk patient, it allows early initiation of primary targeted antifungal therapy. **(strong recommendation, low-quality of evidence)**^{77,198-200}.
8. **In a patient with suspected IFI/IA, how is the diagnostic approach of extrapulmonary aspergillosis performed?**

Recommendation

103. The consensus considers that in a patient with a high suspicion of developing an extrapulmonary IFI/IA, the isolation of an *Aspergillus* species by mycological culture from biopsy of the involved site allows the diagnostic approach of invasive disease. Prior direct microscopic observation with optical brighteners and/or histopathological sta-

ins determines the importance of such fungal isolation. **(strong recommendation, high-quality evidence)** (Table 7)^{4,21,32,34,72-74,77-82,93,99,106,144}.

104. In a patient with a high suspicion of developing an extrapulmonary IFI/IA, it is recommended not to discard any isolation of an *Aspergillus* species obtained from mycological culture, without carefully evaluating the clinical context of the patient. **(strong recommendation, moderate-quality evidence)**^{4,21,32,34,72-74,77-82,93,99,106,144}.
105. It is considered that in a patient with a high suspicion of developing an extrapulmonary IFI/IA, according to the clinical context of the patient, the use of diagnostic tests based on PCR and/or biomarkers (AGA and/or BDG) used in combination, allow an adequate diagnostic approach. **(strong recommendation, moderate-quality evidence)**^{4,21,32,34,72-74,77-82,94,99,106,128,129,135,144}.
106. The consensus considers that in a patient with a high suspicion of developing an extrapulmonary IFI/IA, imaging studies (e.g., computed tomography [CT] and/or magnetic resonance imaging [MRI]) allow to identify areas with specific abnormalities, and to direct the collection of an appropriate clinical specimen, to perform an adequate diagnostic approach. **(strong recommendation, high-quality evidence)** (Table 7)^{4,21,32,34,72-74,77-82,93,94,99,106,135,144}.
9. **In a patient with suspected IA/*Aspergillus* disease, what is the diagnostic value of fibrobronchoscopy (FBC)?**

Recommendation

107. The consensus considers that in order to perform a diagnostic approach in a patient with a high suspicion of developing IA/*Aspergillus* disease, it is necessary to integrate and jointly evaluate the clinical, microbiological and imaging findings. It is considered that a patient at high risk of II/IPA, may present with non-specific symptoms (or subtle and/or absent symptoms) until the invasive disease is very advanced. **(strong recommendation, high-quality evidence)**^{4,21,97,201-209}.
108. In a patient with a high suspicion of developing IA/*Aspergillus* disease, the consensus recommends that if possible a FBC with BAL collection for anatomopathological and microbiological studies should be performed, provided that there are: (a) associated risk factors, (b) respiratory symptoms and/or fever of unknown origin, (c) evidence of hemoptysis, and/or (d) pulmonary involvement identified by chest CT. It is considered that a FBC to take a BAL sample, guided by a chest CT, allows obtaining appropriate samples to perform a diagnostic approach. **(strong recommendation, high-quality evidence)** (Table 7)^{21,77,79,81,205,206}.
109. It is considered that in a patient with a high suspicion of developing an IFI, a FBC with BAL collection using a standardized procedure, and referral of the sample for cytology, mycological culture, AGA measurement and fungal DNA detection, allows a diagnostic approach of a proven/probable IA/IPA. **(strong recommendation, moderate-quality evidence)** (Table 7, Annexes 5 and 7)^{4,21,77,78,81,97,105,110,132,137,152,187,190,195-200,203-206}.

10. In a patient with suspected IA/*Aspergillus* disease, what is the diagnostic value of imaging studies?

Recommendation

110. In a patient with a high suspicion of developing IA/*Aspergillus* disease, the consensus considers that imaging studies are essential for an adequate diagnostic approach. These studies make it possible to identify the site of infection and evaluate the type, number and size of the lesions, as well as the degree of local extension. They are considered to help redirect to the most appropriate area to obtain appropriate diagnostic specimens (e.g., FBC to take a BAL sample and/or CT-guided biopsy). **(strong recommendation, high-quality evidence)**^{4,11,21,97,102,201,202,204,209–231}.

a. In a patient with suspected IFI, what is the imaging approach for the diagnosis of IA/IPA?

