Revision 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

J. Peter Donnelly,1 Sharon C. Chen,2 Carol A. Kauffman,3 William J. Steinbach,4 John W. Baddley,5 Paul E. Verweij,6 Cornelius J. Clancy,7 John R. Wingard,8 Shawn R. Lockhart,9 Tanja C. Sorrell,10 Matteo Bassetti,11 Hamdi Akan,12 Barbara D. Alexander,13 David Andes,14 Elie Azoulay,15 Ralf Bialek,16 Robert W. Bradshaw Jr,17 Stephane Bretagne,18 Thierry Calandra,19 Angela M. Caliendo,20 Elio Castagnola,21 Mario Cruciani,22 Manuel Cuenda-Estrella,23 Catherine F. Decker,24 Sujaí R. Desai,25 Brian Fisher,26 Thomas Harrison,27 Claus Peter Heussel,28 Henrik E. Jensen,29 Christopher C. Kibbler,30 Dimitrios P. Kontoyiannis,31 Bart-Jan Kullberg,32 Katrien Lagrou,33 Frédéric Lamoth,34 Thomas Lehnbacher,35 Jurgen Loeffler,36 Olivier Lortholary,37 Johan Maertens,38 Oscar Marchetti,20 Kieren A. Marr,40 Henry Masur,41 Jacques F. Meis,42 C. Orla Morrisey,43 Marcio Nucci,44 Cornelia Schaefer Prokop,51 Shmuel Shoham,40 Monica A. Slavin,52 David A. Stevens,53 George R. Thompson III,54 Jose A. Vazquez,55 Claudio Viscoli,56 Thomas J. Walsh,57 Adilia Warris,58 L. Joseph Wheat,59 P. Lewis White,60 Theoklis E. Zaoutis,61 and Peter G. Pappas62

1Department of Hematology, Radboudumc, Nijmegen, The Netherlands; 2Centre for Infectious Diseases and Microbiology, Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead Hospital, University of Sydney, Sydney, Australia; Division of Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; 3Division of Infectious Diseases, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; 4Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA; 5Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, Alabama, USA; 6Center of Expertise in Mycology Radboudumc, Nijmegen, The Netherlands; 7Infectious Diseases Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy; 8Infectious Diseases Unit, G. Fraacastoro Hospital, San Bonifacio, Verona, Italy; 9Spanish National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain; 10Division of Infectious Diseases, Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA; 11National Heart & Lung Institute, Imperial College London, the Royal Brompton & Harefield NHS Foundation Trust, London, UK; 12Pediatri¢ Infectious Diseases Division at the Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; 13Centre for Global Health, Institute for Tropical Medicine and m.Ћt¢nc, Geesthacht, Germany; 14Division of Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; 15Institut Pasteur, Molecular Mycology Unit, CNRS UMR 2009, Mycology Laboratory, St. Louis Hospital, Assistance Publique-Hôpitaux de Paris, Université de Paris, Paris, France; 16Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; 17Department of Medicine, Albert Warren Medical School of Brown University, Providence, Rhode Island, USA; 18Infectious Diseases Unit, RCCS Istituto Giannina Gaslini, Genova, Italy; 19Infectious Diseases Unit, G. Fracastoro Hospital, San Bonifacio, Verona, Italy; 20Centre for Medical Microbiology, University College London, London, UK; 21Division of Infectious Diseases, Departments of Medicine, Microbiology and Immunology School of Medicine and Public Health and School of Pharmacy, University of Wisconsin, Madison, Wisconsin, USA; 22Infectious Diseases Unit, VA Ann Arbor Healthcare System, Ann Arbor, Michigan, USA; 23Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA; 24Division of Infectious Diseases, Departments of Medicine, Microbiology and Immunology School of Medicine and Public Health, School of Pharmacy, University of Wisconsin, Madison, Wisconsin, USA; 25Infectious Medicine Intensive and Reanimation Hospital Saint-Louis, APHP: University Paris Diderot, Paris, France; 26Molecular Diagnostics of Infectious Diseases, Microbiology, LAORZ, Zentrallabor Dr. Kramer & Kollegen, Geesthacht, Germany; 27Infectious Diseases Unit, University Hospital Heidelberg, Translational Lung Research Center and Diagnostic and Interventional Radiology, University Hospital Heidelberg, Translational Lung Research Center and Diagnostic and Interventional Radiology with Nuclear Medicine, Thessaloniki, Greece; 28Molecular Diagnostics of Infectious Diseases, Little Rock, Arkansas, USA; 29UT MD Anderson Cancer Center, Houston, Texas, USA; 30Radboud Center for Infectious Diseases and Department of Medicine, Radboudumc, Nijmegen, The Netherlands; 31Department of Microbiology, Immunology and Transplantation and Department of Laboratory Medicine and National Reference Centre for Mycosis, University Hospitals Leuven, Leuven, Belgium; 32Infectious Diseases Service, Department of Medicine and Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland; 33Pediatric Hematology and Oncology, Hospital for Children and Adolescents, University of Genoa, Genoa, Italy; 34Infectious Diseases Unit, 3rd Department of Medicine, University of Thessaloniki, Thessaloniki, Greece; 35Infectious Diseases Service, Department of Medicine, Division of Infectious Diseases, University of Wisconsin, Madison, Wisconsin, USA; 36Infectious Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 37Infectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Pediatric Hematology and Oncology University Children’s Hospital, Münster, Germany; 38Division of Infectious Diseases & Biosecurity, University of Sydney School of Medicine Faculty of Medicine and Health, Westmead Institute for Centre for Infectious Diseases and Microbiology, Westm Row Sydney Local Health District, Sydney, Australia; 39Infectious Disease Clinic, Department of Medicine University of Udine and Department of Health Sciences, DISSAL, University of Udine, Udine, Italy; 40Division of Infectious Diseases, Department of Medicine, Alpert Warren Medical School of Brown University, Providence, Rhode Island, USA; 41Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; 42Division of Infectious Diseases, Department of Internal Medicine, University of California Davis Medical Center, Sacramento, California, USA; 43Division of Infectious Diseases, Medical College of Georgia/ Augusta University, Augusta, Georgia, USA; 44Division of Infectious Disease, University of Genova and San Martino University Hospital, Genova, Italy; 45Division of Infectious Diseases, University of New Mexico School of Medicine, Albuquerque, New Mexico, USA; 46Division of Infectious Diseases, McGovern Medical School, Houston, Texas, USA, 47Istituto di Ematologia, Università Cattolica S. Cuore, Rome, Italy; 48Department of Internal Medicine–Hematology and Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic; 49Infectious Diseases Unit, 3rd Department of Pediatrics, Faculty of Medicine, Aristotle University School of Health Sciences, Hippokration General Hospital, Thessaloniki, Greece; 50Department of Hematology & Oncology, Lurie Hospital, Chicago, Illinois; 51Division of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer, The University of Melbourne, Melbourne, Victoria, Australia; 52Department of Internal Medicine, University of California San Diego School of Medicine, La Jolla, California, USA; 53UT Health San Antonio and South Texas Veterans Health Care System, San Antonio, Texas, USA; 54Department of Internal Medicine–Hematology and Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic; 55Radboud Center for Infectious Diseases and Department of Medicine, Radboudumc, Nijmegen, The Netherlands; 56Infectious Diseases Service, Department of Medicine, Hospital for Children and Adolescents, University of Frankfurt, Frankfurt, Germany; 57Division of Infectious Diseases, University of Wisconsin–Madison, Madison, Wisconsin, USA; 58Institute of Clinical Pathology and Medical Research, Westmead Hospital, University of Sydney, Sydney, Australia; 59Internal Medicine Department NIH Clinical Center, Bethesda, Maryland, USA; 60Division of Medical Microbiology and Infectious Diseases and Centre of Expertise in Mycology Radboudumc, Nijmegen, The Netherlands; 61Pediatric Hematology and Oncology, Hospital for Children and Adolescents, University of Genoa, Genoa, Italy; 62Pediatric Hematology and Oncology, University of Sydney, Sydney, Australia; 63Clinical Infectious Diseases • CID 2020:71(15 September) • 1367

