Title

Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches

Journal

Mycoses, 64(9)

ISSN

0933-7407

Authors

Jenks, Jeffrey D
Nam, Hannah H
Hoenigl, Martin

Publication Date

2021-09-01

DOI

10.1111/myc.13274

Peer reviewed

HHS Public Access

Author manuscript

Mycoses. Author manuscript; available in PMC 2022 December 27.

Published in final edited form as:

Mycoses. 2021 September ; 64(9): 1002–1014. doi:10.1111/myc.13274.

Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches

Jeffrey D. Jenks[1,2,3], **Hannah H. Nam**[4], **Martin Hoenigl**[2,3,5]

[1]Division of General Internal Medicine, Department of Medicine, University of California San Diego, San Diego, CA, USA

[2]Division of Infectious Diseases and Global Public Health, Department of Medicine, University of California San Diego, San Diego, CA, USA

[3]Clinical and Translational Fungal-Working Group, University of California San Diego, La Jolla, CA, USA

[4]Division of Infectious Diseases, Department of Medicine, University of California Irvine, Orange, CA, USA

[5]Section of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Abstract

Invasive aspergillosis (IA) is an increasingly recognised phenomenon in critically ill patients in the intensive care unit, including in patients with severe influenza and severe coronavirus disease 2019 (COVID-19) infection. To date, there are no consensus criteria on how to define IA in the ICU population, although several criteria are used, including the AspICU criteria and new consensus criteria to categorise COVID-19-associated pulmonary aspergillosis (CAPA). In this review, we describe the epidemiology of IA in critically ill patients, most common definitions used to define IA in this population, and most common clinical specimens obtained for establishing a mycological diagnosis of IA in the critically ill. We also review the most common diagnostic tests used to diagnose IA in this population, and lastly discuss the most common clinical presentation and imaging findings of IA in the critically ill and discuss areas of further needed investigation.

Keywords

Aspergillus galactomannan lateral flow assay; Aspergillus-specific lateral flow device; AspICU criteria; COVID-associated invasive pulmonary aspergillosis; critically ill patients; EORTC;

Correspondence: Jeffrey D. Jenks, Department of Medicine, University of California San Diego, 330 Lewis St, Suite 301, San Diego, CA 92103, USA. jjenks@ucsd.edu.

AUTHOR CONTRIBUTIONS

Jeffrey D. Jenks: Conceptualization (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Hannah Nam:** Conceptualization (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Martin Hoenigl:** Conceptualization (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). All the authors contributed equally to this work.

CONFLICT OF INTEREST

JDJ has received research funding from Astellas and Pfizer. HHN has nothing to disclose. M.H has received research funding from Astellas, Gilead and Pfizer.

Galactomannan; influenza-associated invasive aspergillosis; intensive care unit; Invasive aspergillosis; MSG criteria; polymerase chain reaction

1 | INTRODUCTION

Worldwide estimates indicate that over 1.8 million cases of invasive fungal infections occurred in 2017, including around 250,000 cases of invasive aspergillosis (IA).[1] Mould-active prophylaxis has shown some success in reducing IA in patients with traditional risk factors for IA, such as those with underlying haematological malignancy and prolonged neutropenia, although breakthrough infections may occur.[2–7] In contrast, the prevalence of IA continues to increase in non-neutropenic patients with severe underlying diseases, including those in intensive care units,[8–12] those with severe viral infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or influenza virus,[12–16] solid organ transplant recipients,[17] those receiving systemic glucocorticoids,[18] those with solid cancers,[8,19] those with chronic obstructive pulmonary disorder (COPD) and other chronic respiratory disorders, and those who have received ibrutinib.[8,20–22] The immune status, and particularly neutrophil count of the host, determines the pathogenesis of *Aspergillus* disease, which represents a spectrum ranging from allergic and chronic forms to airway-invasive and angio-invasive disease. In contrast with the neutropenic host, where *Aspergillus* grows angio-invasive within hours, there is an extended bronchial phase in the non-neutropenic host, where *Aspergillus* invades in an airway-invasive manner, often over the period of many days, prior to the disease become angio-invasive.[23,24] In line immunological mechanisms differ between the angio-invasive and the primarily airway-invasive type of *Aspergillus* disease,[25] as do radiological findings (often atypical findings in the non-neutropenic host),[14,26,27] and mycological findings (diagnostics in non-neutropenic host primarily from lung, versus blood testing such as with the Aspergillus galactomannan test in neutropenic host).[15,28]

We will here review the clinical definitions of invasive aspergillosis in the critically ill patient and focus specifically on mycological (which samples, which test) and clinical diagnosis of IA in the ICU setting. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required.

2 | EPIDEMIOLOGY

How often does IA occur in the ICU? *Aspergillus* spp. are isolated from lower respiratory tract samples in 0.7%–7% of critically ill patients, with findings suggesting invasive pulmonary aspergillosis in around half of these patients based no criteria including EORTC/MSG criteria and autopsy studies.[29–32] In one retrospective study between 2000 and 2003, of 1,850 admissions to the ICU, 127 patients (6.9%) were diagnosed with invasive aspergillosis, of which 89 patients (70%) of these patients lacked haematological malignancy.[31] A large international, multicentre observational study (*Asp*ICU study) examined the incidence of *Aspergillus* colonisation and IA in 30 ICUs in eight countries, including seven European countries and in India, from January 2000 to January 2011. Over this time period, 563 patients were diagnosed with either *Aspergillus* colonisation

(47%), proven IA (17%) or putative IA (36%) based on the *Asp*ICU criteria.[33] Of these patients, 70% were medical admissions for respiratory diseases (39%) including COPD (31%), cardiovascular disease (26%) and diabetes (16%), and from this total cohort, 11% received immunosuppressive therapy and 45% corticosteroids.[33]

IA is also an increasingly recognised superinfection complicating patients with severe influenza and SARS-CoV-2 infection in the ICU. Influenza-associated aspergillosis is well documented, occurring in 16%–23% of patients with influenza admitted to the ICU, and is associated with a morality rate over 50%.[12,34,35] Recently, IA has been recognised as a severe complication of COVID-19 infection in patients in the ICU, occurring in 18%–39% of patients, and is associated with a mortality rate of up to 50%.[36–47]

Thus, IA occurring in critically ill patients lacking traditional risk factors is an increasingly recognised phenomenon, with non-traditional risk factors including systemic corticosteroid use, underlying respiratory diseases, cardiovascular disease, and diabetes mellitus, as well as severe influenza and severe COVID-19 infection.

3 | IPA DEFINITION IN THE ICU

In the absence of proven infection, which requires histologic evidence or fungal detection from normally sterile body fluids or materials, the diagnosis of IA is based on compatible signs and symptoms of infection in an appropriate host with supportive radiological and mycological findings.[12,15,26,27,48] The newly revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions focus primarily on neutropenic patients with underlying haematological malignancies and a ‘typical’ presentation of IA, and are not applicable to non-neutropenic patients where IA pathogenesis differs,[26] particularly those in the ICU who do not fulfil EORTC/MSG host factors and thereby cannot fulfil criteria of probable disease.[49] Therefore, the newly revised EORTC/MSG criteria are only applicable to the subset of ICU patients with underlying haematological malignancies, solid organ transplant recipients or severe immuno-suppression (as defined by host factors of those criteria, Table 1), but not to the ICU population as a whole. Furthermore, even those non-neutropenic ICU patients who fulfil EORTC/MSG criteria based on host factors and develop IA may present with an atypical clinical presentation or radiological findings, and equivocal diagnostic test results, particularly due to the low sensitivity of galactomannan (GM) and other tests performed in blood.[23,24,26,50–54] Therefore, the newly revised EORTC/MSG criteria have only very limited applicability in the ICU setting.

