Clinical Infectious Diseases

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Revision and Update of the Consensus De nitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium

J. Pe e D (e ,[1] Sha (C. Che (,[2] Ca A. Ka ff a (,[3] Wi ia J. S ei (bach,[4] J h (W. Badd e ,[5] Pa E. Ve ei ,[6] C e i J. C a (c ,[7] J h (R. Wi (ga d,[8] Sha (R. L c ha ,[9] A (d ea H. G ,[10]

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Correspondence: J. P. Donnelly, De Hoefkamp 1096, 6545 MD Nijmegen, The Netherlands (p.donnelly@usa.net).

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Table 1. Criteria for Proven Invasive Fungal Disease

Fungus	Microscopic Analysis: Sterile Material	Culture: Sterile Material	Blood	Serology	Tissue Nucleic Acid Diagnosis
Molds[a]	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Recovery of a hyaline or pigmented mold 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	Blood culture that yields a mold[c] (eg, *Fusarium* species) in the context of a compatible infectious disease process	Not applicable	Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
Yeasts[a]	Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells, for example, *Cryptococcus* species indicating encapsulated budding yeasts or *Candida* species showing pseudohyphae or true hyphae[d]	Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [<24 hours ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process	Blood culture that yields yeast (eg, *Cryptococcus* or *Candida* species) or yeast-like fungi (eg, *Trichosporon* species)	Cryptococcal antigen in cerebrospinal fluid or blood confirms cryptococcosis	Amplification of fungal DNA by PCR combined with DNA sequencing when yeasts are seen in formalin-fixed paraffin-embedded tissue
Pneumocystis	Detection of the organism microscopically in tissue, BAL fluid, expectorated sputum using conventional or immunofluorescence staining	Not applicable	Not applicable	Not applicable	Not applicable
Endemic mycoses	Histopathology or direct microscopy of specimens obtained from an affected site showing the distinctive form of the fungus	Recovery by culture of the fungus from specimens from an affected site	Blood culture that yields the fungus	Not applicable	Not applicable

Abbreviations: BAL, bronchoalveolar lavage; PCR, polymerase chain reaction.

[a]If culture is available, append the identification at the genus or species level from the culture results.

[b]Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Grocott-Gomori methenamine silver stain or periodic acid Schiff stain to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (eg, calcofluor or blankophor).

[c]Recovery of *Aspergillus* species from blood cultures rarely indicates endovascular disease and almost always represents contamination.

[d]*Trichosporon* and yeast-like *Geotrichum* species and *Blastoschizomyces capitatus* may also form pseudohyphae or true hyphae.

and nonspecific surrogate tests, such as increased C-reactive protein or thrombocytopenia, which has been shown to be a predictor of candidemia in infants [16, 17]. In neonates, a positive urine culture has a significance similar to that of a positive blood culture as an indicator of IC [16]. Radiographic findings are less specific in children than those reported in adults [18]. Chest computerized tomography (CT) scans in children with proven invasive pulmonary aspergillosis (IPA) commonly show nonspecific changes and not the halo sign, air crescent formation, or cavitation seen in adults [19].

There are also far fewer data to support the clinical use of nonculture-based fungal biomarkers in neonates and children [20], although the GM assay performs similarly in children and adults when used as an adjunctive tool to diagnose invasive aspergillosis (IA) [20, 21]. Likewise, there are few data regarding the use of BDG, *C* mannan antigen, and anti-mannan antibody biomarkers in pediatrics [22]. Recent data support the utility of BDG in CSF for the diagnosis and therapeutic monitoring of children with *C* meningoencephalitis [23], but the data are sparse regarding the utility of PCR assays and the T2Candida assay for diagnosis [24].

Diagnostic Testing and Imaging

In the previous definitions [1], indirect tests for diagnosing IFD were only included if there was sufficient evidence that they had been standardized and validated. Moreover, commercial tests were included only if criteria for interpretation had been provided. Hence, while tests for GM and BDG were incorporated, tests for detecting fungal nucleic acid were not [1]. Furthermore, there was no agreement about appropriate thresholds, so the manufacturers' analytical thresholds were adopted. The evidence for using GM to diagnose IA has grown considerably since then, and testing for BDG has been extended to a wide range of patients. With respect to *A* PCR, the International Society of Human and Animal Mycology working group Fungal PCR Initiative (FPCRI; www.fpcri.eu) has made significant progress toward setting a standard for the technique after vigorous validation [25].