Received 27 May 2019; editorial decision 29 August 2019; accepted 8 October 2019; published online 5 December 2019.

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

Clinical Infectious Diseases • 2020;71(16):1367–76

© The Author(s) 2019. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1093/cid/ciaa088
The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions of invasive fungal diseases (IFDs) were last updated in 2008 [1]. These definitions achieved their original aim in fostering communication and enabling comparison of study findings among those engaged in research into IFD of patients with cancer and recipients of hematopoietic stem cell transplants (HSCTs) or solid organ transplants (SOTs) [2, 3]. Moreover, they have been adopted by regulatory agencies for evaluation of antifungals and have been used to evaluate diagnostic tests and to conduct epidemiologic studies [4–7]. Importantly, these definitions are specifically intended for these purposes only and not to direct or guide patient care.

The 2008 definitions had their shortcomings, including the facts that the definitions were unsuitable for patients with IFD in the setting of intensive care units (ICUs) or in pediatrics, data were insufficient to establish appropriate thresholds for detecting Aspergillus galactomannan (GM), and there was uncertainty about the role of (1,3)-beta-D glucan (BDG). Furthermore, nucleic acid amplification including polymerase chain reaction (PCR)–based tests were excluded because of lack of standardization and validation. Definitions for cryptococcosis and endemic mycoses also needed clarification, and there were no definitions for pneumocystosis.

**PROCESS**

Volunteers from the EORTC Infectious Diseases Group and the MSGERC were assigned according to their expertise to 10 working groups, each charged with appraising a particular topic (see list of contributors in the Notes section). The chairs of the EORTC and MSG (J. P. D. and P. G. P.) selected leaders for each working group, and S. C. served as executive secretary. After completing the first round of working group assignments, leaders presented each group’s initial deliberations and recommendations at the 7th Trends in Medical Mycology Conference in Lisbon, Portugal, October 2015. A slide set was made available until 31 December 2015 online at www.e-materials.com/trimm2015/invitation/Member and, on request, for public comment. After several iterations, the final draft of the manuscript was circulated to all members for their approval.

**RESULTS.** There is no change in the classifications of “proven,” “probable,” and “possible” IFD, although the definition of “probable” has been expanded and the scope of the category “possible” has been diminished. The category of proven IFD can apply to any patient, regardless of whether the patient is immunocompromised. The probable and possible categories are proposed for immunocompromised patients only, except for endemic mycoses.

**CONCLUSIONS.** These updated definitions of IFDs should prove applicable in clinical, diagnostic, and epidemiologic research of a broader range of patients at high-risk.