Recommendation

111. The consensus recommends that in a patient with a high suspicion of developing an IA/IPA, with risk factors and/or respiratory symptoms present, the imaging approach of choice is through a thin-slice (1 mm/1 mm) chest CT scan, performed, where possible, with multi-detector equipment. This is in order to perform multi-planar reconstructions and image post-processing, as well as to decrease the radiation dose ALARA (As Low As Reasonably Achievable) principle. A chest CT is considered, independent of the results of a previous chest X-ray. **(strong recommendation, high-quality evidence)** (Tables 6 and 7)^{4,21,97,102,135,201,202,204,211–215}.

112. The consensus does not recommend contrast-enhanced chest CT in a patient with a high suspicion of developing an IA/IPA. A contrast-enhanced chest CT is recommended in the presence of a nodule or mass close to a large vessel. **(strong recommendation, high-quality evidence)** (Table 7)^{4,21,81,97,102,135,201,202,204,211–215}.

113. In a patient with a high suspicion of developing an IA/IPA, with risk factors and/or respiratory symptoms present, and in the face of a clinical finding of hemoptysis, the consensus recommends performing a chest angiotomography to identify possible vessel erosion. **(strong recommendation, moderate-quality evidence)**^{4,21,81,102,212–214,220}.

114. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, with persistent fever despite antibiotic treatment and/or clinical symptoms of pneumonia, with high suspicion of developing an IFI, the consensus recommends performing a chest CT scan to detect possible pulmonary abnormalities, where the presence of the "halo sign" is considered highly suggestive of proven/probable IA/IPA. Chest angiotomography is recommended to identify a possible vascular occlusion. **(strong recommendation, moderate-quality evidence)** (Table 7)^{21,77,79,210,211,220,231}.

115. In a critically ill patient with persistent fever despite anti-

biotic treatment and/or clinical symptoms of pneumonia, with high suspicion of developing an IA/IPA, the consensus recommends performing a chest CT scan to detect possible pulmonary abnormalities, where the presence of the "halo sign" and "air-crescent sign" are rare findings. **(strong recommendation, moderate-quality evidence)** (Table 7)^{4,21,81,210,211,231}.

116. In a SOTR patient, especially a lung transplant recipient, with persistent fever despite antibiotic treatment, and/or with clinical symptoms of pneumonia, with high suspicion of developing an IFI, the consensus recommends performing a chest CT scan for possible pulmonary abnormalities, where the presence of bilateral bronchial wall thickening and/or centrolobular opacities, and/or budding tree pattern, and/or ground-glass opacities and/or bilateral areas of consolidation, is considered suggestive of proven/probable IA/IPA. **(strong recommendation, moderate-quality evidence)** (Table 7)^{4,21,77,81,210,211,231}.

117. To evaluate the response to antifungal therapy in a patient diagnosed with an IA/IPA, the consensus recommends a chest CT scan for follow-up after at least 2 weeks of treatment. More frequent follow-up is recommended if the patient deteriorates clinically and/or in the presence of a nodule or mass close to a large vessel. **(strong recommendation, high-quality evidence)**^{4,21,81,97,102,202,204}.

b. In a patient with suspected IFI/IA, what is the imaging approach for the diagnosis of extrapulmonary forms of aspergillosis?

Recommendation

118. The consensus considers that in a patient with a high suspicion of developing an IFI/IA, with risk factors and/or signs and symptoms present, an imaging approach that includes the use of CT, MRI and/or ultrasound, allows a diagnostic approach of a form of extrapulmonary aspergillosis. **(strong recommendation, high-quality evidence)**^{4,21,32,81,211}.