Based on their findings that ‘typical’ signs of IA on computed tomography (CT) in neutropenic patients, such as the ‘halo sign’ or ‘air-crescent sign’, are only found in a subset of non-neutropenic patients with proven disease, where atypical infiltrates and consolidations are most common, Blot and colleagues have created an alternative clinical algorithm for diagnosing IA in the ICU setting, the AspICU algorithm (Table 1), with the aim of overcoming some of limitations of the EORTC/MSG criteria.[27] Originally, this algorithm defined the growth of *Aspergillus* spp. from lower respiratory specimens

on culture as an entry criterion[27] and tried to distinguish colonisation from true infection/disease, relying on clinical signs that typically occur during later stages of invasive pulmonary aspergillosis (IPA) in non-neutropenic patients.[8] However, large studies have shown that sensitivity of culture from lower respiratory tract specimens is imperfect (65% or lower),[22,52,55,56] while bronchoalveolar lavage fluid (BALF) GM testing has significantly higher sensitivity than culture, with both sensitivity and specificity close to 90%.[22,52,55,56] Therefore, the AspICU criteria have been modified in some studies[10,23,50,57] to include positive BALF GM as entry criterion, which is essential to make them applicable to broader cohorts of ICU patients, such as going beyond the subset with cultural detection of *Aspergillus* spp. In addition, the inclusion of newer diagnostic tests such as BALF Aspergillus real-time polymerase chain reaction (PCR), the *Aspergillus*-specific lateral flow device (LFD) and Aspergillus galactomannan lateral flow assay (LFA) point-of-care (POC) tests have been recommended.[57,58] International work on improved definitions of IA in the ICU is currently in progress.[50,59]

Specifically for patients with COPD, Bulpa and colleagues have developed criteria that include acute COPD exacerbation with dyspnoea requiring treatment with systemic corticosteroids as a requirement and classify disease, compatible radiological findings, and *Aspergilllus* spp. growth from BAL culture and serum GM as diagnostic criteria[60] (Table 2). These criteria have not been updated and are rarely used.

IA is emerging as an important complication in patients with severe viral infections who develop acute respiratory distress syndrome (ARDS), including cytomegalovirus, influenza virus and most recently SARS-CoV-2, where IA is associated with high mortality rates.[14–16,40,41,43,45,61] Specific criteria have been developed for patients with severe influenza who develop IA,[12,62] which differentiate between *Aspergillus* tracheobronchitis and IA in patients without tracheobronchitis. These criteria eliminate traditional host factors and use influenza-like-illness, positive influenza PCR or antigen and temporal relationship as entry criterion and use modified mycological and clinical criteria (summarised in Table 2).[62] Very recently, Koehler and other experts from around the world developed the European Confederation of Medical Mycology (ECMM)/International Society for Human and Animal Mycology (ISHAM) consensus criteria for defining *Aspergillus* disease in patients with COVID-19, which were endorsed by medical mycology societies from around the world.[15] These criteria differentiate between the pulmonary form and the tracheobronchial form of COVID-19-associated aspergillosis (CAPA), and use confirmed SARS-CoV-2 infection with ARDS requiring ICU admission as entry criterion.[15] They also use modified clinical, radiological ad mycological criteria that are summarised in Table 2.

For those patients who develop IA while receiving systemic antifungal(s), defining whether or not this infection presents a breakthrough infection and warrants a change of antifungal treatment is important.[4,5] ECMM/MSG consensus criteria for defining breakthrough infections for research have been developed and also cover the ICU setting.[6] However, validation of these criteria for clinical use is currently pending.

4 | MYCOLOGICAL DIAGNOSIS OF IA IN THE ICU

4.1 | Mode of Obtaining clinical specimen for diagnosis

4.1.1 | Biopsy—Biopsy for tissue remains the most definitive way to diagnose IA, particularly when invasive pulmonary aspergillosis is suspected.[63,64] Lung tissue can be obtained through bronchoscopy with trans-bronchial biopsy, surgical biopsy (eg wedge biopsy), or trans-thoracic needle biopsy and sent for fungal stain, culture, and histopathology. Unfortunately, most critically ill patients in the ICU are too unstable to undergo these procedures.

4.1.2 | Blood—Blood is another readily available clinical specimen in patients in the ICU and is often used for the screening of IA in high-risk populations such as those with underlying haematological malignancy and SOT recipients, primarily with GM testing from serum. In non-neutropenic patients such as those in the ICU, the sensitivity of serum GM is around 30%, reflecting the fact that these patients typically develop tissue invasive rather than angio-invasive disease early on in the disease course. In a review of patients with CAPA, the pooled sensitivity of GM from serum was only 21% at an optical density index of 0.5.[14] Neither the *Aspergillus*-specific LFD nor *Aspergillus* galactomannan LFA has been extensively evaluated from blood in the ICU population.

4.1.3 | Sputum—Although sputum is a readily available clinical specimen, the finding of *Aspergillus* spp. in a sputum sample does not necessarily indicate infection and may simply represent colonisation of *Aspergillus*. Culture of *Aspergillus* from sputum has a low sensitivity of around 35% in patients with active infection.[65] Thus, positive testing from sputum should be interpreted based on the entire clinical context including compatible imaging findings or other diagnostic tests that support IA. In one study non-haematology patients admitted to the hospital or ICU, GM from sputum had a sensitivity and specificity of 100% and 62%, respectively, at an optical density index of 1.2.[66] The role of GM testing from sputum in ICU patients is less clear.

4.1.4 | Tracheal aspirate—Tracheal aspirate (TA) is the collection of endotracheal secretions in intubated patients and can be used to diagnose IA using PCR, GM, or from culture.[14,67–69] A positive diagnostic test needs to be interpreted in the context of other clinical signs and symptoms of IA as a positive test can also reflect *Aspergillus* tracheitis or colonisation. Still, TA may be a good option as a screening modality in high-risk patients in the ICU or in patients too clinically unstable to undergo bronchoscopy. For the diagnosis of CAPA, a positive GM or *Aspergillus* culture from TA alone can be used to make a diagnosis of ‘possible’ IA,[15] or a positive test may prompt more extensive testing such as bronchoscopy or further imaging to make a more definitive diagnosis.

4.1.5 | Bronchoalveolar Lavage—Bronchoscopy for BALF involves the insertion of a bronchoscope with a light and small camera through the nose or mouth and down the trachea into the bronchi and bronchioles where secretions can be sampled. This procedure should be considered in a patient stable enough to tolerate this procedure when there is high index of suspicion for IA. Diagnostic tests on BALF fluid should include fungal stain and culture,

GM, and possibly PCR where this assay is available. The overall sensitivity of culture from BALF is between 30% and 60% and specificity 50% in intubated patients.[70] As with testing of other clinical specimens, results need to be interpreted in the clinical context given possible background colonisation with *Aspergillus* spp. Similarly, a positive *Aspergillus* PCR from BALF may represent colonisation, especially in patients with structural or functional lung disease, or may represent contamination.