**KEYWORDS.** consensus; definitions; invasive fungal diseases; diagnosis; research.
Table 1. Criteria for Proven Invasive Fungal Disease

| Fungus        | Microscopic Analysis: Sterile Material                  | Culture: Sterile Material | Blood                                      | Serology                  | Tissue Nucleic Acid Diagnosis |
|---------------|---------------------------------------------------------|---------------------------|--------------------------------------------|---------------------------|------------------------------|
| Molds*        | Histopathologic, cytopathologic, or direct microscopic examination 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* (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*       | Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained 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† | 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.
*†Histopathologic and cytopathologic findings are considered positive if the mycosis is identified in tissue or sputum. For molds, this finding includes identification of the specific species from the culture results.
‡Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Giemsa 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).
§Recovery of Aspergillus species from blood cultures rarely indicates endovascular disease and almost always represents contamination.
¶Trichosporon and yeast-like Geotrichum species and Blastomyces 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, Candida 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 Candida meningocencephalitis [23], but the data are sparse regarding the utility of PCR assays and the T2Candida assay for diagnosis [24].

Diagnostic Tests 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 Aspergillus PCR, the International Society of Human and Animal Mycology working group Fungal PCR Initiative (FPCR; www.fpcr.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
Aspergillus Galactomannan: Group 3
We evaluated Aspergillus 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 (Plateia Aspergillus (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 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 than 10) 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].

| Table 2. Probable Invasive Pulmonary Mold Diseases |
|-----------------------------------------------|
| **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 |
| 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 T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-α blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days |
| Treatment with recognized B-cell immunosuppressants, such as Bruton’s tyrosine kinase inhibitors, eg, ibritinib |
| Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency) |
| Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids |
| **Clinical features**                          |
| **Pulmonary aspergillosis**                    |
| The presence of 1 of the following 4 patterns on CT: |
| Dense, well-circumscribed lesion(s) with or without a halo sign |
| Air crescent sign                              |
| Cavity                                         |
| Wedge-shaped and segmental or lobar consolidation |
| Other pulmonary mold diseases                  |
| As for pulmonary aspergillosis but also including a reverse halo sign |
| **Tracheobronchitis**                          |
| Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis |
| **Sino-nasal diseases**                        |
| Acute localized pain (including pain radiating to the eye) |
| Nasal ulcer with black eschar                  |
| Extension from the paranasal sinus across bony barriers, including into the orbit |
| Central nervous system infection               |
| 1 of the following 2 signs:                    |
| Focal lesions on imaging                       |
| Meningeal enhancement on magnetic resonance imaging or CT |
| **Mycological evidence**                      |
| Any mold, for example, Aspergillus, Fusarium, Scedosporium species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate |
| Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold |
| **Tracheobronchitis**                          |
| Aspergillus recovered by culture of BAL or bronchial brush |
| **Sino-nasal diseases**                        |
| Mold recovered by culture of sinus aspirate samples |
| Microscopic detection of fungal elements in sinus aspirate samples indicating a mold |
| **Aspergillosis only**                         |
| **Galactomannan antigen**                     |
| **Antigen detected in plasma, serum, BAL, or CSF** |
| Any 1 of the following:                        |
| Single serum or plasma: ≥1.0                   |
| BAL fluid: ≥1.0                                |
| Single serum or plasma: ≥0.7 and BAL fluid ≥0.8 |
Table 2. Continued

| Plasma, serum, or whole blood 2 or more consecutive PCR tests positive |
|--------------------------|--------------------------|
| Plasma                  | Plasma                  |
| Serum                   | Serum                   |
| Whole blood             | Whole blood             |
| 2 or more duplicate PCR tests positive  |
| At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid |
| Aspergillus species recovered by culture from sputum, BAL, bronchial brush, or aspirate |

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 IFD can apply to any patient, regardless of whether the patient is immunocompromised. Probable IFD requires the presence of a host factor, a clinical feature, and mycologic evidence. 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. (1,3)-beta-D glucan was not considered to provide mycological evidence of any invasive mold disease.

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; CT, computed tomography; PCR, polymerase chain reaction.

Aspergillus PCR: Group 5

In considering Aspergillus 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 Aspergillus PCR on serum, plasma, whole blood, and BAL fluid in adults. The group acknowledged that Aspergillus PCR data have been evaluated most extensively for adults with hematologic malignancies and HSCT. Systematic reviews of Aspergillus PCR methods on blood and BAL fluid conclude that PCR provides a robust diagnostic test for screening and confirming the diagnosis of Aspergillus infection [22, 48–53].

There are relatively few commercial PCR assays, and most investigators have developed methods in-house. As such, the FPCRI was established to develop criteria for Aspergillus 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 Aspergillus. PCR is also capable of identifying certain mutations associated with trizole 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].

Other Disease Entities

Pneumocystosis: Group 7

The inclusion of Pneumocystis jirovecii 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. jirovecii 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.

Definitions of Invasive Fungal Disease • CID 2020:71 (15 September) • 1371
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 Cryptococcus spp. is no longer included in the definitions as it is poorly understood and its natural history is unknown.

