

REVIEW ARTICLE

Diagnostic challenges and recent advances in the early management of invasive fungal infections

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Abstract

During the past 20 yr, the population of immunocompromized patients at risk of developing invasive fungal infections (IFIs) has increased, and there has been a shift in fungal epidemiology, with more infections caused by non-*Aspergillus* molds and yeasts, which are often resistant to one or more antifungal drugs. Traditional diagnostic methods, such as culture and the histopathology of infected tissue, often fail to detect IFIs until the later stages. Furthermore, invasive diagnostic methods to obtain tissue may be contraindicated in severely ill patients; even when tissue is available, the morphology of several filamentous fungi is identical, or the cultures may fail to grow the pathogen. Recently developed non-invasive diagnostic techniques, such as tests for serum markers and polymerase chain reaction assays, may allow for earlier and more accurate diagnoses – crucial in the effort to reduce morbidity and the risk of mortality. This article reviews current approaches to diagnosis and treatment, focusing on how an early and accurate diagnosis can guide treatment and improve outcomes. Strategies for improving the management of IFIs also are discussed.

Key words invasive fungal infections; diagnostic tools; clinical presentation; management of IFIs

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Accepted for publication 27 November 2009

doi:10.1111/j.1600-0609.2009.01391.x

Invasive fungal infections (IFIs) are a significant cause of morbidity and mortality. Although mortality from candidiasis has generally decreased, after a peak in the early 1990s, the overall number of deaths from fungal infections has risen substantially as a result of diseases caused by other fungal pathogens, such as *Aspergillus* (1, 2). One study estimated an overall case-fatality rate of 58% for invasive aspergillosis (3). Over the past two decades, there has been an increase in the number of immunocompromized patients at risk of developing IFIs (4–6), including patients receiving aggressive chemotherapy and immunosuppressive drugs. The economic cost of treating IFIs in the United States is high. The estimated mean increase in hospital charges related to candidemia is \$39 331 (7). The average hospital costs because of aspergillosis was earlier estimated at \$62 426 (8), but a recent analysis of hospital discharge data estimated a mean total hospital charge of \$96 731

for patients diagnosed with primary or secondary aspergillosis (9). Thus, prevention or early diagnosis and treatment of IFIs are crucial to decreasing the clinical and economic burden.

Clinicians face many challenges in diagnosing and treating IFIs. Non-specific symptoms present difficulties in establishing an early definitive diagnosis. In addition, microbiological cultures may be negative, particularly in the early stages of infection (10). Antifungal drugs should be chosen carefully, taking into account the spectrum of activity and possible drug resistance. New diagnostic tools may allow for rapid diagnosis and identification of specific fungal pathogens; these tools are still being validated in different patient populations (11, 12). For clinicians managing at-risk patients, it is important to recognize the followings: (i) factors that contribute to the misdiagnosis of IFIs, (ii) the utility of improved diagnostic methods, (iii) the benefits of early

diagnosis, (iv) primary reasons for the mismanagement of IFIs, and (v) therapeutic options that can safely and effectively manage IFIs.

Traditional methods of diagnosing fungal infections

Although other methods of diagnosing IFIs have been developed, laboratory culture remains the gold standard for a definitive diagnosis. However, there are considerable difficulties in using culture as a diagnostic tool. Obtaining a biopsy for histopathology or culture from a sterile site, such as the lung, would require invasive techniques, which may be contraindicated in severely ill patients (e.g. patients with hypoxia and/or thrombocytopenia). Furthermore, it can be difficult to determine whether a positive culture from a non-sterile site is attributed to colonization or active infection (10). Nonetheless, improvements in culture techniques, such as the development of lysis centrifugation, new formulations of culture media, and automated blood culturing and monitoring systems, have led to a higher frequency and a more rapid detection of *Candida* species from blood samples (13–16). Unfortunately, sensitivity remains low, with the rate of positive samples from patients with invasive candidiasis at approximately 50% (17, 18).