119. The consensus considers that in a patient with a high suspicion of developing IFI/IA, with risk factors and/or signs and symptoms present, the characteristics of the images have a fundamental role in the evaluation of disease activity, response to treatment and/or related complications, which can affect a variety of systems and/or organs (CNS, paranasal sinuses, bone and joints, cardiac, gastrointestinal, genitourinary, etc.), and allow a timely and accurate diagnostic approach to a form of extrapulmonary aspergillosis. **(strong recommendation, moderate-quality evidence)** (Table 7)^{4,21,32,81,211,221–230}.

c. In a patient with suspected *Aspergillus* disease, what is the imaging approach for the diagnosis of chronic and/or allergic forms associated with *Aspergillus* spp?

Recommendation

120. In a patient with a high suspicion of developing a chronic and/or allergic form associated with *Aspergillus* spp. with risk factors and/or respiratory symptoms present,

the consensus recommends that the imaging approach of choice for the diagnostic approach is a contrast-enhanced chest CT and/or a chest X-ray. **(strong recommendation, high-quality evidence)** (Tables 6 and 7)^{9,21,32,34,35,92,97,102,168,202,204,212}.

121. In a patient with a high suspicion of developing CPA, with risk factors and/or respiratory symptoms present, the consensus recommends performing a chest CT scan to detect possible pulmonary abnormalities, where the presence of cavitation and/or a fungus ball and/or pleural thickening and/or upper lobe fibrosis is considered suggestive of a chronic form associated with *Aspergillus* spp. **(strong recommendation, moderate-quality evidence)** (Table 7)^{9,21,32,34,35,92,97,102,168,202,204,212}.
122. In a patient with high suspicion of developing CPA, with risk factors and/or respiratory symptoms present, the consensus recommends performing a chest angiotomography to evaluate the manifestation of hemoptysis and/or possible therapeutic failure. **(strong recommendation, moderate-quality evidence)**^{4,21,34,81,211}.
123. In a patient diagnosed with CPA, the consensus recommends performing a chest CT (low dose) and/or a chest X-ray to evaluate the response to antifungal therapy. It is recommended to start follow-up at 3-6 months and/or in the face of any important clinical change. **(strong recommendation, moderate-quality evidence)**^{4,21,34,81,211}.

11. In a patient with IA/*Aspergillus* disease, what is the value of using diagnostic tests in combination?

Recommendation

124. In a severely or mildly immunocompromised patient, during each risk period, the consensus recommends implementing a surveillance strategy based on different diagnostic tools that are available and/or accessible in a timely manner, used in combination. It is considered that, according to the clinical context of the patient, the use of different diagnostic approaches based on direct microscopy, mycological cultures, serological tests, fungal biomarkers, fungal DNA detection and/or imaging studies provides an accurate diagnostic approach. **(strong recommendation, high-quality evidence)** (Annex 7)^{78,83,84,94,128,129,232-237}.

a. In a patient with suspected IA/*Aspergillus* disease, when should we use a test-based approach?

Recommendation

Invasive infection

125. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, without antifungal prophylaxis against filamentous fungi, the consensus recommends performing the diagnostic approach of a proven/probable IA, by: (a) histopathology and/or culture positive for *Aspergillus* spp. from a respiratory tract sample (induced sputum, tracheal aspirates, BAL, etc.) and/or biopsy of the involved site and/or contiguous site, (b) positive PCR test from res-

piratory tract and/or biopsy specimen from the involved site and/or from serum, (c) positive AGA test from serum (x2) and/or from BAL (x1), and (d) abnormal CT and/or MRI findings (from clinically indicated sites). FBC with BAL collection is considered to increase the diagnostic yield for an IA/IPA, and allows ruling out other possible etiologic agents. **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-82,94,129,234-236}.