4.2 | Diagnostic tests

4.2.1 | Histology and culture—Histopathological diagnosis of IA relies on the identification of hyphae forms in tissue biopsied from a normally sterile site. On direct microscopic examination, *Aspergillus* is narrow (3–12 μm wide) with septated, hyaline, acute angle branching hyphae with 45-degree branching.[71] Although rare, the presence of conidial heads is pathognomonic for the diagnosis of aspergillosis.[72] On microscopy, *Aspergillus* can be confused with several other filamentous fungi including *Scedosporium* spp. and *Fusarium* spp. so definitive identification of the pathogen by culture is desirable.[73] When recovered *Aspergillus* begins to develop within 24–48 h on fungal media and sheep blood agar, with colonies appearing as velutinous, grey-blue-green colonies.[73]

Although microscopy and culture have traditionally been the cornerstone for the diagnosis of IA, the diagnostic yield varies based on host factors and is typically rather low. Even in patients with ‘classic’ risk factors for IA such as patients with underlying haematological malignancy or SOT recipients, the majority of patients are diagnosed with IA by other means than microscopy or culture. The yield can be even lower in ICU patients who may lack traditional clinical signs and symptoms of infection and have atypical radiological findings of IA.[20] Furthermore, microscopy and culture alone cannot distinguish between colonisation and infection and lung biopsies are often difficult to perform in critically ill patients who may have other comorbid conditions, may be hemodynamically unstable or have respiratory distress, or coagulation disorders making biopsy challenging.[74] Thus, more non-invasive strategies are often preferred in this population.

Lastly, the susceptibility profiles of *Aspergillus fumigatus* are changing with increased resistance against triazole antifungals, including voriconazole and isavuconazole,[75–77] which are commonly used to treat these infections. Culture-based methods can determine antifungal resistance but is time-consuming, and delayed diagnosis of resistance *Aspergillus* infections can lead to poor patient outcomes. Increasingly, new molecular-based approaches for detecting triazole resistance to *Aspergillus*, including PCR to detect mutations to the *Cyp51A* protein, have been developed to overcome some of the limitations of culture.[78]

4.2.2 | Galactomannan—Antigen-based testing, such as with the conventional GM test, has now become the ‘gold-standard’ test for the diagnosis of IA, particularly in critically ill patients. GM is a polysaccharide found in the cell wall of *Aspergillus* spp. and is released by growing hyphae and germinating spores or conidia. In immunocompromised patients with angio-invasive growth, GM can be detectable in serum, although GM is often not present in the serum of non-neutropenic patients, in which airway-invasive growth is more typical.[11,26,79] Thus, GM testing from BALF is preferred in this setting. For conventional

GM testing, a positive result is based on an optical density (OD) cut-off GM index of ≥0.5 from serum and >1.0 from BALF. Still, GM testing has some limitations including the potential for false-positive results, such as in the setting of concurrent medications. False-positive serum and BALF results have been found in patients who received amoxicillin–clavulanate, piperacillin–tazobactam, and cefepime, as well as false-positive BALF results in patients receiving carbapenems and ceftriaxone.[80–82] False-negative results are particularly common in patients on mould-active prophylaxis[53,83] and can be found in settings with delayed turnaround times.

4.2.3 | Polymerase chain reaction—Molecular methods such as PCR and polymerase chain reaction–enzyme-linked immunosorbent assay (PCR-ELISA) have been available for over two decades. Overall, the pooled sensitivity and specificity of PCR from blood are 79% and 80% for a single positive test result and 60% and 95% for two consecutive positive test results.[84] Still, PCR has several limitations. First, PCR testing varies in methodology, standardisation and performance across settings. In addition, like the GM test from blood, PCR from blood has decreased utility in patients on mould-active prophylaxis.[85] Lastly and perhaps most importantly, PCR from serum has a sensitivity as low as 11% in ICU patients,[86] although the sensitivity improved to 56% in BALF specimens.[86]

4.2.4 | Lateral flow assay and lateral flow device—Both the LFA and LFD assays are POC diagnostic tests for the diagnosis of IA. These assays are simple to use, do not require advanced laboratory equipment, with results available in under an hour. Thus, they obviate the need for complex laboratory equipment required by PCR and avoid varying turnaround times that sometimes limit conventional GM testing. In the ICU setting, the LFD from BALF has a pooled sensitivity of 64% and specificity of 85%, which is slightly inferior to its performance in patients with haematological malignancies, where its pooled sensitivity and specificity are 70% and 88%, respectively.[51] In a recent multicentre study, the LFA from BALF had a sensitivity and specificity of 74% and 83%, respectively, at an optical density index cut-off of 1.5, with comparable performance to the conventional GM assay.[10] In another recent study, the LFA from BALF had a sensitivity that ranged from 88% to 94%, depending on whether the EORTC/MSG, AspICU or modified AspICU definitions for IPA were used, and a specificity of 81%.[87] The performance of neither the LFA nor LFD assays has specifically been evaluated from blood in ICU patients, so more investigation is necessary to determine the role of testing blood in ICU patients.

4.2.5 | Role of beta-D-glucan testing—(1–3)-beta-D-glucan (BDG) is a fungal cell wall component that is currently used as a serum marker for the presumptive diagnosis and treatment monitoring of invasive fungal infections (IFI) ICU and has been proposed as a marker of IA.[36,86,88–90] In contrast, BALF BDG levels are non-specific and often represent *Candida* colonisation of the respiratory tract, although they have prognostic potential in the ICU.[91,92] However, in the absence of IFI, blood levels of BDG also emerged as candidate biomarker of gut fungal translocation.[93–99] Fungal translocation is the passage of fungal components through a compromised intestinal epithelial barrier due to immune dysfunction, gut damage or altered gut microbiota composition. Translocation may include only fungal components or—much more rarely—fungal pathogens that may cause infection and sepsis,

as recently outlined in a report of two patients with severe COVID-19 developing fungemia due to *Saccharomyces cerevisiae* after receiving probiotics containing the same strains.[100] Elevated serum BDG levels have been frequently reported in patients with diseases and conditions associated with a leaky gut who do not have other evidence of systemic fungal infection.[101–105] In fact, it has been shown that serum BDG levels correlate strongly with sequential organ failure assessment (SOFA) scores in patients with sepsis.[101] While the value of serum BDG for diagnosis and treatment stratification of systemic *Candida* infections in the ICU has shown some promise,[89,106] the role of BDG for diagnosis IA remains unclear, as elevated levels may simply represent fungal translocation of *Candida* components form the gut and not necessarily pulmonary *Aspergillus* infection with airway invasion.

5 | CLINICAL AND R ADIOLOGICAL PRESENTATION OF IA IN THE ICU

5.1 | Clinical presentation

Clinical presentation of IA differs between non-neutropenic and neutropenic patients. These differences are explained by different immunological mechanism. In murine models of IA, immunopathology of non-neutropenic mice on glucocorticoids shows extensive inflammation with minimal angio-invasion and low fungal burden, in contrast with extensive angio-invasion and necrosis with minimal inflammation in neutropenic mice.[18] These findings are supported by autopsy studies in humans.[107] In non-neutropenic patients with more airway-invasive IA, fever is present in around 70% of patients compared to over 95% of neutropenic patients. Cough and chest pain are also less frequent among non-neutropenic patients (28% and 11%, respectively, versus 67% and 33% in neutropenic patients).[8] Interestingly, despite angio-invasion occurring more frequently in neutropenic patients, hemoptysis may not occur more frequently in neutropenic compared to non-neutropenic patients.[8] Clinical findings of IA (ie fever, shortness of breath, cough) strongly overlap with those observed in severe influenza and COVID-19.[14] IA of the paranasal sinuses that may progress rapidly to cause CNS IA is seen rarely in non-neutropenic patients, except those with profound immuno-suppression or uncontrolled diabetes.