Identification to the species level for Cryptococcus neoformans and Cryptococcus gattii has become increasingly important based on reports that suggest different clinical presentations, outcomes, and responses to antifungal therapy between these 2 species [71, 72]. Verification of species that use CGB (L-canavanine, glycine, bromthymol blue) agar or matrix-assisted laser desorption ionization—time of flight mass spectrometry is recommended. Outcomes for HIV-associated cryptococcosis due to C. neoformans and C. gattii appear to be similar, and identification 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 Histoplasma capsulatum var. capsulatum and H. capsulatum var. duboisii, Blastomyces species complex (eg, B. dermatitidis, B. gilchristii, B. helicus, B. silvaticus, and B. parvus), Coccidioides immitis/Coccidioides posadasii, Paracoccidioides brasiliensis/Paracoccidioides lutzii, Sporothrix species complex (S. brasiliensis, S. schenckii sensu stricto, S. globosa, and S. luriei), Talaromyces (formerly Pencilliun) marneffi, and Emergomyces species (E. pasteurianus, E. africanus, E. orientalis, E. canadensis, and E. europaeus) [75–80]. Probable endemic mycoses are defined by evidence of environmental exposure to the fungus, a compatible clinical illness, and the presence of either Histoplasma or Blastomyces antigen in any body fluid or antibody to Coccidioides species in serum or CSF as recovery by culture and histopathologic evidence of infection is generally lacking. There are no approved serologic tests for T. marneffi.

**Table 3. Other Probable Invasive Diseases**

| 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 |
|             | Treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-α blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days |
|             | Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, CARD9 deficiency, STAT1 gain of function, or severe combined immunodeficiency) |
|             | Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids |
|             | Clinical features |
|             | At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks: |
|             | Small, target-like abscesses in liver or spleen (bull’s-eye lesions) or in the brain, or, meningeal enhancement |
|             | Progressive retinal exudates or vitreal opacities on ophthalmologic examination |
|             | Mycological evidence |
|             | ß-D-glucan (Fungitell) ≥80 ng/L (pg/mL) detected in at least 2 consecutive serum samples provided that other etiologies have been excluded |
|             | Positive T2Candida |
| Cryptococcosis | Host factors |
|             | Human immunodeficiency virus infection |
|             | Solid organ or stem cell transplant recipient |
|             | Hematologic malignancy |
|             | Antibody deficiency (eg, common variable immunoglobulin deficiency) |
|             | Immunosuppressive therapy (including monoclonal antibodies) |
|             | End-stage liver or renal disease |
|             | Idiopathic CD4 lymphocytopenia |
|             | Clinical features |
|             | Meningeal inflammation |
|             | Radiological lesion consistent with cryptococcal disease |
|             | Mycological evidence |
|             | Recovery of Cryptococcus from a specimen obtained from any nonsterile site |
| Pneumocystis | Host factors |
|             | Low CD4 lymphocyte counts <200 cells/mm^3 (200 × 10^6 cells/L) for any reason |
|             | Exposure to medication (antineoplastic therapy, antimflammatory, or immunosuppressive treatment) associated with T-cell dysfunction |
|             | Use of therapeutic doses of ≥0.3 mg/kg prednisone equivalent for ≥2 weeks in the past 60 days |
|             | Solid organ transplant |
|             | Clinical features |
|             | Any consistent radiographic features particularly bilateral ground glass opacities, consolidations, small nodules or unilateral infiltrates lobar infiltrate, nodular infiltrate with or without cavitation, multifocal infiltrates, miliary pattern |
|             | Respiratory symptoms with cough, dyspnea, and hypoxemia accompanying radiographic abnormalities including consolidations, small nodules, unilateral infiltrates, pleural effusions, or cystic lesions on chest X-ray or computed tomography scan |
 Definitions of Invasive Fungal Disease • CID 2020;71 (15 September) • 1373

Table 3. Continued

| Mycological evidence | 8-D-glucan (Fungitell) >280 ng/L (pg/mL) detection in ≥2 consecutive serum samples provided other etiologies have been excluded |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Detection of Pneumocystis jiroveci 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

Possible Invasive Fungal Disease

S. schenckii species complex, or P. brasiliensis. 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.

Proven Invasive Fungal Disease

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

Probable Invasive Fungal Disease

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 prior cut points for GM with new whole blood detectable limits. For cases defined as requiring further evaluation, GM results in whole blood are now required to be confirmed in serum. 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.