126. In a high-risk patient with hematologic malignancy and/or HSCT, with/without profound and prolonged neutropenia, receiving antifungal prophylaxis against filamentous fungi, the consensus recommends performing the diagnostic approach of a proven/probable IA, by: (a) positive culture for *Aspergillus* spp. from BAL, (b) positive PCR test from BAL, (c) positive AGA test from BAL (x1), (d) positive BDG test from serum, and (e) abnormal CT and/or MRI findings (from clinically indicated sites). It is considered that if all microbiological tests of a patient with a high suspicion of developing an IA/IPA are negative, a biopsy of the involved site should be performed for an accurate diagnostic approach and to rule out other possible etiologic agents. **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-82,94,129,146,234-237}.
127. In a non-neutropenic oncology patient, especially a patient with a solid lung tumor and/or pulmonary metastatic disease, in whom CPA has been ruled out, the consensus recommends making the diagnostic approach of proven/probable IA by: (a) nonspecific clinical presentation (or subtle and/or absent symptoms), (b) positive culture for *Aspergillus* spp. from BAL, (c) positive PCR test from BAL, (d) positive AGA test from BAL (x1), (e) abnormal findings on chest CT (or clinically indicated sites). **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-83,94,102,129}.
128. In a SOTR patient, especially a lung transplant recipient, the consensus recommends performing the diagnostic approach of a proven/probable IA/IPA by: (a) positive culture for *Aspergillus* spp. from BAL, (b) positive PCR test from BAL, (c) positive AGA test from BAL (x1), (d) positive *Aspergillus*-LFD test from serum, (e) abnormal findings on chest CT (or clinically indicated sites). FBC with BAL collection is considered the key to diagnosis; chest CT is of limited value, as classical imaging findings are rare, and measurement of AGA from serum is less sensitive. **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,8,21,32,34,77-83,94,102,232}.
129. In a patient in the ICU, the consensus recommends making the diagnostic approach of proven/probable IA/IPA by: (a) positive culture for *Aspergillus* spp. from BAL, (b) positive PCR test from BAL, (c) positive AGA test from BAL (x1), (d) positive BDG test from serum, (e) abnormal findings on chest CT (or from clinically indicated sites). Findings of "halo sign" or "air-crescent sign" on chest CT are considered rare. **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-83,94,129,236}.
130. In a patient diagnosed with COPD, the consensus recommends making the diagnostic approach of a proven/probable IA/IPA by: (a) positive culture for *Aspergillus* spp.

Table 9. Systemic antifungal agents for treatment of IA. ADME, Doses.