5.2 | Imaging findings

Radiological findings of IA are variable and differ significantly depending on host factors. Chest X-ray can rarely differentiate between IA and other aetiologies of disease; therefore, early computed tomography (CT) of the chest is the imaging modality of choice to diagnose IA. Classically, in neutropenic patients IA presents as pulmonary nodules with surrounding ground-glass infiltrates (termed the ‘halo sign’), which reflect angio-invasion and haemorrhage into the area surrounding the fungal infection. These nodules may cavitate and produce the ‘air-crescent sign’. These two typical signs of neutropenic IA on imaging are rarely observed in non-neutropenic patients,[8,27] with other typical radiological signs of IPA such as solitary nodules near the pleura only occurring in about 30% of non-neutropenic patients, where unspecific infiltrates and consolidations are the most frequently observed finding.[24,27] Radiological findings of IA such as unspecific infiltrates and halo sign may also overlap with those of severe COVID-19.[14,15]

6 | CONCLUSION

IA affecting critically ill patients in the ICU is an increasingly recognised phenomenon, particularly in patients receiving systemic corticosteroids, with underlying respiratory or cardiovascular disease, as well as in patients with severe influenza and severe COVID-19 infection. The diagnosis of IA can be challenging given the lack of consensus on how to define IA in this population, the non-specific symptoms of IA in critically ill patients, and the non-specific signs of IA on imaging. Furthermore, diagnostic assays such as PCR and GM— particularly from blood—suffer from low sensitivity, and bronchoscopy and biopsy are often difficulty in these patients as they are often too clinically unstable to perform these procedures.

Studies evaluating the LFA and LFD POC tests have shown good sensitivity and specificity from BALF, and these may be good options in settings where GM is unavailable or long turnaround times may make GM less useful. Further evaluation of these tests from blood is needed in the critically ill population. Non-CT-based imaging modalities such as antibody-guided PET/MR imaging (immunoPET/MRI) have shown promise in murine models but need further investigation, particularly in immunocompetent patients in the ICU. Lastly, further consensus on how best to define IA in the ICU, including in patients with breakthrough invasive fungal infections, is important so clear definitions are being used across different settings.

Funding information

This work was partially funded by NIH UL1TR001442-061, and an investigator initiated research study from Pfizer (Nr 60278533).

REFERENCES

1. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi. 20173(4):57. 10.3390/jof3040057
2. Lenczuk D, Zinke-Cerwenka W, Greinix H, et al. Antifungal prophylaxis with posaconazole delayed-release tablet and oral suspension in a real-life setting: plasma levels, efficacy, and tolerability. Antimicrob Agents Chemother. 2018;62(6):e02655–17. [PubMed: 29581116]
3. Duarte RF, Sanchez-Ortega I, Cuesta I, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis. 2014;59(12):1696–1702. [PubMed: 25165088]
4. Jenks JD, Cornely OA, Chen SC, Thompson GR 3rd, Hoenigl M. Breakthrough invasive fungal infections: who is at risk? Mycoses. 2020;63(10):1021–1032. [PubMed: 32744334]
5. Jenks JD, Gangneux J-P, Schwartz IS, et al. Diagnosis of breakthrough fungal infections in the clinical mycology laboratory: an ECMM consensus statement. J Fungi (Basel). 2020;6(4):216. [PubMed: 33050598]
6. Cornely OA, Hoenigl M, Lass-Flörl C, et al. Defining breakthrough invasive fungal infection-Position paper of the my-coses study group education and research consortium and the European Confederation of Medical Mycology. Mycoses. 2019;62(9):716–729. [PubMed: 31254420]
7. Hoenigl M, Salmanton-García J, Walsh TJ, et al. Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology. Lancet Infect Dis. 2021;16:S1473–3099(20)30784–2.

8. Cornillet A, Camus C, Nimubona S, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. Clin Infect Dis. 2006;43(5):577–584. [PubMed: 16886149]

9. Bassetti M, Peghin M, Vena A. Challenges and solution of invasive aspergillosis in non-neutropenic patients: a review. Infect Dis Ther. 2018;7(1):17–27. [PubMed: 29273978]

10. Jenks JD, Prattes J, Frank J, et al. Performance of the bronchoalveolar lavage fluid aspergillus galactomannan lateral flow assay with cube reader for diagnosis of invasive pulmonary aspergillosis: a Multicenter Cohort Study. Clin Infect Dis. 2020.

11. Eigl S, Prattes J, Lackner M, et al. Multicenter evaluation of a lateral-flow device test for diagnosing invasive pulmonary aspergillosis in ICU patients. Crit Care. 2015;19(1):178. [PubMed: 25927915]

12. Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med. 2018;6(10):782–792. [PubMed: 30076119]

13. Thompson GR III, Cornely OA, Pappas PG, et al. Invasive aspergillosis as an under-recognized superinfection in COVID-19. Open Forum Infect Dis. 2020;7(7):ofaa242.

14. Arastehfar A, Carvalho A, van de Veerdonk FL, et al. COVID-19 Associated Pulmonary Aspergillosis (CAPA)-from immunology to treatment. J Fungi. 2020;6(2):91.

15. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis. 2020.

16. Hoenigl M Invasive Fungal Disease complicating COVID-19: when it rains it pours. Clin Infect Dis; 2020.

17. Pappas P, Alexander B, Andes D, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101–1111. [PubMed: 20218876]

18. Lewis RE, Kontoyiannis DP. Invasive aspergillosis in glucocorticoid-treated patients. Med Mycol. 2009;47(Suppl 1):S271–S281. [PubMed: 18654923]

19. Yan X, Li M, Jiang M, Zou LQ, Luo F, Jiang Y. Clinical characteristics of 45 patients with invasive pulmonary aspergillosis: retrospective analysis of 1711 lung cancer cases. Cancer. 2009;115(21):5018–5025. [PubMed: 19637340]

20. Guinea J, Torres-Narbona M, Gijón P, et al. Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. Clin Microbiol Infect. 2010;16(7):870–877. [PubMed: 19906275]

21. Ghez D, Calleja A, Protin C, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. Blood. 2018;131(17):1955–1959. [PubMed: 29437588]

22. Prattes J, Flick H, Prüller F, et al. Novel tests for diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. Am J Respir Crit Care Med. 2014;190(8):922–929. [PubMed: 25203869]

23. Jenks JD, Mehta SR, Taplitz R, Aslam S, Reed SL, Hoenigl M. Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: Aspergillus Galactomannan Lateral Flow Assay versus Aspergillus-specific Lateral Flow Device test in bronchoalveolar lavage. Mycoses. 2019;62(3):230–236. [PubMed: 30565352]

24. Nucci M, Nouer SA, Cappone D, Anaissie E. Early diagnosis of invasive pulmonary aspergillosis in hematologic patients: an opportunity to improve the outcome. Haematologica. 2013;98(11):1657–1660. [PubMed: 24186309]

25. Jenks J, Rawlings S, Garcia-Vidal C, et al. Immune Parameters for Diagnosis and Treatment Monitoring in Invasive Mold Infection. J Fungi. 2019;5(4):E116.

26. Bergeron A, Porcher R, Sulahian A, et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. Blood. 2012;119(8):1831–1837.quiz 956. [PubMed: 22010103]

27. Blot SI, Taccone FS, Van den Abeele A-M, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med. 2012;186(1):56–64. [PubMed: 22517788]