Histopathology is the cornerstone for diagnosing and identifying fungal pathogens. However, as with culture, it may not be possible to obtain a tissue sample from critically ill patients. In addition, identifying the specific pathogen based solely on morphological characteristics can be difficult or impossible, because several different organisms

can display similar histopathological characteristics. For example, pathogens such as *Fusarium* species, *Pseudallescheria* species, and *Penicillium* have hyaline, narrow septate hyphae, indistinguishable from *Aspergillus* in tissue biopsies (11, 19). As *Aspergillus* is far more commonly encountered than the other pathogens mentioned, a pathologist often may describe an organism as *Aspergillus* or *Aspergillus*-like based upon morphological features alone. This can hinder diagnosis, particularly with the recognition that the incidence of non-*Aspergillus* infections caused by *Fusarium* species and Zygomycetes may be increasing (5, 20, 21). Given the differences in susceptibility profiles among fungal pathogens, misdiagnosis can lead to inappropriate therapy.

Two serological tests have been developed and standardized for the detection of *Cryptococcus* and *Histoplasma*. Infections with *Cryptococcus* species can be determined by the detection of cryptococcal capsular polysaccharide antigen in blood or cerebrospinal fluid. This test is highly accurate, with a sensitivity of 90% and a specificity of 95% (12, 22, 23). Detecting *Histoplasma* antigen in body fluids is also a reliable test, with a sensitivity of 82–95%, and a specificity of 98%. This test is most often used in urine samples, to diagnose disseminated histoplasmosis (24–26).

While the clinical utility of the serological tests for *Cryptococcus* and *Histoplasma* is well established, traditional methods are not reliable for diagnosing infections with *Candida*, *Aspergillus*, and emerging pathogens such as *Fusarium* species and Zygomycetes. Table 1 summarizes traditional diagnostic methods and non-invasive methods used to detect IFIs.

Table 1 Summary of diagnostic methods

Method	Advantages	Disadvantages
Culture	Long history of use in diagnosis Accurate Specific Considered gold standard	May require invasive techniques to obtain a sterile site culture May be falsely negative
Histopathology	Direct visualization of fungal pathogens Presence of infection can be proven (if specimen from sterile site)	Requires invasive techniques to acquire a tissue sample Difficulty in identifying the specific pathogen because of histological similarities among fungal pathogens
High-resolution computed tomography scans	Non-invasive High predictive value for diagnosis of pulmonary infections	Non-specific Difficulty in differentiating aspergillosis from other filamentous fungal infections (e.g. mucormycosis)
β -D-glucan test	Distinct features, which indicate the stage of infection Non-invasive High negative-predictive value for most fungi	Prone to false-positives Positive results may be seen with <i>Candida</i> , <i>Aspergillus</i> , and other fungi Cannot be used for detection of Zygomycetes
Galactomannan test	Non-invasive High specificity	Prone to false-positives and false-negatives
Polymerase chain reaction	Non-invasive Can determine specific genus and/or species High specificity	Prone to false-positives Not standardized Not commercially available

Non-invasive methods for diagnosing IFIs

Several non-invasive methods for detecting IFIs have been developed and refined in the past few years. These methods have improved the accuracy of diagnosis of IFIs and help to identify the infectious pathogen. High-resolution computer tomography (CT) scan has a 100% predictive value for the diagnosis of pulmonary fungal infections, such as aspergillosis (27, 28). It also has distinct features that indicate the stage of the infection. High-resolution CT scans of pulmonary infections in the early stage (<5 d) exhibit a halo sign, and infections in the late stages exhibit an air-crescent sign, which frequently correlates with neutrophil recovery (27, 28). Caillot *et al.* (29) recently published results of a prospective study in neutropenic patients with probable or proven invasive pulmonary aspergillosis (IPA), in which they found that sequential chest CT scans can be useful in assessing treatment outcome by monitoring volume changes in pulmonary lesions over time. The volume of pulmonary lesions increased significantly from day 0 to day 7 and steadily decreased by day 14. Although CT scans are critical to guiding lung biopsies (30), the findings may be non-specific; features suggestive of invasive aspergillosis can be caused by other pathogens, such as mucormycosis, other fungi, bacteria, and non-infectious etiologies (19, 28). As cases of mucormycosis are on the rise (5, 20, 21), CT scans must be carefully evaluated. A reverse halo sign (ground-glass attenuation surrounded by a solid ring) in patients with invasive pulmonary mycoses, for example, may be a sign of mucormycosis (31).