POLYENES	ANPHOTERICIN B	ADME				
		A	D	E		
		A	It is not absorbed PO.			
		D	It has little CNS penetration.			
		M	Degradation in tissue.			
		E	Renal (<10% unmodified); Biliary (15%)			
		Adjustment	<u>Kidney failure</u> : no changes, no dose adjustment required. On HD or CAPD it dialyzes <5%. <u>Liver failure</u> : no changes, no dose adjustment required.			
		Pregnancy	It can be used in cases of strict necessity.			
		Lactation	Contraindicated			
		Formulations	D-AmB	L-AmB	LC-AmB	
		Dosage for adults	IV. 0,4-1 mg/kg/d	IV. 3-5 mg/kg/d	IV. 3-5 mg/kg/d	
		Dosage for children	IV. 0,4-1 mg/kg/d	IV. 3-5 mg/kg/d	IV. 3-5 mg/kg/d	
ECHINOCANDINS	CASPOFUNGIN	A	IV only.			
		D	Widespread, although it decreases in CNS.			
		M	Hepatic and spontaneous chemical degradation.			
		E	Renal (41% inactive metabolites); Fecal (35% inactive metabolites).			
		Adjustment	<u>Kidney failure</u> : No changes. On HD: does not dialyze. <u>Liver failure</u> : <i>Child-Pugh A</i> : no changes, no dose adjustment required, <i>Child-Pugh B</i> : 70 mg 1st d, then 35 mg/d, <i>Child-Pugh C</i> : no studies available in this population.			
		Pregnancy	Avoid it if there is an alternative.			
		Lactation	Should be avoided.			
		Dosage for adults	IV, 70 mg 1st dose, then 50 mg/d (70 mg/d if >80 kg), perfuse the doses in 60 min.			
		Dosage for children	IV, <3 months of age, 25 mg/m ² /d, one dose. > 3 months 70 mg/m ² , then 50 mg/m ² /d, one dose, not to exceed the adult dose.			
	ANIDULAFUNGIN	A	IV only.			
		D	Widespread, although it decreases in CNS.			
		M	Spontaneous chemical degradation.			
E		Renal (<1%); Fecal (>90% inactive metabolites).				
Adjustment		<u>Kidney failure</u> : no changes. On HD: does not dialyze. <u>Liver failure</u> : no changes, no dose adjustment required.				
Pregnancy		Avoid it if there is an alternative.				
	Lactation	Should be avoided.				
	Dosage for adults	IV, 200 mg 1st dose (in 3h), then 100 mg/d (in 1.5h).				
	Dosage for children	IV, 3 mg/kg 1st dose, then 1.5 mg/kg/d.				
MICAFUNGIN	A	IV only.				
	D	Widespread, although it decreases in CNS.				
	M	Hepatic (via catechol-O-methyltransferase), CYP3A <i>in vitro</i> .				
	E	Renal [10-30% (<1% unmodified)]; Fecal (70% as metabolites).				
	Adjustment	<u>Kidney failure</u> : no changes. On HD: does not dialyze. <u>Liver failure</u> : <i>Child-Pugh A</i> and <i>B</i> : no changes, no dose adjustment required, <i>Child-Pugh C</i> : no data.				
	Pregnancy	Avoid it if there is an alternative.				
	Lactation	Should be avoided.				
	Dosage for adults	IV. 100-150 mg/d (in perfusion for 1 h).				
	Dosage for children	Newborn: 4 to 10 mg/kg/d in one dose. > 4 months (<40 kg): 2-4 mg/kg/d in one dose. > 40 kg: 100 mg/d.				
AZOLES	FLUCONAZOLE	A	IV and PO (high).			
		D	Very wide. High CNS penetration			
		M	Hepatic. [10% (CYP3A4)].			
		E	Renal [70-80% (glomerular filtration and tubular reabsorption)].			
		Adjustment	<u>Kidney failure</u> : GF > 50: 100-400 mg/kg/d; GF 10-50: 50% of dose; GF <10: 50% of dose. In HD, it dialyzes 50%: 100-400 mg/kg/d (post-HD); In CAPD: 50-200 mg/kg/d; In CRRT: 200-400 mg/kg/d. <u>Liver failure</u> : <i>Child-Pugh A</i> : no dose adjustment required. <i>Child-Pugh B</i> , <i>Child-Pugh C</i> : use it as a last option, monitor liver function and assess dosage adjustment.			
		Pregnancy	Avoid it if there is an alternative.			
			Lactation	It can be used.		
			Dosage for adults	PO 50-800 mg/d; IV. 50-800 mg/d. Requires loading dose in severe shock/sepsis: 800 mg (12 mg/kg).		
	Dosage for children	> 1 year, 3-12 mg/kg/d; neonates 6-12 mg/kg/d.				