28. Petraitiene R, Petraitis V, Bacher JD, Finkelman MA, Walsh TJ. Effects of host response and antifungal therapy on serum and BAL levels of galactomannan and (1–>3)-beta-D-glucan in experimental invasive pulmonary aspergillosis. Med Mycol. 2015;53(6):558–568. [PubMed: 26129890]
29. Vandewoude KH, Blot SI, Depuydt P, et al. Clinical relevance of Aspergillus isolation from respiratory tract samples in critically ill patients. Crit Care. 2006;10(1):R31. [PubMed: 16507158]
30. Dimopoulos G, Piagnerelli M, Berré J, Eddafali B, Salmon I, Vincent JL. Disseminated aspergillosis in intensive care unit patients: an autopsy study. J Chemother. 2003;15(1):71–75. [PubMed: 12678418]
31. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med. 2004;170(6):621–625. [PubMed: 15229094]
32. Janssen JJ, Strack van Schijndel RJ, van der Poest Clement EH, Ossenkoppele GJ, Thijs LG, Huijgens PC. Outcome of ICU treatment in invasive aspergillosis. Intensive Care Med. 1996;22(12):1315–1322. [PubMed: 8986479]
33. Taccone F, Van den Abeele A-M, Bulpa P, et al. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Crit Care. 2015;19(1):7. [PubMed: 25928694]
34. Wauters J, Baar I, Meersseman P, et al. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. Intensive Care Med. 2012;38(11):1761–1768. [PubMed: 22895826]
35. van de Veerdonk FL, Kolwijck E, Lestrade PPA, et al. Influenza-associated aspergillosis in critically Ill patients. Am J Respir Crit Care Med. 2017;196(4):524–527. [PubMed: 28387526]
36. Alanio A, Dellière S, Fodil S, Bretagne S, Mégarbane B. Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19. Lancet Respir Med. 2020;8(6):e48–e49. [PubMed: 32445626]
37. van Arkel ALE, Rijpstra TA, Belderbos HNA, van Wijngaarden P, Verweij PE, Bentvelsen RG. COVID-19-associated pulmonary aspergillosis. Am J Respir Crit Care Med. 2020;202(1):132–135. [PubMed: 32396381]
38. Rutsaert L, Steinfort N, Van Hunsel T, et al. COVID-19-associated invasive pulmonary aspergillosis. Ann Intensive Care. 2020;10(1):71. [PubMed: 32488446]
39. Nasir N, Farooqi J, Mahmood SF, Jabeen K. COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: An observational study from Pakistan. Mycoses. 2020;63(8):766–770. [PubMed: 32585069]
40. Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study. Clin Infect Dis. 2020.
41. Gangneux J-P, Reizine F, Guegan H, et al. Is the COVID-19 pandemic a good time to include aspergillus molecular detection to categorize aspergillosis in ICU patients? A monocentric experience. J Fungi. 2020;6(3):105.
42. Chen X, Zhao B, Qu Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. Clin Infect Dis. 2020;71(8):1937–1942. [PubMed: 32301997]
43. White PL, Dhillon R, Cordey A, et al. A national strategy to diagnose COVID-19 associated invasive fungal disease in the ICU. Clin Infect Dis. 2020.
44. Dupont D, Menotti J, Turc J, et al. Pulmonary aspergillosis in critically ill patients with Coronavirus Disease 2019 (COVID-19). Med Mycol. 2021;59(1):110–114. [PubMed: 32914189]
45. Koehler P, Cornely OA, Böttiger BW, et al. COVID-19 associated pulmonary aspergillosis. Mycoses. 2020;63(6):528–534. [PubMed: 32339350]
46. Salmanton-García J, Sprute R, Stemler J, et al. COVID-19–associated pulmonary aspergillosis, March–August 2020. Emerg Infect Dis. 2021;27(4):1077–1086. [PubMed: 33539721]
47. Permpalung N, Chiang T-Y, Massie AB, et al. COVID-19 associated pulmonary aspergillosis in mechanically ventilated patients. Clin Infect Dis. 2021.

48. Nucci M, Nouer SA, Grazziutti M, Kumar NS, Barlogie B, Anaissie E. Probable invasive aspergillosis without prespecified radiologic findings: proposal for inclusion of a new category of aspergillosis and implications for studying novel therapies. Clin Infect Dis. 2010;51(11):1273–1280. [PubMed: 21034199]
49. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis. 2020;71(6):1367–1376. [PubMed: 31802125]
50. Bassetti M, Giacobbe DR, Grecchi C, Rebuffi C, Zuccaro V, Scudeller L. Performance of existing definitions and tests for the diagnosis of invasive aspergillosis in critically ill, adult patients: a systematic review with qualitative evidence synthesis. J Infect. 2020.
51. Jenks JD, Hoenigl M. Point-of-care diagnostics for invasive aspergillosis: nearing the finish line. Expert Rev Mol Diagn. 2020;20(10):1009–1017. [PubMed: 32902359]
52. Eigl S, Hoenigl M, Spiess B, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Med Mycol. 2017;55(5):528–534. [PubMed: 27744310]
53. Heldt S, Prattes J, Eigl S, et al. Diagnosis of invasive aspergillosis in hematological malignancy patients: Performance of cytokines, Asp LFD, and Aspergillus PCR in same day blood and bronchoalveolar lavage samples. J Infect. 2018;77(3):235–241. [PubMed: 29972764]
54. Centers for Disease Control and Prevention (CDC). Shigella flexneri serotype 3 infections among men who have sex with men--Chicago, Illinois, 2003–2004. MMWR Morb Mortal Wkly Rep. 2005;54(33):820–822. [PubMed: 16121121]
55. Meersseman W, Lagrou K, Maertens J, et al. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. Am J Respir Crit Care Med. 2008;177(1):27–34. [PubMed: 17885264]
56. Cordonnier C, Botterel F, Ben Amor R, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. Clin Microbiol Infect. 2009;15(1):81–86.
57. Salzer HJF, Lange C, Honigl M. Aspergillus in airway material : Ignore or treat? Der Internist. 2017;58(11):1150–1162. [PubMed: 28986661]
58. Jenks JD, Miceli MH, Prattes J, Mercier T, Hoenigl M. The aspergillus lateral flow assay for the diagnosis of invasive aspergillosis: an update. Curr Fungal Infect Rep. 2020;14(4):378–383. [PubMed: 33312332]
59. Bassetti M, Scudeller L, Giacobbe DR, et al. Developing definitions for invasive fungal diseases in critically ill adult patients in intensive care units. Protocol of the FUNgal infections Definitions in ICU patients (FUNDICU) project. Mycoses. 2019;62(4):310–319. [PubMed: 30426598]
60. Bulpa P, Dive A, Sibille Y. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J. 2007;30(4):782–800. [PubMed: 17906086]
61. Marr KA, Platt A, Tornheim JA, et al. Aspergillosis complicating severe coronavirus disease. Emerg Infect Dis J. 2021;27(1):18–25.
62. Verweij PE, Rijnders BJA, Brüggemann RJM, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Med. 2020;46(8):1524–1535. [PubMed: 32572532]
63. Lass-Flörl C How to make a fast diagnosis in invasive aspergillosis. Med Mycol. 2019;57(Supplement_2):S155–S160. [PubMed: 30816965]
64. Lass-Florl C, Resch G, Nachbaur D, et al. The value of computed tomography-guided percutaneous lung biopsy for diagnosis of invasive fungal infection in immunocompromised patients. Clin Infect Dis. 2007;45(7):e101–e104. [PubMed: 17806041]
65. Schelenz S, Barnes RA, Barton RC, et al. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. Lancet Infect Dis. 2015;15(4):461–474. [PubMed: 25771341]
66. Xiao W, Gong D-Y, Mao B, et al. Sputum signatures for invasive pulmonary aspergillosis in patients with underlying respiratory diseases (SPARED): study protocol for a prospective diagnostic trial. BMC Infect Dis. 2018;18(1):271. [PubMed: 29890956]