Two serum tests have been developed that detect components of the fungal cell wall to diagnose IFIs: the β -D-glucan and galactomannan tests. The test for β -D-glucan, a common fungal cell wall component, is useful for detecting a variety of fungal pathogens, including *Candida*, *Aspergillus*, and *Fusarium* species, but not *Zygomycetes* (32). In patients with acute myelogenous leukemia and myelodysplastic syndrome, the β -D-glucan assay has been shown to have a high degree of accuracy, with a sensitivity of 97% and a specificity of 93% (32). The sensitivity of this test is lower in patients with neutropenia (88%), but specificity is comparable (90%) (33). As glucan is ubiquitous in the environment, the β -D-glucan assay is prone to false-positive results caused by, for example, contact with cotton during surgery, the administration of plasma protein fractions or coagulation factors, hemolysis, treatment with intravenous immunoglobulins, and hemodialysis with cellulose membranes. False-positives are also common in patients with bacteremia, especially those with gram-positive bacteremia, and in patients receiving β -lactam antibiotics (12, 34). The negative-predictive value of the test for candidiasis is

excellent (32–35). The test is most useful for exclusion of invasive candidiasis in suspected cases. Of interest, the β -D-glucan assay is useful in the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with cancer or patients with HIV infection (36). Standardization of the test by determining cutoff values and validation of the β -D-glucan assay is crucial to using this assay as a routine diagnostic tool for IFIs. The test is not available in most institutions.

The use of galactomannan as a serological test for invasive aspergillosis is growing (12, 37, 38). The galactomannan assay is highly sensitive (89.7%) and specific (98.1%) for the diagnosis of *Aspergillus* infections in patients with prolonged neutropenia and in stem cell transplantation recipients (37). A meta-analysis of 27 studies from 1996–2005 evaluated the accuracy of the galactomannan assay in the diagnosis of invasive aspergillosis. The overall sensitivity and specificity were found to be 71% and 89%, respectively (39). However, enteral feeding with soybean protein, gastrointestinal colonization with *Bifidobacterium* in neonates, or therapy with β -lactam antibiotics such as piperacillin–tazobactam can result in false-positives (37–39). Antifungal drug therapy, which causes a low-level release of fungal galactomannan, can affect the accuracy of the galactomannan assay by causing false-negative results (12, 37, 39). Moreover, the galactomannan assay is not available at all institutions. To improve the accuracy of serological tests, serial samples should be tested to confirm positive or negative results (37, 40). More data are needed for the β -glucan and the galactomannan assays to be used in solid organ recipients with suspected IFIs.

Other non-invasive methods use molecular biological analysis of fungal DNA to determine the presence of a fungal infection and identify the genus/species of the pathogen. Reverse transcription polymerase chain reaction (PCR) can be used to detect genus- or species-specific DNA markers, such as heat shock protein 90 (10). There are also pan fungal PCR tests for highly conserved genomic sequences, such as 18S rRNA, 28S rRNA, or mitochondrial genes, which are found in multiple copies in nearly all fungal species (10). Studies have shown varying sensitivity (45–92%) but high specificity (>90%) with PCR analysis (12, 41–43). However, PCR is highly susceptible to contamination by airborne spores, resulting in false-positives (12, 44). Furthermore, PCR detects the presence of the organism but does not distinguish colonization from invasive infection. Importantly, the use of PCR for diagnosis of IFIs is limited by the lack of commercially available standardized assays.

A peptide nucleic acid fluorescent *in situ* hybridization (PNA FISH) test has been developed to differentiate between *Candida albicans* and non-*C. albicans* yeast infections (45). After a blood culture is confirmed as

positive for yeast by gram-stain morphology, blood samples are subjected to *in situ* hybridization with fluorescently labeled *C. albicans*-specific rRNA probes. The results are available in 2–3 h, in contrast to the conventional method of identification by culture, which takes 36–48 h or longer. The PNA FISH test is highly accurate; both sensitivity and specificity are reported to be 100% (45, 46). Although infections with *C. albicans* are the most common, infections caused by other species are becoming more prevalent (1, 47, 48). Recently, a highly accurate PNA FISH test for identifying *Candida glabrata* was licensed by the US Department of Agriculture for commercial use (46). Varied antifungal susceptibility among *Candida* species makes exact identification of the pathogen useful for selecting an appropriate antifungal drug.