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. Pediatrics: William Steinbach (Chair, Mycoses Study Group [MSG]), Brian Fisher (MSG), Andreas Groll (European Organization for Research and Treatment of Cancer [EORTC]) Thomas Lehnenreber (EORTC), Emmanuel Rolides (EORTC), Thomas J Walsh (MSG), Adilia Warris (EORTC), Theo Zaoutis (MSG). Guidance on imaging: John W. Baddley (Chair, MSG), Barbara Alexander (MSG), Sujal Desai (EORTC), Klaus Peter Heussel (EORTC), Frédéric Lamothe (EORTC), Orla Morrissey (MSG), Cornelia Schaefer Prokop (EORTC). Update of
galactomannan: Paul Verweij (Chair, EORTC), Elio Castagnola (EORTC), Johan Maertens (EORTC), Kieren Marriott (MSG), Joseph Wheat (MSG). Update on beta-D-glucan, T2Candida: Cornelius J. Clancy (Chair, MSG), Hamdi Akan (EORTC), David Andes (MSG), Mario Cruciani (EORTC), Frédéric Lamoth (EORTC), Oscar Marchetti (EORTC), Luis Ostrosky-Zeicher (MSG), Zdenek Racil (EORTC). Update of polymerase chain reaction: John R. Wingard (Chair, MSG), Stephane Bretagne (EORTC), Angela Caliendo (MSG), Jurgen Loeffler (EORTC), Tom Patterson (MSG), Monica Slavin (MSG), P. Lewis White (EORTC). Tissue diagnosis: Shawn Lockhart (Chair, MSG), Ralf Bialek (EORTC), Manuel Cuencas (EORTC), Henrik Jensen (EORTC), Chris Kibbler (EORTC), Dimitrios Kontoyiannis (MSG). Pneumocystis: Andreas Groll (Chair, EORTC), Sharon Chen (MSG), Catherine Decker (MSG), Katrin Lagrou (EORTC), Henry Masur (MSG), Livio Pagano (EORTC), Claudio Viscoli (EORTC). Cryptococcosis: Tania Sorrell (Chair, MSG), Peter Pappas (MSG), Tom Harrison (EORTC), Olivier Lortholary (EORTC), John Perfect (MSG). Endemic mycosis: Carol A. Kaufman (Chair, MSG), Robert W. Bradley (MSG), Jacques F. Meis (EORTC), Marcio Nucci (MSG), David Stevens (MSG), George R Thompson (MSG). Inclusion of patients in the intensive care unit: Matteo Bassetti (Chair, EORTC), Elie Azoulay (EORTC), Thierry Calandra (EORTC), Bart-Jan Kullberg (EORTC), Frédéric Lamoth (EORTC), Marcus Ruhnke (EORTC), Shmuel Shoham (MSG), Jose Vazquez (MSG).