67. Borman AM, Palmer MD, Fraser M, et al. COVID-19-associated invasive aspergillosis: data from the UK National Mycology Reference Laboratory. J Clin Microbiol. 2020;59(1).
68. Roman-Montes CM, Martinez-Gamboa A, Diaz-Lomelí P, et al. Accuracy of galactomannan testing on tracheal aspirates in COVID-19-associated pulmonary aspergillosis. Mycoses. 2021;64(4):364–371. [PubMed: 33217784]
69. Contou D, Dorison M, Rosman J, et al. Aspergillus-positive lower respiratory tract samples in patients with the acute respiratory distress syndrome: a 10-year retrospective study. Ann Intensive Care. 2016;6(1):52. [PubMed: 27294891]
70. Hage CA, Carmona EM, Epelbaum O, et al. Microbiological laboratory testing in the diagnosis of fungal infections in pulmonary and critical care practice. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2019;200(5):535–550. [PubMed: 31469325]
71. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev. 2011;24(2):247–280. [PubMed: 21482725]
72. Lanzarin LD, Mariano LC, Macedo MC, Batista MV, Duarte AN Sr. Conidial heads (Fruiting Bodies) as a hallmark for histopathological diagnosis of angioinvasive aspergillosis. Autops Case Rep. 2015;5(4):9–18.
73. McClenny N Laboratory detection and identification of Aspergillus species by microscopic observation and culture: the traditional approach. Med Mycol. 2005;43(Suppl 1):S125–S128. [PubMed: 16110804]
74. Kaziani K, Mitrakou E, Dimopoulos G. Improving diagnostic accuracy for invasive pulmonary aspergillosis in the intensive care unit. Ann Transl Med. 2016;4(18):352. [PubMed: 27761456]
75. Pfaller MA, Rhomberg PR, Wiederhold NP., et al. In vitro activity of isavuconazole against opportunistic fungal pathogens from two mycology reference laboratories. Antimicrob Agents Chemother. 2018;62(10):e01230–18. [PubMed: 30061288]
76. Pinto E, Monteiro C, Maia M, et al. Aspergillus species and antifungals susceptibility in clinical setting in the north of Portugal: Cryptic species and emerging azoles resistance in A. fumigatus. Front Microbiol. 2018;9:1656. [PubMed: 30083151]
77. Buil JB, Brüggemann RJM, Wasmann RE, et al. Isavuconazole susceptibility of clinical Aspergillus fumigatus isolates and feasibility of isavuconazole dose escalation to treat isolates with elevated MICs. J Antimicrob Chemother. 2018;73(1):134–142. [PubMed: 29048485]
78. Jenks JD, Spiess B, Buchheidt D, Hoenigl M. (New) Methods for detection of Aspergillus fumigatus resistance in clinical samples. Curr Fungal Infect Rep. 2019;13(3):129–136. [PubMed: 31552129]
79. Maertens J, Maertens V, Theunissen K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. Clin Infect Dis. 2009;49(11):1688–1693. [PubMed: 19886801]
80. Boonsarngsuk V, Niyompattama A, Teosirimongkol C, Sriwanichrak K. False-positive serum and bronchoalveolar lavage Aspergillus galactomannan assays caused by different antibiotics. Scand J Infect Dis. 2010;42(6–7):461–468. [PubMed: 20192889]
81. Park SY, Lee S-O, Choi S-H, et al. Aspergillus galactomannan antigen assay in bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. J Infect. 2010;61(6):492–498. [PubMed: 20833201]
82. Brownback KR, Pitts LR, Simpson SQ. Utility of galactomannan antigen detection in bronchoalveolar lavage fluid in immunocompromised patients. Mycoses. 2013;56(5):552–558. [PubMed: 23496321]
83. Hoenigl M, Seeber K, Koidl C, et al. Sensitivity of galactomannan enzyme immunoassay for diagnosing breakthrough invasive aspergillosis under antifungal prophylaxis and empirical therapy. Mycoses. 2013;56(4):471–476. [PubMed: 23432536]
84. Cruciani M, Mengoli C, Barnes R, et al. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev. 2019;9(9):CD009551.
85. Egger M, Jenks JD, Hoenigl M, Prattes J. Blood Aspergillus PCR: the good, the bad, and the ugly. J Fungi. 2020;6(1):18.

86. Boch T, Reinwald M, Spiess B, et al. Detection of invasive pulmonary aspergillosis in critically ill patients by combined use of conventional culture, galactomannan, 1–3-beta-D-glucan and Aspergillus specific nested polymerase chain reaction in a prospective pilot study. J Crit Care. 2018;47:198–203. [PubMed: 30015290]
87. Mercier T, Dunbar A, Veldhuizen V, et al. Point of care aspergillus testing in intensive care patients. Crit Care. 2020;24(1):642. [PubMed: 33168049]
88. Prattes J, Schneditz D, Pruller F, et al. 1,3-ss-d-Glucan testing is highly specific in patients undergoing dialysis treatment. J Infect. 2017;74(1):72–80. [PubMed: 27717781]
89. Prattes J, Hoenigl M, Rabensteiner J, et al. Serum 1,3-beta-d-glucan for antifungal treatment stratification at the intensive care unit and the influence of surgery. Mycoses. 2014;57(11):679–686. [PubMed: 25040144]
90. Giacobbe DR, Mikulska M, Tumbarello M, et al. Combined use of serum (1,3)-β-D-glucan and procalcitonin for the early differential diagnosis between candidaemia and bacteraemia in intensive care units. Crit Care. 2017;21(1):176. [PubMed: 28693606]
91. Reischies FMJ, Prattes J, Prüller F, et al. Prognostic potential of 1,3-beta-d-glucan levels in bronchoalveolar lavage fluid samples. J Infect. 2016;72(1):29–35. [PubMed: 26416472]
92. Mutschlechner W, Risslegger B, Willinger B, et al. Bronchoalveolar lavage fluid (1,3)beta-D-Glucan for the diagnosis of invasive fungal infections in solid organ transplantation: a Prospective Multicenter Study. Transplantation. 2015;99(9):e140–e144. [PubMed: 25710608]
93. Hoenigl M Fungal translocation: a driving force behind the occurrence of non-AIDS events? Clin Infect Dis. 2020;70(2):242–244. [PubMed: 30861074]
94. Mehraj V, Ramendra R, Isnard S, et al. Circulating (1->3)-beta-D-Glucan is associated with immune activation during HIV infection. Clin Infect Dis. 2019.
95. Hoenigl M, Lin J, Finkelman M, et al. Glucan rich nutrition does not increase gut translocation of beta-glucan. Mycoses. 2021;64(1):24–29. [PubMed: 32780885]
96. Ramendra R, Isnard S, Lin J, et al. CMV seropositivity is associated with increased microbial translocation in people living with HIV and uninfected controls. Clin Infect Dis. 2019.
97. Ramendra R, Isnard S, Mehraj V, et al. Circulating LPS and (1->3)-beta-D-Glucan: a Folie a Deux contributing to HIV-associated immune activation. Front Immunol. 2019;10:465. [PubMed: 30967860]
98. Gianella S, Letendre SL, Iudicello J, et al. Plasma (1 ->3)-beta-D-glucan and suPAR levels correlate with neurocognitive performance in people living with HIV on antiretroviral therapy: a CHARTER analysis. J Neurovirol. 2019;25(6):837–843. [PubMed: 31297727]
99. Hoenigl M, Moser CB, Funderburg N, et al. Soluble urokinase plasminogen activator receptor is predictive of non-AIDS events during antiretroviral therapy-mediated viral suppression. Clin Infect Dis. 2019;69(4):676–686. [PubMed: 30418519]
100. Ventoulis I, Sarmourli T, Amoiridou P, et al. Bloodstream infection by Saccharomyces cerevisiae in two COVID-19 patients after receiving supplementation of saccharomyces in the ICU. J Fungi. 2020;6(3):98.
101. Leelahavanichkul A, Worasilchai N, Wannalerdsakun S, et al. Gastrointestinal leakage detected by serum (1–>3)-beta-D-Glucan in mouse models and a Pilot Study in patients with sepsis. Shock. 2016;46(5):506–518. [PubMed: 27172153]
102. Issara-Amphorn J, Surawut S, Worasilchai N, et al. The synergy of endotoxin and (1→3)-β-D-Glucan, from gut translocation, worsens sepsis severity in a lupus model of Fc gamma receptor IIb-deficient mice. J Innate Immun. 2018;10(3):189–201. [PubMed: 29393221]
103. Yang A-M, Inamine T, Hochrath K, et al. Intestinal fungi contribute to development of alcoholic liver disease. J Clin Invest. 2017;127(7):2829–2841. [PubMed: 28530644]
104. Hoenigl M, Perez-Santiago J, Nakazawa M, et al. (1–>3)-beta-d-Glucan: a biomarker for microbial translocation in individuals with acute or early HIV infection? Front Immunol. 2016;7:404. [PubMed: 27752257]
105. Szyszkowitz A, Zurl C, Herzeg A, et al. Serum 1,3-beta-D-Glucan values during and after laparoscopic and open intestinal surgery. Open Forum Infect Dis. 2018;5(12):ofy296.