ITRACONAZOLE	A	IV and PO.
	D	Low. Does not penetrate CNS.
	M	Hepatic, extensive via CYP3A4, CYP3A5, hydroxy-itraconazole metabolite (fluconazole-like activity).
	E	Renal (< 1% unmodified, 40% metabolites); Biliary (55% metabolites).
	Adjustment	<u>Kidney failure</u> : IV formulation contains cyclodextrin, which accumulates in kidney failure (not +2 weeks). GF > 10: no changes (IV formulation should not be used if GF < 30, use oral formulation, 50-100 mg/d), GF < 10: 50% of PO formulation. On HD: it dialyzes < 5%, 100 mg/12-24h PO formulation; In CAPD it dialyzes < 5%, 100 mg/12-24h PO formulation; In CRRT: 100-200 mg/12-24h of PO formulation. <u>Liver failure</u> : there are few data available for PO use. Caution should be exercised when administering it, and should be monitored in patients with hepatic dysfunction. In patients with increased liver enzymes or active liver disease, or in those who have experienced liver toxicity with other drugs, do not administer unless the expected benefits outweigh the risk of liver injury.
	Pregnancy	Avoid it if there is an alternative.
	Lactation	Should be avoided.
	Dosage for children	> 5 years, 2.5 mg/kg/12h.
	Dosage for children	> 5 years, 2.5 mg/kg/12h.
	VORICONAZOLE	A
D		Very wide. High CNS penetration
M		Hepatic. They are P-450 inhibitors. IV. CYP2C19, CYP3A4, CYP2C9; P.O. CYP3A4
E		Renal (85% inactive metabolites, 2% unmodified); Fecal (20% inactive metabolites).
Adjustment		<u>Kidney failure</u> : PO, no changes. With IV use, the diluent (cyclodextrin) may accumulate; GF > 50: 4 mg/kg/12h; GF 10-50: Do not use the IV formulation; GF < 50 (accumulation of cyclodextrin with IV formulation), use the PO formulation 200 mg/12h; GF < 10: use the PO formulation 200 mg/12h. On HD: does not dialyze, use the PO formulation 200 mg/12h; CAPD: does not dialyze, use the PO formulation 200 mg/12h; CRRT: use the PO formulation: 200 mg/12h. <u>Liver failure</u> : IV: <i>Child-Pugh</i> A and B: 6 mg/kg/12h for 2 doses, then 2 mg/kg/12h (50% dose reduction). PO: <i>Child-Pugh</i> A and B: 400 mg/kg /12h for 2 doses (> 40 kg weight), then 100 mg/12h (50% dose reduction). <i>Child-Pugh</i> C: avoid it, no studies are available in this population.
Pregnancy		Avoid it if there is an alternative.
Lactation		Should be avoided.
Dosage for adults		IV. 6 mg/kg/12h 1st dose, then 4 mg/kg/12h. PO > 40 kg, 400 mg/12h 1st dose, then 200 mg/12h; < 40 kg, 200 mg/12h 1st dose, then 100 mg/12h. Bioavailability of 95%, administration with food decreases it by 20-30% (administer it on an empty stomach).
Dosage for children		IV. 2-12 years or 12-14 years and weight < 50 kg, 9 mg/kg/12h. 1st dose, then 8 mg/kg/12h. PO. 9 mg/kg/12h (maximum dose 350 mg/12h). Child > 12 years and weight ≥ 50 kg or > 15 years, same as adult.
POSACONAZOLE		A
	D	Widespread.
	M	Hepatic (glucuronconjugation); Inactive metabolisms, CYP3A4.
	E	Renal (14% inactive metabolites); Fecal (77%, 66% unmodified).
	Adjustment	<u>Kidney failure</u> : GF > 50: 300 mg/d; GF 10-50: 300 mg/d; GF < 10: 300 mg/d. On HD: does not dialyze, 300 mg/d; In CAPD: 300 mg/d; In CRRT: 300 mg/d. <u>Liver failure</u> : no changes, no dose adjustment required.
	Pregnancy	Avoid it if there is an alternative.
	Lactation	Contraindicated.
	Dosage for adults	PO suspension (40 mg/mL): 400 mg/12h, with meals (if no meals are taken, 200 mg/6h). PO. 200 mg/8h (with food), for prophylaxis. Delayed-release tablets ([DRT] 100 mg): 300 mg/12h 1st dose, then 300 mg/d, for prophylaxis. IV: 300 mg/12h 1st dose, then 300 mg/d (prophylaxis). It takes 7-10 d to achieve steady state. It takes 7-10 d to reach steady state. No IV formulation. Administration with food (preferably fatty) significantly increases absorption. On the other hand, an increase in gastric pH (antacids, H antagonists, proton pump inhibitors) and grade I-II mucositis decrease it.
	Dosage for children	Children > 13 years old, same as in adults. Children < 13 years, there are no specific recommendations. Children 2-16 years with CGD for 30 d: 10-14 kg: 120 mg/12h; 15-19 kg: 160 mg/12h; 20-24 kg: 200 mg/12h; 25-29 kg: 220 mg/12h; 30-34 kg: 260 mg/12h; 35-39kg: 280 mg/12h; ≥40 kg: 300 mg/12h.