It is likely that combinations of diagnostic techniques may facilitate the rapid and accurate diagnosis of IFIs and ultimately help formulate appropriate treatment strategies. Serological methods that detect fungal antigens, and PCR assays that detect fungal genomic DNA, may assist clinicians in making earlier and more specific diagnoses. Analyzing bronchoalveolar lavage (BAL) fluid with these methods may facilitate the early diagnosis of IFIs or help to confirm a diagnosis in patients with pulmonary infiltrates (12, 44, 49). Detection of *Aspergillus* galactomannan in BAL fluid has been shown to have superior sensitivity to serum galactomannan in the diagnosis of pulmonary aspergillosis (50). High-resolution CT scan and the galactomannan assay in patients with neutropenia are highly effective in diagnosing fungal infections (51). The two serological assays, along with culture and microscopic evaluation, were successfully used as guides for treating patients with neutropenia (51). Another study has reported the use of the galactomannan and β -D-glucan assays to diagnose patients with invasive aspergillosis. Using both assays, the study found a significantly higher specificity (100%) and positive predictive value (100%) than either test alone (33). The use of molecular techniques in combination with biopsy may also be of value. For instance, the use of PCR on CT-guided percutaneous lung biopsy samples was shown to have high sensitivity and specificity for the detection of *Aspergillus* (52). In another study, Rickerts *et al.* (53) found PCR to be superior to tissue culture in detecting *Aspergillus* species and *Zygomycetes* in respiratory tract biopsy samples of patients suspected of having a mold infection.

Clinical/radiological presentation of fungal pathogens

The symptoms of IFIs are typically non-specific and similar to those of other infections. Although clinical signs

of fungal infections are mostly non-descript relative to the infecting pathogen, there are some pathogen-specific aspects of clinical presentation that can serve as clues for diagnosis.

In patients with *Candida* infections, signs of candidemia or candidiasis may include weight loss, abdominal pain, and hepatic and/or spleen enlargement in addition to prolonged antibiotic-resistant fever. In cases of hepatosplenic candidiasis, after recovery from neutropenia, small radiolucent lesions in the liver or spleen may be seen on a CT scan (54). Examining the fundus, with or without usual symptoms, is a must during candidemia; positive finding of endophthalmitis may occasionally be seen (55). Skin lesions – papular/nodular, erythematous, non-tender, and widely scattered – may be seen in disseminated candidiasis; a biopsy helps to prove the diagnosis, particularly with a negative blood culture (56).

Cryptococcal infection in the central nervous system (CNS) usually presents as meningitis and on rare occasions as single or multiple focal mass lesions or cryptococcomas (57). The presentation of pulmonary cryptococcosis can range from asymptomatic nodular disease to severe acute respiratory distress syndrome. Classic symptoms of pneumonitis, including cough, fever, and sputum production, may be present, or pleural symptoms may predominate (57). The organism has a predilection to invade skin, bone, and prostate. Skin lesions, if present, need to be biopsied for prompt diagnosis.

Aspergillus infections are often recognized relatively late, and the first signs of infection, such as prolonged antibiotic-resistant fever, may indicate that the infection has spread to several organs (19). Aspergilloma and semi-invasive (chronic necrotizing, pulmonary) aspergillosis may manifest with hemoptysis (58). Patients with IPA typically present with cough or pleuritic chest pain (19). The presence of pleuritic pain during neutropenic fever is highly suggestive of an IFI such as aspergillosis. Clinical manifestations include cough, hypoxia, acute tracheobronchitis, bronchiolitis, and bronchopneumonia. Patients with acute tracheobronchitis usually have normal radiologic findings (19, 58). The presence of halo or air-crescent signs on chest CT scans and a positive galactomannan serum test is indicative of IPA. In patients with disseminated aspergillosis, nodular, erythematous skin lesions resembling ecthyma gangrenosum may be seen (59, 60).

Infections with *Zygomycetes* may be rhinocerebral, pulmonary, gastrointestinal or cutaneous. Disseminated disease is found most frequently in the brain, with possible metastatic lesions in the spleen, heart, and other organs (54). A reverse halo observed on chest CT scan is often a sign of mucormycosis, not aspergillosis (31). Mucormycosis, more so than aspergillosis, should be

suspected in patients, particularly those with diabetes, presenting with sinus involvement in addition to pulmonary disease (21). Breakthrough infections in patients receiving voriconazole may indicate mucormycosis (61–65). Infections with *Fusarium* species often begin with skin lesions; unlike with most filamentous fungi, blood cultures are positive in at least 50% of patients with fusariosis (66). Scedosporiosis should be suspected in cases where there is involvement of the CNS and sometimes the skin, in addition to pulmonary symptoms and positive blood cultures (67).