106. Cento V, Alteri C, Mancini V, et al. Quantification of 1,3-β-d-glucan by Wako β-glucan assay for rapid exclusion of invasive fungal infections in critical patients: A diagnostic test accuracy study. Mycoses. 2020;63(12):1299–1310. [PubMed: 32810888]

107. Chamilos G, Luna M, Lewis RE, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989–2003). Haematologica. 2006;91(7):986–989. [PubMed: 16757415]

TABLE 1

IPA definitions[12,27,49]

Diagnostic criteria	Revised EORTC/MSG criteria (2019)[49]	AspICU criteria[27]	Modified AspICU[12]
Host factors	**1** Recent history of neutropenia (<0.5 × 10^9 neutrophils/L for more than 10 days) that is temporally related to the onset of fungal disease. **2** Haematological malignancy **3** Receipt of an allogeneic stem cell transplant. **4** Receipt of a solid organ transplant **5** Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of ≥0.3 mg/kg corticosteroids for ≥3 weeks in the past 60 days **6** Treatment with other recognised T-cell immunosuppressants, such as calcineurin inhibitors, tumour necrosis factor-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days **7** Treatment with recognised B-cell immunosuppressants, such as Bruton's tyrosine kinase inhibitors, eg ibrutinib **8** Acute graft-versus-host disease grade III or IV involving the gut, lungs or liver that is refractory to first-line treatment with steroids **9** Inherited severe immunodeficiency.	**1** Neutropenia (<0.5 × 10^9 neutrophils/L) preceding or at the time of ICU admission. **2** Underlying haematological or oncological malignancy treated with cytotoxic agents. **3** Glucocorticoid treatment (prednisone equivalent >20 mg/day) **4** Congenital or acquired immunodeficiency	None
Clinical Data	*For tracheobronchitis, bronchoscopic findings with:* **1** Tracheobronchial ulceration **2** Nodule **3** Pseudomembrane **4** Plaque **5** Eschar *For sino-nasal diseases* **1** Acute localised pain (including pain radiating to the eye) **2** Nasal ulcer with black eschar extension from the paranasal sinus across bony barriers, including into the orbit	*One of the following signs or symptoms:* **1** Fever refractory to at least 3 days of appropriate antibiotic therapy. **2** Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause. **3** Dyspnoea. **4** Haemoptysis. **5** Pleural friction rub or pleuritic chest pain. **6** Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support.	*One of the following signs or symptoms:* **1** Fever refractory to at least 3 days of appropriate antibiotic therapy. **2** Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause. **3** Dyspnoea. **4** Haemoptysis. **5** Pleural friction rub or chest pain. **6** Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support.

Diagnostic criteria	Revised EORTC/MSG criteria (2019)[49]	AspICU criteria[27]	Modified AspICU[12]
Radiological findings	*For LRT, patients must have subjected to at least one CT scan and must exhibit 1 of the following 4 signs:* **1** Dense, well-circumscribed lesion(s) with or without a halo sign. **2** An air-crescent sign. **3** A cavity. **4** Wedge-shaped and segmental or lobar consolidation *For Central nervous system infection* **1** Of the following 2 signs: Focal lesions on imaging Meningeal enhancement on magnetic resonance imaging or CT	Any infiltrate or abnormal pulmonary imaging by portable chest XR or CT scan of the lungs.	Any infiltrate or abnormal pulmonary imaging by portable chest XR or CT scan of the lungs.
Mycological findings	**1** Positive culture and/or microscopy result for *Aspergillus* from sputum, BAL, bronchial brush or aspirate **2** Galactomannan antigen detected in plasma, serum, BALF or CSF with any 1 of the following: **1.** Single serum or plasma: ≥1.0 **2.** BAL fluid: ≥1.0 **3.** Single serum or plasma: >0.7 and BAL fluid ≥0.8 **4.** CSF: ≥1.0 **3** Aspergillus PCR: **1.** Plasma, serum, or whole blood 2 or more consecutive PCR tests positive **2.** BAL fluid 2 or more duplicate PCR tests positive **3.** At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid	*Aspergillus*-positive lower respiratory tract specimen culture (= entry criterion) In the absence of a host factor: Semiquantitative *Aspergillus*-positive culture of BALF (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae.	*One or more has to be present:* **1** Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for *Aspergillus* from tissue. **2** A positive *Aspergillus* culture from a BALF. **3** Galactomannan optical index on BALF ≥1. **4** Galactomannan optical index on serum ≥0.5.
Categories	**Proven IFD:** Histopathological, cytopathologic or direct microscopic evidence for *Aspergillus* spp. in a specimen obtained by needle aspiration or biopsy accompanied by evidence of associated tissue damage OR Recovery of *Aspergillus* spp. by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine OR Amplification of fungal DNA by PCR combined with DNA sequencing when moulds are seen in formalin-fixed paraffin-embedded tissue **Probable IFD**: Host factor +Clinical feature/Radiological findings +Mycological findings **Possible IFD:** Host factor +Clinical feature/Radiological findings	**Proven IPA**: Identical to EORTC/MSG criteria **Putative IPA**: *Aspergillus*-positive lower respiratory tract specimen culture + Clinical Data + Radiological Findings + Host Factors OR Semiquantititative *Aspergillus*-positive culture plus positive cytological smear **Respiratory Tract Colonisation:** when ≥1 criterion necessary for a diagnosis of putative IPA is not met.	**Proven IPA**: Identical to EORTC/MSG criteria **Putative IPA**: Clinical data + Radiological findings + Mycological findings **Respiratory Tract Colonisation:** when ≥1 criterion necessary for a diagnosis of putative IPA is not met.

Note: Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; CT, computed tomography; DNA, deoxyribonucleic acid; EORTC./MSG, European Organization for the Research and Treatment of Cancer/Mycoses Study Group; h, hours; ICU, intensive care unit; IFD, invasive fungal disease; IPA, invasive pulmonary aspergillosis; kg, kilogram; L, litre; LRT, lower respiratory tract; mg, milligram; PCR, polymerase chain reaction; spp, species; XR, X-ray.