Imaging: Group 2

There is mounting evidence that the radiologic manifestations of invasive mold disease are more varied than previously recognized. The increased sensitivity of newer imaging techniques enables a greater number and depth of abnormalities to be seen in different anatomic regions. Recent data relating to the role of imaging in the diagnosis of IPA and pulmonary mucormycosis (PM) in adults suggest that a high-resolution CT scan (HRCT) is preferred to chest radiographs, magnetic resonance imaging (MRI), and positron emission tomography (PET), likely reflecting that HRCT is more sensitive than a chest

radiograph, more widely available than MRI, and the experience with HRCT is much larger than with PET [26, 27]. Among patients with IPA, nodules or infiltrates with a halo sign remain useful among neutropenic patients but they are nonspecific for IPA in other groups [28]. Furthermore, the air crescent sign is a late and nonspecific sign. Among nonneutropenic patients, multiple pulmonary nodules and various nonspecific findings including bronchopneumonia, consolidation, cavitation, pleural effusions, ground glass opacities, tree-in-bud opacities, and atelectasis are found [29]. Overall, consolidation is the most frequent presentation of PM, followed by mass lesions, nodules, and cavitation [30]. Multiple nodules (more than10) and pleural effusions appear to be more frequent in PM than in IPA [31]. Moreover, the reverse halo sign is more specific for PM than IPA, although the differential diagnosis also includes other diseases including tuberculosis [32].

Aspergillus Galactomannan: Group 3

We evaluated *A* galactomannan for both adults and children and specific patient groups and its utility and validity for different clinical specimens. We adopted different thresholds for different specimens rather than for different host groups [33–35] (Table 2). These differ from those recommended by the manufacturer of the GM assay (Platelia *A* (Bio-Rad, CA), validated only for use in serum and bronchoalveolar lavage (BAL) fluid; however, detection of GM in plasma and CSF should support a diagnosis of IA [36, 37]. Exposure to mold-active antifungals compromises the utility of the GM test for IA [38] by reducing its sensitivity [39]. Therefore, caution should be exercised when GM is found to be absent from serum or plasma in patients receiving mold-active antifungals. There was consensus that similar GM thresholds are appropriate for adults and children.

BDG and T2Candida Assays: Group 4

The group considers detection of BDG to be suitable for diagnosing probable IFD in the appropriate clinical setting. This includes patients with hematologic malignancies with and without neutropenia, neutropenia following HSCT, and certain patients in the ICU who are at higher risk (>10%) for IC as a result of gastrointestinal surgery with recurrent anastomotic leaks, perforations of the upper gastrointestinal tract, or necrotizing pancreatitis when there is clinical suspicion of infection [40, 41]. A single threshold (>80 pg/mL) using the Fungitell test (Associates of Cape Cod, Falmouth, MA) is recommended; there is insufficient evidence to include assays produced by other manufacturers [42]. Confidence for true positive results increases with repeated positive tests and for values that greatly exceed the positivity threshold [43]. There may be variability in positive predictive value (PPV) and negative predictive value (NPV) based on patient population, but a single threshold is favored at this time. The group did not support the use of

Tab e 2. P bab e l \ a i \ e P⊠ r ta M d Di ea e

Host factors
Recent history of neutropenia (<0.5 × 10^9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of invasive fungal disease
Hematologic malignancy[a]
Receipt of an allogeneic stem cell transplant
Receipt of a solid organ transplant
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
Treatment with other recognized 88 3307 eneic stem cell transplant6onary ascalci6 Tr(s)inh

CSF: ≥1.0	
Aspergillus PCR	
Any 1 of the following:	
Plasma, serum, or whole blood 2 or more consecutive PCR tests positive	
BAL fluid 2 or more duplicate PCR tests positive	

serum BDG to rule in patients for clinical trials or for defining IA or IC, as BDG detection is not specific for any one IFD. It was agreed that this test should only be used on serum samples, although the test has been used for CSF samples with some success to support a diagnosis of central nervous system (CNS) IFD in certain circumstances when other diagnostic tests are negative or inconclusive [44].

e T2Candida panel has been cleared by the US Food and Drug Administration for the detection of common *C* species from whole blood specimens. e test has a very high NPV but, as with all such tests, the PPV is variable and depends upon disease prevalence in a given patient population [45, 46]. e PPV increases from 62% among patients with sepsis, shock, or lengths of stay greater than 3–7 days in an ICU to 92% for bone marrow transplant recipients and patients with leukemia who are neutropenic but not receiving any antifungal prophylaxis. e test has been included as mycologic evidence to support a diagnosis of candidemia in selected clinical trials [47].