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Potential conflicts of interest. H. Akan reports being a primary investigator in a clinical trial for MSD. B. D. Alexander reports grants from Astellas, Cidara, Sycinex, F2G, and Viamet and personal fees from Cidara, Scynexis, and UpToDate outside the submitted work. D. Andes reports grants and consulting fees from Amplyxx, Matinas, Cidara, and Merck outside the submitted work. E. Aozayl reports personal fees from Gilead, Pfizer, Albylnx, Alexion, and Baxter; grants and personal fees from MSD; and conference invitation from Gilead outside the submitted work. M. Bassetti reports grants from Pfizer, MSD, and Cidara and personal fees from Pfizer, MSD, Astellas, ThermoFisher, Gilead, Cidara, Biorimexeu, and Menarini outside the submitted work. S. Bretagne reports grants from MSD and Pfizer and personal fees from Gilead outside the submitted work. T. Calandra reports advisory board membership from Astellas, Basilea, Cidara, MSD, Sobi, ThermoFisher, and GE Healthcare and data monitoring board membership from Novartis. A. M. Caliendo reports grants from T2 Biosystems outside the submitted work. E. Castagnola reports personal fees from Gilead outside the submitted work. S. C. Chen reports grants from MSD Australia outside the submitted work. C. J. Clancy reports consultancy fees from Merck, Cidara, Astellas, The Medicine Company, Scynexis, and T2Biosystems; grants/pending grants from T2Biosystems, Merck, Cidara, and Astellas; and personal fees from Merck and T2 Biosystems outside the submitted work. M. Cuenca-Estrella reports grants and personal fees from Gilead Sciences and grants from F2G, Scynexis, Cidara, and Amplyxx outside the submitted work. S. R. Desai reports paid honorarium and travel expenses from Intersstitial Lung Disease MD—Boehringer-Ingelheim. J. P. Donnelly reports personal fees from F2G Ltd, Gilead Sciences, and Pfizer outside the submitted work. B. Fisher reports grants from Pfizer, Merck, and the National Institutes of Health and personal fees from Astellas outside the submitted work. T. Harrison reports grants from Gilead Sciences and personal fees from Gilead Sciences, Pfizer, and Viamet outside the submitted work. C. P. Heussel reports personal fees from Basilea, Bayer, Boehringer-Ingelheim, Gilead, Interimmune, MSD, Novartis, and Pfizer; grants from the German Center for Lung Research, MeVis, Pfizer, and Siemens; and consultancy fees from Fresenius outside the submitted work. C. A. Kaufmann reports data safety monitoring board membership for Cidara Therapeutics outside the submitted work. D. P. Kontoyiannis reports personal fees from Merck & Co, Gilead Sciences, United Medical, Astellas Pharma, Pharma, Cidara Therapeutics, Amplyx Pharmaceuticals, and Mayne Pharma outside the submitted work. B.-J. Kullberg reports personal fees from Amplyxx, Astellas, Cidara, Pfizer, and Scynexis outside the submitted work. K. Lagrou reports personal fees and travel support from Pfizer and MSD and personal fees from Abbott, SMB Laboratoires Brussels, Gilead, and Roche outside the submitted work. F. Lamothe reports personal fees from MSD and Basilea outside the submitted work. T. Lehrnbecher reports grants, personal fees, and nonfinancial support from Gilead Sciences; personal fees and nonfinancial support from MSD/Merck and Astellas; and personal fees from Basilea outside the submitted work. J. Maertens reports personal fees and nonfinancial support from Basilea Pharmaceuticals, Bio-Rad Laboratories, Cidara, F2G Ltd, Gilead Sciences, Merck, and Pfizer Inc and grants from Gilead Sciences outside the submitted work. K. A. Marr reports personal fees from Amulyx, Cidara, and Merck; licensing royalties from MycoMed Technologies; and patents from MycoMed outside the submitted work. J. F. Meis reports grants from F2G Ltd, personal fees from Gilead Sciences, other from Pulmocide, grants and personal fees from Scynexis, personal fees and other from TEVA, and personal fees and other from United Medical outside the submitted work. C. O. Morrissey reports grants from Gilead Sciences, Merck, Sharp and Dohme and advisory board membership from Merck, Sharp, and Dohme outside the submitted work. M. Nucci reports personal fees from Pfizer, MSD, Basilea, Gilead, Biotoscana, Teva, Abbvie, Astellas, and Jansen outside the submitted work. L. Ostrosky-Zeicher reports personal fees from Merck, F2G, Mayne, Viracor, and Gilead; grants and personal fees from Pfizer, Astellas, Scynexis, and Cidara; grants from Amplyxx; and personal fees from Realtime outside the submitted work. L. Pagano reports personal fees from MSD and Pfizer and grants and personal fees from Gilead outside the submitted work. T. F. Patterson reports personal fees from Basilea; grants from Cidara; and personal fees from Gilead, Merck, Scynexis, Toyama, and Pfizer outside the submitted work. J. R. Perfect reports grants from Pfizer and Mayne; grants and other from Merck, Minnetronix, and Amplyxx and other from Viamet, F2G, Vical, Matinas, Cidara during the conduct of the study. E. Rolides reports grants from Astellas Pharma, Gilead Sciences, MSD Sharp & Dohme, Pfizer outside the submitted work. M. Ruhnke reports personal fees from SCYNEXIS, Basilea, Kedplasma, and Daiichi outside the submitted work. S. Shoham reports grants from Merck, Astellas, Shinogoi, Cidara, Scynexis, Shire, T2 Microsystems, Ansum, Emergent, and Gilead and personal fees from Merck outside the submitted work. M.A. Slavin reports grants from Merck; personal fees from Merck; and other from Pfizer and Gilead outside the submitted work. D. A. Stevens reports grants from Astellas and Riptide and consulting fees from Pulmatrix, the US Department of Justice, and Fresenius outside the submitted work. G. R. Thompson reports grants and other from Astellas, Cidara, Vical, Scynexis, F2G, and Mayne and grants from Realtime Labs outside the submitted work. J. A. Vazquez reports personal fees from Astellas, Cidara, Amplyxx, and F2G outside the submitted work. C. Viscosi reports personal fees from MSD Int, Gilead, Pfizer, Angelini, Astellas, and Basilea outside the submitted work. P. E. Verweij reports grants from Gilead Sciences, Merck, Pfizer, and F2G and nonfinancial support from OLM and IMMY outside the submitted work. T. J. Walsh has received grants for experimental and clinical antifungal pharmacology and molecular diagnostics to his institution from Amplyxx, Astellas, Lediant, Merck, Scynexis, and T2 Biosystems and has served as a consultant to Amplyxx, Astellas, Gilead, Lediant, Merck, and Scynexis. A. Warris reports grants and personal fees from Gilead outside the submitted work. P. L. White reports nonfinancial support from Bruker, Dynamikera, and Lauchain; personal fees and nonfinancial support from Gilead; and personal fees from MSD outside the submitted work. J. R. Wingard reports personal fees from Ansun, Astellas, Behring, Celgene, Cidara, Merck, and Shire outside the submitted work. T. E. Zaoutis reports personal fees from Pfizer outside the submitted work. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. De Paauw B, Walsh TJ, Donnelly JP, et al; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group Consensus Group. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008; 46:1813–21.
2. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mold disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. Lancet 2016; 387:760–9.

3. Herbrecht R, Patterson TF, Slavin MA, et al. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus amphotericin B for therapy of invasive aspergillosis: a collaborative study of the Mycoses Study Group (MSG 05) and the European Organization for Research and Treatment of Cancer Infectious Diseases Group. Clin Infect Dis 2015; 60:213–20.

4. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis 2010; 50:1101–11.

5. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. Clin Infect Dis 2010; 50:1091–100.

6. Park BJ, Pappas PG, Warnemuehler KA, et al. Invasive non-Aspergillus mold infections in transplant recipients, United States, 2001–2006. Emerg Infect Dis 2011; 17:1855–64.

7. Kaufman CA, Freifeld AG, Andes DR, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis 2014; 16:213–24.

8. Bassetti M, Scudeller L, Giacobbe DR, et al. Developing definitions for invasive fungal diseases in critically ill adults in intensive care units. Protocol of the FUNGal infections Definitions in ICU patients (FUNDICU) project. Mycoses 2019; 62:310–9.

9. Fisher BT, Ross RK, Localio AR, Prasad PA, Zaoutis TE. Decreasing rates of invasive candidiasis in pediatric hospitals across the United States. Clin Infect Dis 2014; 58:74–7.

10. Steinbach WJ, Rolides E, Berman D, et al; International Pediatric Fungal Collaborative Network on Infections in Canada (PICNIC) study. BMC Infect Dis 2017; 17:123–33.

11. van Asbeck EC, Clemons KV, Stevens DA. Candida parapsilosis: a review of its epidemiology, pathogenesis, clinical aspects, typing and antifungal susceptibility. Crit Rev Microbiol 2009; 35:283–309.