Early treatment options for IFIs

There are several approaches to the treatment of IFIs (Table 2). Prophylactic therapy is often initiated in patients at high risk of developing fungal infections, for example, allogeneic stem cell transplant patients, patients undergoing chemotherapy that will result in severe neutropenia, patients with immunodeficiency disorders (e.g. chronic granulomatous disease), and patients who are undergoing a period of immunosuppression (68, 69). The recently revised 2009 Infectious Disease Society of America (IDSA) guidelines for candidiasis recommend fluconazole, posaconazole, and caspofungin or micafungin for prophylaxis against invasive candidiasis for patients with chemotherapy-induced neutropenia or stem cell transplant recipients with neutropenia. Fluconazole is optimal for prevention of *C. albicans* infections, while other drugs may be useful in the setting of resistant *Candida* species such as *C. glabrata* and *Candida krusei* (70).

IDSA guidelines recommend posaconazole for prophylaxis against invasive aspergillosis (69). In a study comparing prophylactic posaconazole with fluconazole in patients who had severe graft-versus-host disease and who were receiving immunosuppressive therapy, posaconazole was more effective in preventing proven or probable invasive aspergillosis, as well as decreasing the

number of deaths from IFIs (71). In another trial, patients with neutropenia and acute leukemia who were receiving prophylactic posaconazole had fewer proven or probable fungal infections and higher rates of survival compared with those treated with either fluconazole or itraconazole (72). Voriconazole, when compared with fluconazole, had similar fungal-free survival rates in allogeneic stem cell recipients (73).

Instead of widespread, perhaps excessive prophylactic use of an antifungal drug for early intervention, an empiric or preemptive therapy is initiated only when a fungal infection is suspected. Empiric therapy is initiated after the appearance of non-specific symptoms (e.g. fever) but before a positive culture or other diagnostic assays indicate the presence of a fungal infection. Clinical practice guidelines for the use of antimicrobial agents in patients with neutropenia and cancer recommend all formulations of amphotericin B, itraconazole, and caspofungin for the empiric treatment of patients with febrile (fever unresponsive to antibiotics) neutropenia whose neutropenia lasts >10 d (68). Studies have shown that delaying specific antifungal therapy decreases the effectiveness of treatment and the likelihood of recovery (1, 74–78), thus supporting early intervention. However, because empiric therapy is administered before adequate evidence for the diagnosis of a fungal infection, antifungal drugs may be administered to patients without an IFI. Some studies have shown that IFIs are documented in only ≤5% of patients who receive empiric therapy (79, 80). In contrast to empiric therapy, preemptive therapy is initiated when there are signs/symptoms of an infection with supportive laboratory markers in patients at high risk for invasive mycoses or who are suspected of having a fungal infection. A high index of suspicion is determined by one or more diagnostic techniques, including high-resolution CT scans, serum antigen tests, and PCR assays (49). Pre-emptive therapy has been referred to by some clinicians as presumptive treatment (81) or targeted prophylaxis (49). There are no standardized criteria for the initiation of pre-emptive therapy; different studies use different laboratory tests and different cutoff points as a basis for pre-emptive therapy. For instance, in the study by Hebart *et al.*, pre-emptive therapy was initiated after one positive PCR result, whereas in a report by Lin *et al.*, pre-emptive therapy was initiated after two consecutive positive PCR results (82, 83). Similarly, Maertens *et al.* and Cordonnier *et al.* (51, 84) used different galactomannan cutoff values to determine administration of pre-emptive therapy. Nonetheless, pre-emptive treatment of fungal infections has been associated with lower mortality rates (35, 51, 83, 84).

There is no clear evidence supporting the use of either pre-emptive or empiric therapy over the others (81, 84, 85). Maertens *et al.* showed that pre-emptive therapy

Table 2 Antifungal treatment approaches

Treatment paradigm	Definition
Prophylactic	Administered to high-risk patients; no infection
Empiric	Administered to high-risk patients with possible fungal infection; based on persistent fever despite antibacterial therapy
Pre-emptive	Administered to high-risk patients with probable fungal infection; based on results of one or more diagnostic methods (e.g. CT scan, serum β -glucan or galactomannan)
Targeted therapy ¹	Administered to patients with proven fungal infection

¹Not discussed in this review.