Aspergillus PCR: Group 5

In considering *A* PCR, target species, patient populations, appropriate specimens for testing, technical issues, comparison with other biomarker assays, and unique attributes of PCR assays were reviewed. The data were sufficiently robust for performing *A* PCR on serum, plasma, whole blood, and BAL fluid in adults. The group acknowledged that *A* PCR data have been evaluated most extensively for adults with hematologic malignancies and HSCT. Systematic reviews of *A* PCR methods on blood and BAL fluid conclude that PCR provides a robust diagnostic test for screening and confirming the diagnosis of *A* infection [22, 48–53].

ere are relatively few commercial PCR assays, and most investigators have developed methods in-house. As such, the FPCRI was established to develop criteria for *A* PCR rather than a standardized method per se. Despite technologic variability, PCR performance was comparable with that for detecting GM and BDG [54]. Moreover, commercial PCR tests performed using methodology in line with the FPCRI recommendations provide a standardized approach that has been independently associated with improved performance. A unique feature of PCR is its ability to detect both genus and species of *A* . PCR is also capable of identifying certain mutations associated with triazole resistance directly from clinical specimens [55–57].

Tissue Diagnosis: Group 6

Tissue diagnosis requires the presence of fungal elements in formalin-fixed paraffin-embedded tissue and signifies proven fungal disease but not the identity of the fungus involved. To achieve this, we recommend amplification of fungal DNA by PCR combined with DNA sequencing, but only when fungal elements are seen by histopathology. PCR would add value by allowing identification of the fungus to genus and possibly species levels. Because the technique used should be rigorously quality controlled, only laboratories with a proven record in performing DNA extraction from formalin-fixed tissue should undertake this. The identity of the fungus should be consistent with the histopathologic findings [58–60].

O he Di ea e E i ie

Pneumocystosis: Group 7

The inclusion of *P* pneumonia (PCP) diagnosis in the updated definitions was limited to patients not living with human immunodeficiency virus (HIV). Diagnosing PCP has been more difficult among these patients possibly due to a more focal pulmonary involvement, lower suspicion of disease, and lower sensitivity of traditional histologic and microscopy diagnostic tests [61]. As such, it is important to more fully define host factors for patients at increased risk for PCP. We selected receipt of therapeutic doses of corticosteroids for at least 2 weeks within the past 60 days; antineoplastic, antiinflammatory, or immunosuppressive treatment; and low CD4 lymphocyte counts due to a medical condition. This includes, but is not limited to, patients with primary immunodeficiencies, hematologic malignancies, SOTs, and allogeneic HSCT recipients [62, 63]. Clinical criteria in this population tend to be nonspecific and include cough, dyspnea, and hypoxemia. Radiographic abnormalities include bilateral ground-glass opacities and, less frequently, consolidation, small nodules, unilateral infiltrates, pleural effusions, and cystic lesions [61, 64, 65]. Amplification of *P.* DNA by quantitative real-time PCR on BAL fluid, expectorated sputum, or oral wash specimens is preferred to qualitative PCR and is helpful to establish probable disease. However, further studies are needed

to validate thresholds for positivity [66, 67]. Similarly, 2 or more serum BDG levels of ≥80 ng/L are useful for diagnosing probable disease in appropriate clinical context provided other IFDs have been excluded [68, 69].

Cryptococcosis: Group 8

A broader understanding of the natural history and host factors associated with cryptococcal disease warrants updating these definitions. We support the previous definitions of proven and probable cryptococcal disease in any host. However, we also recognize cryptococcal infection among individuals in high-risk host groups who have few, if any, symptoms and only a positive serum cryptococcal antigen test (asymptomatic cryptococcal antigenemia). This condition may be more common than symptomatic disease, and patients may develop clinical cryptococcal disease unless treated and so are now included in these definitions [70]. The term "disseminated cryptococcosis as distinct from CNS cryptococcosis has been abandoned in favor of the terms "pulmonary, "CNS and "other extrapulmonary sites. "Colonization with *C* spp. is no longer included in the definitions as it is poorly understood and its natural history is unknown.