12. Henriet SS, Verweij PE, Warris A. Aspergillus nidulans and chronic granulomatous disease: a unique host-pathogen interaction. J Infect Dis 2012; 206:1128–37.

13. Robinson JL, Davies HD, Barton M, et al. Characteristics and outcome of infants with candiduria in neonatal intensive care—a Paediatric Investigative Collaborative Network on Infections in Canada (PICNIC) study. BMC Infect Dis 2009; 9:183.

14. Katragkou A, Fisher BT, Groll AH, Rolides E, Walsh TJ. Neonatal candidemia and end-organ damage: a critical appraisal of the literature using meta-analytic techniques. Pediatrics 2013; 112:634–40.

15. McCarthy MW, Kalasauskas D, Petraitis V, Petraitiene R, Walsh TJ. Fungal infections of the central nervous system in children. J Pediatric Infect Dis Soc 2017; 6:e123–33.

16. Marchiori E, Zanetti G, Escussato DL, et al. Reversed halo sign: high-resolution CT scan findings in 79 patients. Chest 2012; 141:1260–6.

17. D’Haese J, Theunissen K, Vermeulen E, et al. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. J Clin Microbiol 2012; 50:1258–63.

18. Leeffang MM, Debets-Ossenkopp TJ, Wang J, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. Cochrane Database Syst Rev 2015; CD007394.

19. Bennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect Dis 2004; 4:349–57.

20. Chong GM, Maertens JA, Lagrou K, Driessen GJ, Cornelissen JJ, Rijnders BJ. Diagnostic performance of galactomannan antigen testing in cerebrospinal fluid. J Clin Microbiol 2016; 54:428–31.

21. Klont RR, Bennink-Kersten MA, Verweij PE. Utility of Aspergillus antigen detection in specimens other than serum specimens. Clin Infect Dis 2004; 39:1467–47.

22. Duarte RE, Sánchez-Ortega I, Cuesta I, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematolology patients receiving effective antifungal prophylaxis. Clin Infect Dis 2014; 59:1696–702.

23. Marr KA, Laverdere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis 2005; 40:1762–9.

24. Clancy CJ, Nguyen MH. Diagnosing invasive candidiasis. J Clin Microbiol 2018; 56. doi: 10.1128/JCM.01909-17.

25. Lamoth F, Cruciani M, Mengoli C, et al; Third European Conference on Infections in Leukemia. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin Infect Dis 2012; 54:633–43.

26. White SK, Walker BS, Hanson KE, Schmidt RL. Diagnostic Accuracy of beta-D-Glucan Surveillance with preemptive anidulafungin for invasive candidiasis in intensive care unit patients: a randomized pilot study. PLoS One 2012; 7:e42282.

27. Stevens DA, Zhang Y, Finkelman MA, Pappagianis D, Clemons KV, Martinez M. Cerebrospinal fluid (1,3)-beta-D-glucan testing is useful in diagnosis of coccidiodial meningitis. J Clin Microbiol 2016; 54:2707–10.

28. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 2015; 60:892–9.

29. Clancy CJ, Pappas PG, Vazquez J, et al. Detecting infections rapidly and easily for candidemia trial, part 2 (DIRECT2): a prospective, multicenter study of the T2Candida panel. Clin Infect Dis 2018; 66:1678–86.

30. Clancy CJ, Nguyen MH. T2 magnetic resonance for the diagnosis of bloodstream infections: charting a path forward. J Antimicrob Chemother 2018; 73(suppl_4):iv2–iv5.