AZOLES ISAVUCONAZOLE	A	IV and PO.
	D	Widespread, although it decreases in CNS.
	M	Hepatic. CYP 3A4. CYP3A4 - CYP3A5.
	E	<1% urine. Degradation products in urine.
	Adjustment	<u>Kidney failure</u> : no changes. IV. GF > 50: 200 mg/d; GF 10-50: 200 mg/d; GF <10: 200 mg/d. On HD: 200 mg/d; In CAPD: 200 mg/d; In CRRT: 200 mg/d. <u>Liver failure</u> : No dose adjustment is required in patients with mild or moderate liver failure (<i>Child-Pugh A and B</i>). There is no experience in severe liver failure (<i>Child-Pugh C</i>).
	Pregnancy	Teratogenic.
	Lactation	Contraindicated.
	Dosage for adults	IV and PO: 200 mg/8h, first 48 h (6 doses), then 200 mg/d, started 12-24h after loading dose.
	Dosage for children	No data available.

IA: invasive aspergillosis; A: Administration; D: Distribution; M: Metabolism; E: Excretion; D-AmB: Amphotericin B deoxycholate; L-AmB: Liposomal amphotericin B; LC-AmB: Amphotericin B lipid complex; GF: Glomerular filtration; IV: Intravenous route; PO: Oral route; d: Day/days; h: Hour/hours; g: Grams; mg: Milligrams; kg: Kilograms; HD: Hemodialysis; CAPD: Continuous Ambulatory Peritoneal Dialysis; CRRT: Continuous Renal Replacement Therapy; CGD: Chronic Granulomatous Disease; CNS: Central Nervous System.

from BAL and/or sputum, (b) positive PCR test from BAL, (c) positive AGA test from BAL (x1), (d) abnormal findings on chest CT (or clinically indicated sites). **(strong recommendation, moderate-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-83,94,102,129,236}.

131. In an HIV+ patient, the consensus recommends making the diagnostic approach of proven/probable IA by: (a) histopathology and/or positive culture for *Aspergillus* spp. from a respiratory tract specimen (induced sputum, tracheal aspirates, BAL, etc.) and/or from a biopsy of the involved site and/or contiguous site, and (b) AGA positive test from serum (x2) and/or BAL (x1). **(strong recommendation, high-quality evidence)** (Table 7, Annex 7)^{4,21,32,34,77-82}.

Aspergillus disease

132. In an immunocompetent or mildly immunocompromised patient with a high suspicion of developing CPA, the consensus considers performing the diagnostic approach of an *Aspergillus* disease, by means of: (a) detection of elevated titers of *Aspergillus*-specific IgG Abs and/or *Aspergillus* precipitins, and (b) abnormal findings on chest CT (e.g., cavitory or nodular pulmonary infiltrate). **(strong recommendation, moderate-quality evidence)**^{9,34,92,102,168}.

Ethical disclosures

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Confidentiality of data. In this consensus there are not data from patients.

Right to privacy and informed consent. No data from patients is published.

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Protection of human and animal subjects. There are not experimental data from humans and animals in this work.

Supplementary material online

The Tables that are described as annex on the text, are available at the link for supplementary material online, of this manuscript, at the website of journal.

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