Identi cation to the species level for *C* and *C* has become increasingly important based on reports that suggest di erent clinical presentations, outcomes, and responses to antifungal therapy between these 2 species [71, 72]. Veri cation of species that use CGB (L-canavanine, glycine, bromthymol blue) agar or matrix-assisted laser desorption ionization–time of ight mass spectrometry is recommended. Outcomes for HIV-associated cryptococcosis due to *C.* and *C.* appear to be similar, and identi cation to the species level may be unnecessary [73, 74].

Endemic Mycoses: Group 9

The endemic mycoses are caused by environmental fungi that are usually restricted geographically and cause disease in immunocompetent and immunocompromised hosts. Causative agents include *H* var. and *H.* var. , *B* species complex (eg, *B.* , *B.* , *B.* , *B.* , and *B.*), *C* /*C* , *P* /*P* , *S* species complex (*S.* , *S.* , *S.* , and *S.*), (formerly *P*) , and *E* species (*E.* , *E.* , *E.* , *E.* , and *E.*) [75–80]. Probable endemic mycoses are defined by evidence of environmental exposure to the fungus, a compatible clinical illness, and the presence of either *H* or *B* antigen in any body fluid or antibody to *C* species in serum or CSF as recovery by culture and histopathologic evidence of infection is generally lacking. There are no approved serologic tests for . ,

Tab e 3. O he P bab e l a i e Di ea e

Candidiasis
Host factors
Recent history of neutropenia <0.5 × 10^9 neutrophils/L (<500 neutrophils/mm^3 for >10 days) temporally related to the onset of invasive fungal disease
Hematologic malignancy
Receipt of an allogeneic stem cell transplant
Solid organ transplant recipient
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

Table 3. C i ed

Mycological evidence
ß-D-glucan (Fungitell) ≥80 ng/L (pg/mL) detection in ≥2 consecutive serum samples provided other etiologies have been excluded
Detection of *Pneumocystis jirovecii* DNA by quantitative real-time polymerase chain reaction in a respiratory tract specimen
Endemic mycoses
Host factors
Not applicable as these diseases affect both healthy and less healthy hosts
Clinical features
Evidence for geographical or occupational exposure (including remote) to the fungus and compatible clinical illness
Mycological evidence
Histoplasma or *Blastomyces* antigen in urine, serum, or body fluid
Antibody to *Coccidioides* in cerebrospinal fluid or 2-fold rise in 2 consecutive serum samples

Probable invasive fungal diseases (IFD)requires the presence of at least 1 host factor, a clinical feature and mycologic evidence and is proposed for immunocompromised patients only, whereas proven invasive fungal disease can apply to any patient, regardless of whether the patient is immunocompromised. Except for endemic mycoses, probable IFD requires the presence of a host factor, a clinical feature, and mycologic evidence, whereas cases that meet the criteria for a host factor and a clinical feature but for which mycological evidence has not been found are considered possible IFD.

[a]T2Candida is US Food and Drug Administration approved for the detection of *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* in blood.

[b]Cryptococcosis also occurs in phenotypically normal hosts.

[c]Definitions for human immunodeficiency virus–associated pneumocystosis are not included here.

[d]Bilateral, diffuse ground glass opacities with interstitial infiltrates are more common than other features such as consolidations, small nodules, thin-walled cavities, and unilateral infiltrates.

S. species complex, or *P.* . Exposure to 1 of these fungi is defined as a history of residence in an endemic area, no matter how remote, travel to an endemic area, or contact with fomites such as soil or vegetation that is derived from an endemic area.

P e l a i e F ga Di ea e

The revised definitions of proven IFD are shown in Table 1.

P bab e l a i e F ga Di ea e

Several changes were made to the definitions of probable IFD (Tables 2 and 3). For example, host factors were expanded to include inherited severe immunodeficiency and low CD4 lymphocyte counts. Radiographic features were expanded to include wedge-shaped and segmental or lobar consolidation and a reverse halo sign to indicate mold disease of the lower respiratory tract. Revised thresholds for GM now replace those of the manufacturer. *A* PCR is now included, and there are mycologic criteria for non-HIV–associated pneumocystosis.