31. Arvanitis M, Ziaakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol 2014; 52:3731–42.

32. Guo YL, Chen YQ, Wang K, Qin SM, Yu C, Kong JL. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate metaanalysis and systematic review. Chest 2010; 138:817–24.
50. Heng SC, Morrissey O, Chen SC, et al. Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematologic patients: a systematic review and meta-analysis. Crit Rev Microbiol 2015; 41:124–34.
51. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 2009; 9:89–96.
52. Sun W, Wang K, Gao W, et al. Evaluation of PCR on bronchoalveolar lavage fluid for diagnosis of invasive aspergillosis: a bivariate metaanalytic and systematic review. PLoS One 2011; 6:e28467.
53. Zou M, Tang L, Zhao S, et al. Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. PLoS One 2012; 7:e43347.
54. White PL, Wingard JR, Bretagne S, et al. Aspergillus polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis 2015; 61:1293–303.
55. Schauwvliegh AFAD, Vonk AG, Buddingh EP, et al. Detection of azole-susceptible and azole-resistant Aspergillus coinfection by cyp51A PCR amplicon melting curve analysis. J Antimicrob Chemother 2017; 72:3047–50.
56. Chong GL, van de Sande W, Dingemans GJ, et al. Validation of a new Aspergillus real-time PCR assay for direct detection of Aspergillus and azole resistance of Aspergillus fumigatus on bronchoalveolar lavage fluid. J Clin Microbiol 2015; 53:868–74.
57. White PL, Posso RB, Barnes RA. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs directly from plasma samples. J Clin Microbiol 2017; 55:2356–66.
58. Buttrago MJ, Bernal-Martinez L, Castelli MV, Rodriguez-Tudela JL, Cuenca-Estrella M. Performance of panfungal- and specific-PCR-based procedures for etiological diagnosis of invasive fungal diseases on tissue biopsy specimens with proven infection: a 7-year retrospective analysis from a reference laboratory. J Clin Microbiol 2014; 52:1737–40.
59. Moncada PA, Budvytiene I, Ho DY, Deresinski SC, Montoya JG, Banaei N. Utility of DNA sequencing for direct identification of invasive fungi from fresh and formalin-fixed specimens. Am J Clin Pathol 2015; 140:203–8.
60. McKinnell JA, Cannella AP, Kunz DF, et al. Pneumocystis pneumonia in hospitalised patients: a detailed examination of symptoms, management, and outcomes in human immunodeficiency virus (HIV)-infected and HIV-uninfected persons. Transpl Infect Dis 2012; 14:510–8.
61. Sepkowitz KA. Pneumocystis carinii pneumonia in patients without AIDS. Clin Infect Dis 1993; 17 Suppl 2:S416–22.
62. Messiaen PE, Cuyx S, Dejager T, van der Hilst JC. The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against Pneumocystis jiroveci pneumonia in human immunodeficiency virus-negative immunocompromised patients: a systematic review. Transpl Infect Dis 2017; 19: doi: 10.1111/tid.12651.
63. Messiaen PE, Cuyx S, Dejager T, van der Hilst JC. The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against Pneumocystis jiroveci pneumonia in human immunodeficiency virus-negative immunocompromised patients: a systematic review. Transpl Infect Dis 2017; 19: doi: 10.1111/tid.12651.
64. Pagano L, Fianchi L, Mele L, et al. Pneumocystis carinii pneumonia in patients with malignant haematological diseases: 10 years’ experience of infection in GIMEMA centres. Br J Haematol 2002; 117:379–86.
65. Roux A, Gonzalez F, Roux M, et al. Groupe de recherche respiratoire en réanimation en onco-hématologie (Grr-OH). Update on pulmonary Pneumocystis jiroveci infection in non-HIV patients. Med Mal Infect 2014; 44:185–98.
66. Alanio A, Bretagne S. Diagnosis of Pneumocystis jiroveci pneumonia: role of β-D-glucan detection and PCR. Curr Fungal Infect Rep 2014; 8:322–30.
67. Fan LC, Lu HW, Cheng KR, Li HP, Xu JF. Evaluation of PCR in bronchoalveolar lavage fluid for diagnosis of Pneumocystis jiroveci pneumonia: a bivariate meta-analysis and systematic review. PLoS One 2013; 8:e73099.
68. Onishi A, Sugiyama D, Kogata Y, et al. Diagnostic accuracy of serum 1,3-β-D-glucan for Pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol 2012; 50:7–15.
69. Karageorgopoulos DE, Qi JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of β-D-glucan for the diagnosis of Pneumocystis jiroveci pneumonia: a meta-analysis. Clin Microbiol Infect 2013; 19:39–49.
70. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis 2009; 49:856–62.
71. Chen S, Sorrell T, Nimmoh G, et al. Epidemiology and host- and variety-dependent characteristics of infection due to Cryptococcus neoformans in Australia and New Zealand. Australasian Cryptococcal Study Group. Clin Infect Dis 2000; 31:499–508.
72. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of Cryptococcus neoformans. Clin Infect Dis 1995; 21:28–34; discussion 5–6.
73. Morgan J, McCarthy KM, Gould S, et al. Cryptococcus gattii infection: characteristics and epidemiology of cases identified in a South African province with high HIV seroprevalence, 2002–2004. Clin Infect Dis 2006; 43:1077–80.
74. Steele KT, Thakur R, Nthobatsang R, Steenhoff AP, Bisson GP. In-hospital mortality of HIV-infected cryptococcal meningitis patients with C. gattii and C. neoformans infection in Gaborone, Botswana. Med Mycol 2010; 48:1112–5.
75. Gast KB, van der Hoveen A, de Boer MGI, et al. Two cases of Emergomyces pasteurianus infection in immunocompromised patients in the Netherlands. Med Mycol Case Rep 2019; 24:5–8.
76. Schwartz IS, Sanche S, Wiederhold NP, Patterson TF, Sigler L. Emergomyces canadenis, a dimorphic fungus causing fatal systemic disease in North America. Emerg Infect Dis 2018; 24:758–61.
77. Crombie K, Spengane Z, Locketz M, et al. Paradoxical worsening of Emergomyces africanus infection in an HIV-infected male on itraconazole and antiretroviral therapy. PLoS Negl Trop Dis 2018; 12:e0006173.
78. Wang P, Kenyon C, de Hoog S, et al. A novel dimorphic pathogen, Emergomyces orientalis (Omygenales), agent of disseminated infection. Mycoses 2017; 60:310–9.
79. Schwartz IS, Wiederhold NP, Hanson KE, Patterson TF, Sigler L. Blastomyces helicus, a new dimorphic fungus causing fatal pulmonary and systemic disease in humans and animals in Western Canada and the United States. Clin Infect Dis 2019; 68:188–95.
80. Brown EM, McGaggart LR, Zhang SX, Lowe DE, Stevens DA, Richardson SE. Phylogenetic analysis reveals a cryptic species Blastomyces glichertii, sp. nov. within the human pathogenic fungus Blastomyces dermatitidis. PLoS One 2013; 8:e59237.