based on serum galactomannan levels and high-resolution CT scans resulted in a 78% decrease in the number of patients receiving unnecessary exposure to expensive and potentially toxic antifungal drugs. In addition, the pre-emptive approach identified 10 cases of fungal infections that clinically were not suspected of being IFIs. Using the empiric therapy paradigm, these patients would not have received antifungal therapy. However, one case of zygomycosis was missed using the pre-emptive approach (51). Using data from CT scans, Greene *et al.* (75) measured the clinical utility of the halo sign for early detection and treatment of IPA. Patients whose treatment was initiated on the basis of the halo sign had better response and survival rates compared with patients who had other imaging findings, suggesting that early pre-emptive therapy based on the halo sign is associated with an improved response. In two other studies, pre-emptive treatment was based on PCR in patients with neutropenia and cancer (83) and β -D-glucan levels in liver transplant patients (35). Although there were false-positives in both studies, the negative-predictive values were >90%, thus reducing the number of patients receiving unnecessary antifungal treatment. Oshima *et al.* (81) conducted a retrospective study in hematopoietic stem cell transplant recipients comparing empiric therapy with pre-emptive therapy, based on chest CT scans, β -D-glucan levels, and galactomannan levels. In the pre-emptive group, 93% fewer patients were given antifungal treatment with amphotericin B deoxycholate, itraconazole, micafungin, or voriconazole than in the empiric group.

Cordonnier *et al.* recently reported the results of a randomized, controlled study comparing empiric with pre-emptive therapy in patients with febrile neutropenia treated for hematological malignancies with either amphotericin B deoxycholate or liposomal amphotericin B. The administration of pre-emptive therapy was based on CT scans, galactomannan levels, and clinical presentation (84). Although pre-emptive therapy identified more proven and probable IFIs, the strategy did not increase mortality compared with empiric strategy. As with the other studies, pre-emptive therapy reduced the number of patients receiving antifungal drugs (84). In a recently published randomized trial of allogeneic stem cell transplant patients, pre-emptive therapy with liposomal amphotericin B was administered to patients with a positive PCR result, and empirical therapy was administered to patients experiencing five or more days of febrile neutropenia refractory to broad-spectrum antibiotics (82). The authors reported no difference in the incidence of IFIs or survival between the two groups. More randomized clinical studies are needed to fully evaluate the advantages and disadvantages of pre-emptive therapy versus empiric therapy.

Conclusions

The diagnosis and treatment of IFIs remain a great challenge. There is a greater occurrence of infections with rare and emerging pathogens that are often resistant to one or more antifungal drugs. Etiologic diagnosis during early infection is important for a favorable outcome. Treatment outcomes may be improved by utilizing recently developed diagnostic techniques, which are non-invasive and allow for earlier and more accurate diagnoses. However, these techniques are not universally accepted, and it is not recommended that they entirely replace the more standard diagnostic approaches, such as culture and histology. Combinations of diagnostic assays may ultimately prove to be necessary to make a rapid, accurate, and definitive diagnosis.

Pre-emptive therapy guided by diagnostic assays may decrease the number of patients receiving unnecessary antifungal treatment without adversely affecting mortality rates and may be an alternative treatment approach to empiric therapy. Further studies of treatment strategies, and the development and standardization of diagnostic tools, are needed reduce the morbidity and mortality associated with IFIs.

Acknowledgements

The author has received speaker honoraria from Schering-Plough Corporation, Pfizer Incorporated, Merck & Co., Inc., Enzon Pharmaceuticals, Inc., and Astellas Pharmaceuticals, Inc; research grants from Schering-Plough Corporation and Pfizer Incorporated; and has been on advisory boards for Enzon Pharmaceuticals, Inc. and Astellas Pharmaceuticals, Inc. Editorial support was provided by Kakuri Omari, PhD, Phase Five Communications Inc., New York, NY, with funding from Enzon Pharmaceuticals, Inc.

References

1. Chamilos G, Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, Safdar A, Raad II, Kontoyiannis DP. 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**:986–9.
2. McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BD, Warnock DW. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. *Clin Infect Dis* 2001;**33**:641–7.
3. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001;**32**:358–66.
4. Baddley JW, Stroud TP, Salzman D, Pappas PG. Invasive mold infections in allogeneic bone marrow transplant recipients. *Clin Infect Dis* 2001;**32**:1319–24.

5. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909–17.
6. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004;**39**:309–17.
7. Zaoutis TE, Argon J, Chu J, Berlin JA, Walsh TJ, Feudtner C. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 2005;**41**:1232–9.
8. Dasbach EJ, Davies GM, Teutsch SM. Burden of aspergillosis-related hospitalizations in the United States. *Clin Infect Dis* 2000;**31**:1524–8.
9. Tong KB, Lau CJ, Murtagh K, Layton AJ, Seifeldin R. The economic impact of aspergillosis: analysis of hospital expenditures across patient subgroups. *Int J Infect Dis* 2009;**13**:24–36.
10. Stevens DA. Diagnosis of fungal infections: current status. *J Antimicrob Chemother* 2002;**49**(Suppl. 1):11–9.
11. Alexander B, Pfaller M. Contemporary tools for the diagnosis and management of invasive mycoses. *Clin Infect Dis* 2006;**43**:S15–27.
12. Wheat LJ. Antigen detection, serology, and molecular diagnosis of invasive mycoses in the immunocompromised host. *Transpl Infect Dis* 2006;**8**:128–39.
13. Archibald LK, McDonald LC, Addison RM, McKnight C, Byrne T, Dobbie H, Nwyanwu O, Kazembe P, Reller LB, Jarvis WR. Comparison of BACTEC MYCO/F LYTIC and WAMPOLE ISOLATOR 10 (lysis-centrifugation) systems for detection of bacteremia, mycobacteremia, and fungemia in a developing country. *J Clin Microbiol* 2000;**38**:2994–7.
14. Horvath LL, George BJ, Murray CK, Harrison LS, Hospenthal DR. Direct comparison of the BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for candida growth detection. *J Clin Microbiol* 2004;**42**:115–8.
15. McDonald LC, Weinstein MP, Fune J, Mirrett S, Reimer LG, Reller LB. Controlled comparison of BacT/ALERT FAN aerobic medium and BATEC fungal blood culture medium for detection of fungemia. *J Clin Microbiol* 2001;**39**:622–4.
16. Procop GW, Cockerill FR III, Vetter EA, Harmsen WS, Hughes JG, Roberts GD. Performance of five agar media for recovery of fungi from isolator blood cultures. *J Clin Microbiol* 2000;**38**:3827–9.
17. Berenguer J, Buck M, Witebsky F, Stock F, Pizzo PA, Walsh TJ. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. Disseminated versus single-organ infection. *Diagn Microbiol Infect Dis* 1993;**17**:103–9.
18. Creger RJ, Weeman KE, Jacobs MR, Morrissey A, Parker P, Fox RM, Lazarus HMI. Lack of utility of the lysis-centrifugation blood culture method for detection of fungemia in immunocompromised cancer patients. *J Clin Microbiol* 1998;**36**:290–3.
19. Marr KA, Patterson T, Denning D. Aspergillosis. Pathogenesis, clinical manifestations, and therapy. *Infect Dis Clin North Am* 2002;**16**:875–94.
20. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008;**47**:503–9.
21. Kontoyiannis DP, Lionakis MS, Lewis RE, et al. Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005;**191**:1350–60.
22. Gade W, Hinnefeld SW, Babcock LS, Gilligan P, Kelly W, Wait K, Greer D, Pinilla M, Kaplan RL. Comparison of the PREMIER cryptococcal antigen enzyme immunoassay and the latex agglutination assay for detection of cryptococcal antigens. *J Clin Microbiol* 1991;**29**:1616–9.
23. Powderly WG, Cloud GA, Dismukes WE, Saag MS. Measurement of cryptococcal antigen in serum and cerebrospinal fluid: value in the management of AIDS-associated cryptococcal meningitis. *Clin Infect Dis* 1994;**18**:789–92.
24. Wheat LJ, Connolly-Stringfield P, Williams B, Connolly K, Blair R, Bartlett M, Durkin M. Diagnosis of histoplasmosis in patients with the acquired immunodeficiency syndrome by detection of Histoplasma capsulatum polysaccharide antigen in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1992;**145**:1421–4.
25. Wheat LJ. Current diagnosis of histoplasmosis. *Trends Microbiol* 2003;**11**:488–94.
26. Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. *Clin Infect Dis* 2005;**40**:844–52.
27. Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001;**19**:253–9.
28. Erjavec Z, Verweij PE. Recent progress in the diagnosis of fungal infections in the immunocompromised host. *Drug Resist Updat* 2002;**5**:3–10.
29. Caillot D, Latrabe V, Thiébaud A, Herbrecht R, De Botton S, Pigneux A, Monchecourt F, Mahi L, Alfandari S, Couaillier JF. Computer tomography in pulmonary invasive aspergillosis in hematological patients with neutropenia: a useful tool for diagnosis and assessment of outcome in clinical trials. *Eur J Radiol* 2009 Jul 3. [Epub ahead of print].
30. Lass-Flörl C, Resch G, Nachbaur D, Mayr A, Gastl G, Auberger J, Bialek R, Freund MC. The value of computed tomography-guided percutaneous lung biopsy for diagnosis of invasive fungal infection in immunocompromised patients. *Clin Infect Dis* 2007;**45**:e101–4.