P ib e l a i e F ga Di ea e

While definitions of proven and probable disease have been shown to be reliable in research and attracted little controversy among the group, this cannot be said of the possible IFD category. There is much confusion about the difference between a host factor and a risk factor. As before, a host factor has been defined as a characteristic of individuals clearly predisposed to, and not simply at risk of, an IFD [1]. For example, while impaired gut wall integrity through surgery or illness may increase the risk of candidiasis, it was not considered specific enough to warrant inclusion as a host factor. Pulmonary abnormalities such as tree-in-bud opacities and interstitial abnormalities were excluded from the clinical features as they can be due to a wide range of pathologies in addition to IFD.

GENERAL POINTS

Throughout this process, we have emphasized the need to differentiate between definitions of IFD required for clinical research from those that influence clinical practice. In clinical practice, many would administer an antifungal agent to any patient at risk of IFD when fungi are detected by biomarkers in serum, plasma, whole blood, or relevant body site fluid without there being sufficient evidence to satisfy the consensus definitions of IFD. We also recognize that our definition of a host factor errs on the side of conservatism given the increasing use of drugs such as monoclonal antibodies for treating a variety of conditions.

Other controversial issues included distinguishing between the performance characteristics of tests for screening and con- rmation, the impact of exposure to antifungal agents used for prophylaxis or treatment on imaging and diagnostic tests, and the use of biomarkers to monitor therapeutic outcome. We agree that further research will be required to evaluate the evidence for each of these assays. Finally, there was consensus that diagnostic strategies to determine the relative e ciency of an available test, alone or in combination with other diagnostic tests, should be considered further.

CONCLUSIONS

In summary, these revised definitions represent consensus expert opinion based on the best available evidence. As such, they will need to be reviewed regularly for their utility and relevance and, where possible, extended to other populations affected by IFDs. We acknowledge the limitations of these definitions, including the exclusion of certain cases of IFD. However, the reliance on host factors, clinical features, and mycologic evidence to define IFD in selected populations has proven its value for clinical trials, epidemiologic studies, and the evaluation of diagnostic tests.

Notes

Author contributions. *P* : William Steinbach (Chair, Mycoses Study Group [MSG]), Brian Fisher (MSG), Andreas Groll (European Organization for Research and Treatment of Cancer [EORTC]) omas Lehrnbecher (EORTC), Emmanuel Roilides (EORTC), omas J Walsh (MSG), Adilia Warris (EORTC), eo Zaoutis (MSG). *G* - : John W. Baddley (Chair, MSG), Barbara Alexander (MSG), Sujal Desai (EORTC), Klaus Peter Heussel (EORTC), Fr d ric Lamoth (EORTC), Orla Morrisey (MSG), Cornelia Schaefer Prokop (EORTC).

: Paul Verweij (Chair, EORTC), Elio Castagnola (EORTC), Johan Maertens (EORTC), Kieren Marr (MSG), Joseph Wheat (MSG). -D- , 2C : Cornelius J. Clancy (Chair, MSG), Hamdi Akan (EORTC), David Andes (MSG), Mario Cruciani (EORTC), Fr d ric Lamoth (EORTC), Oscar Marchetti (EORTC), Luis Ostrosky-Zeichner (MSG), Zdenek Racil (EORTC). -
: John R. Wingard (Chair, MSG), Stephane Bretagne (EORTC), Angela Caliendo (MSG), Jurgen Loe er (EORTC), Tom Patterson (MSG), Monica Slavin (MSG), P Lewis White (EORTC). : Shawn Lockhart (Chair, MSG), Ralf Bialek (EORTC), Manuel Cuenca (EORTC), Henrik Jensen (EORTC), Chris Kibbler (EORTC), Dimitrios Kontoyiannis (MSG). P : Andreas Groll (Chair, EORTC), Sharon Chen (MSG), Catherine Decker (MSG), Katrien Lagrou (EORTC), Henry Masur (MSG), Livio Pagano (EORTC), Claudio Viscoli (EORTC). C : Tania Sorrell (Chair, MSG), Peter Pappas (MSG), Tom Harrison (EORTC), Olivier Lortholary (EORTC), John Perfect (MSG). E : Carol A. Kau man (Chair, MSG), Robert W. Bradsher (MSG), Jacques F. Meis (EORTC), Marcio Nucci (MSG), David Stevens (MSG), George R ompson (MSG). I : Matteo Bassetti (Chair, EORTC), Elie Azoulay (EORTC), ierry Calandra (EORTC), Bart-Jan Kullberg (EORTC), Fr d ric Lamoth (EORTC), Marcus Ruhnke (EORTC), Shmuel Shoham (MSG), Jose Vazquez (MSG).

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