TABLE 2

IPA in special populations[15,60,62]

Disease	Classification	Host factors/Entry criterion	Clinical factors	Radiographic findings	Mycological evidence
COPD	Proven	History of COPD		Any pulmonary lesion present for <3 months	Histopathological or cytopathological examination, from needle aspiration or biopsy specimen obtained from pulmonary lesion showing hyphae consistent with *Aspergillus* and evidence of associated tissue damage if *accompanied by one of the following:* **1** Positive culture of *Aspergillus* spp. from any LRT sample **2** Positive serum antibody/antigen testing for A *fumigatus* (including precipitins). **3** Confirmation that hyphae observed are those of *Aspergillus* by a direct molecular, immunological method and/or culture.
	Probable	As for proven IPA but without confirmation that *Aspergillus* is responsible. **1** Patients with a pulmonary functional level of stage III or IV according to the GOLD guidelines. **2** Recent exacerbation of dyspnoea **3** Patients treated with steroids, with no strict requirement regarding the usage, dosage or duration.	Exacerbation of dyspnoea or bronchospasm resistant to appropriate treatment including antibiotics.	Pulmonary lesions on chest imaging (radiograph or CT scan) findings <3 months, unresponsive to appropriate treatment including antibiotics	*One of the following:* **1** Positive culture and/or microscopy findings for *Aspergillus* from the LRTs. **2** Positive *serum antibody/antigen test* for *A fumigatus* (including precipitin). **3** Two consecutive positive serum galactomannan tests. **4** Confirmation that the hyphae observed are those of Aspergillus by a direct molecular, immunological method and/or culture.
	Possible	**a1** Patients with a pulmonary functional level of stage III or IV according to the GOLD guidelines. **e2** Recent exacerbation of dyspnoea **a3** Patients treated with steroids, with no strict requirement	Exacerbation of dyspnoea or bronchospasm resistant to appropriate treatment including antibiotics.	Pulmonary lesions on chest imaging (radiograph or CT scan) findings <3 months, unresponsive to appropriate treatment including antibiotics	Without positive *Aspergillus* culture or serology.

Disease	Classification	Host factors/Entry criterion	Clinical factors	Radiographic findings	Mycological evidence
		regarding the usage, dosage or duration.			
	Colonisation	History of COPD	No exacerbation of dyspnoea, bronchospasm	No new pulmonary infiltrate	Positive *Aspergillus* culture from LRT.
Influenza	Tracheobronchitis, Proven	Admission to ICU with positive influenza test (PCR or rapid Ag) within 1 week prior to or 72–96 h post-admission to ICU.	ICU admission for respiratory distress with positive influenza test temporally related to ICU admission.	No requirements	Biopsy or brush specimen of airway plaque, pseudomembrane or ulcer *showing one of the following:* **1** Hyphal elements and *Aspergillus* growth on culture. **2** Positive *Aspergillus* PCR in tissue.
	Tracheobronchitis, Probable	Admission to ICU with positive influenza test (PCR or rapid Ag) within 1 week prior to or 72–96 h post-admission to ICU.	ICU admission for respiratory distress with positive influenza test temporally related to ICU admission.	No requirements	Biopsy or brush specimen of airway plaque, pseudomembrane or ulcer *showing one of the following:* **1** Serum GM index >0.5 **2** BAL GM index ≥1.0 **3** Positive BAL culture **4** Positive tracheal aspirate culture **5** Positive sputum culture **6** Hyphae consistent with *Aspergillus*
	Influenza-Associated Pulmonary Aspergillosis (IAPA), Proven	Admission to ICU with positive influenza test (PCR or rapid Ag) within 1 week prior to or 72–96 h post-admission to ICU.	ICU admission for respiratory distress with positive influenza test temporally related to ICU admission.	Pulmonary infiltrate	Lung biopsy showing invasive fungal elements and *Aspergillus* growth on culture or positive *Aspergillus* PCR in tissue.
	Influenza-Associated Pulmonary Aspergillosis (IAPA), Probable	Admission to ICU with positive influenza test (PCR or rapid Ag) within 1 week prior to or 72–96 h post-admission to ICU.	ICU admission for respiratory distress with positive influenza test temporally related to ICU admission.	Pulmonary infiltrate	*At least one of the following:* **1** Serum GM index >0.5 **2** BAL GM index ≥1.0 **3** Positive BAL culture
		Admission to ICU with positive influenza test (PCR or rapid Ag) within 1 week prior to or 72–96 h post-admission to ICU.	ICU admission for respiratory distress with positive influenza test temporally related to ICU admission.	Cavitating infiltrate not attributed to another cause	At least one of the following: **1** Positive sputum culture **2** Positive tracheal aspirate culture

Disease	Classification	Host factors/Entry criterion	Clinical factors	Radiographic findings	Mycological evidence
COVID-19	Tracheobronchitis or other pulmonary form, Proven	**1** Respiratory insufficiency requiring intensive care. **2** Positive SARS-CoV-2 RT-PCR anytime during 2 weeks between hospital admission or positive RT-PCR within 72–96 h after ICU admission	Respiratory insufficiency requiring intensive care with clinical symptoms compatible with COVID-19.		*At least one of the following:* **1** Histopathological or direct microscopic detection of fungal hyphae, showing invasive growth with associated tissue damage. **2** *Aspergillus* recovered by culture or microscopy or histology **3** PCR obtained by a sterile aspiration or biopsy from a pulmonary site, showing an infectious disease process
	Tracheobronchitis, Probable	**1** Respiratory insufficiency requiring intensive care. **2** Positive SARS-CoV-2 RT-PCR anytime during 2 weeks between hospital admission or positive RT-PCR within 72–96 h after ICU admission	**1** Respiratory insufficiency requiring intensive care with clinical symptoms compatible with COVID-19. **2** Tracheobronchitis, indicated by tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis.		*At least one of the following:* **1** Microscopic detection of fungal elements in BAL, indicating a mould **2** Positive BAL culture or PCR **3** Serum GM index >0.5 **4** Serum LFA index >0.5 **5** BAL GM index ≥1.0 **6** BAL LFA index ≥1.0
	Pulmonary forms, Probable	**1** Respiratory insufficiency requiring intensive care. **2** Positive SARS-CoV-2 RT-PCR anytime during 2 weeks between hospital admission or positive RT-PCR within 72–96 h after ICU admission	Respiratory insufficiency requiring intensive care with clinical symptoms compatible with COVID-19.	Pulmonary infiltrate, preferable documented by chest CT, or cavitating infiltrate (not attributed to another cause).	*At least one of the following:* **1** Microscopic detection of fungal elements in BAL, indicating a mould **2** Positive BAL culture **3** Serum GM index >0.5 **4** Serum LFA index >0.5 **5** BAL GM index ≥1.0 **6** BAL LFA index ≥1.0 **7** Two or more positive aspergillus PCR tests in plasma, serum, or whole blood[a] **8** Single positive *Aspergillus* PCR in BALF (<36 cycles)[a] **9** Single positive *Aspergillus* PCR in plasma, serum, or whole blood AND

Disease	Classification	Host factors/Entry criterion	Clinical factors	Radiographic findings	Mycological evidence
					Single positive *Aspergillus* PCR in BALF (any cycle threshold)[a]
	Pulmonary forms, Possible	**1** Respiratory insufficiency requiring intensive care. **2** Positive SARS-CoV-2 RT-PCR anytime during 2 weeks between hospital admission or positive RT-PCR within 72–96 h after ICU admission	Respiratory insufficiency requiring intensive care with clinical symptoms compatible with COVID-19.	Pulmonary infiltrate, preferable documented by chest CT, or cavitating infiltrate (not attributed to another cause).	*At least one of the following:* **1** Microscopic detection of fungal elements in non-BAL, indicating a mould **2** Positive non-BAL culture **3** Single non-BAL GM index >4.5 **4** Non-BAL GM index >1.2 twice or more **5** Non-BAL GM index >1.2 plus another non-BAL mycology test positive (non-BAL PCR or LFA)

Note: Non-BAL is considered a blind application of 10–20 ml saline recovered by aspiration via the closed suction system in an intubated patient. BAL and non-BAL are not currently considered equal for diagnosing CAPA.

Abbreviations: Ag, antigen; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disorder; COVID-19, coronavirus disease 2019;CT, computed tomography; GM, galactomannan; GOLD, Global Initiative for Obstructive Lung Disease; IAPA, influenza-associated pulmonary aspergillosis; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; LFA, lateral flow assay; LRT, lower respiratory tract; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; spp, species.

[a]In case of patients with COPD or chronic respiratory disease, the PCR or culture should be confirmed by galactomannan testing to rule out colonisation or chronic aspergillosis.