31. Wahba H, Truong MT, Lei X, Kontoyiannis DP, Marom EM. Reversed halo sign in invasive pulmonary fungal infections. *Clin Infect Dis* 2008;**46**:1733–7.
32. Odabasi Z, Mattiuzzi G, Estey E, Kantarjian H, Saeki F, Ridge RJ, Ketchum PA, Finkelman MA, Rex JH, Ostrosky-Zeichner L. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004;**39**:199–205.
33. Pazos C, Ponton J, Del Palacio A. Contribution of (1->3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005;**43**:299–305.
34. Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1->3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* 2005;**43**:5957–62.
35. Akamatsu N, Sugawara Y, Kaneko J, Tamura S, Makuuchi M. Preemptive treatment of fungal infection based on plasma (1-3)beta-D-glucan levels after liver transplantation. *Infection* 2007;**35**:346–51.
36. Marty FM, Koo S, Bryar J, Baden LR. (1->3) beta-D-glucan assay positivity in patients with *Pneumocystis (carinii) jirovecii* pneumonia. *Ann Intern Med* 2007;**147**:70–2.
37. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a non-invasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001;**97**:1604–10.
38. Sulahian A, Touratier S, Ribaud P. False positive test for aspergillus antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* 2003;**349**:2366–7.
39. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006;**42**:1417–27.
40. Patterson TF. Advances and challenges in management of invasive mycoses. *Lancet* 2005;**366**:1013–25.
41. Buchheidt D, Hummel M, Schleiermacher D, et al. Prospective clinical evaluation of a LightCycler-mediated polymerase chain reaction assay, a nested-PCR assay and a galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. *Br J Haematol* 2004;**125**:196–202.
42. Kawazu M, Kanda Y, Nannya Y, Aoki K, Kurokawa M, Chiba S, Motokura T, Hirai H, Ogawa S. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1->3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004;**42**:2733–41.
43. White PL, Linton CJ, Perry MD, Johnson EM, Barnes RA. The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. *Clin Infect Dis* 2006;**42**:479–86.
44. Willinger B. Laboratory diagnosis and therapy of invasive fungal infections. *Curr Drug Targets* 2006;**7**:513–22.
45. Forrest GN, Mankes K, Jabra-Rizk MA, Weekes E, Johnson JK, Lincalis DP, Venezia RA. Peptide nucleic acid fluorescence in situ hybridization-based identification of *Candida albicans* and its impact on mortality and antifungal therapy costs. *J Clin Microbiol* 2006;**44**:3381–3.
46. Shepard JR, Addison RM, Alexander BD, et al. Multicenter evaluation of the *Candida albicans*/*Candida glabrata* peptide nucleic acid fluorescent in situ hybridization method for simultaneous dual-color identification of *C. albicans* and *C. glabrata* directly from blood culture bottles. *J Clin Microbiol* 2008;**46**:50–5.
47. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. *J Infect Dis* 2000;**181**:309–16.
48. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989–1999. *Clin Infect Dis* 2002;**35**:627–30.
49. Jones BL, McLintock LA. Impact of diagnostic markers on early antifungal therapy. *Curr Opin Infect Dis* 2003;**16**:521–6.
50. Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S, Spriet I, Verbeken E, Van Wijngaerden E. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008;**177**:27–34.
51. Maertens J, Theunissen K, Verhoef G, Verschakelen J, Lagrou K, Verbeken E, Wilmer A, Verhaegen J, Boogaerts M, Van Eldere J. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* 2005;**41**:1242–50.
52. Lass-Flörl C, Günsilius E, Gastl G, Bonatti H, Freund MC, Gschwendtner A, Kropshofer G, Dierich MP, Petzer A. Diagnosing invasive aspergillosis during antifungal therapy by PCR analysis of blood samples. *J Clin Microbiol* 2004;**42**:4154–7.
53. Rickerts V, Mousset S, Lambrecht E, Tintelnot K, Schwerdtfeger R, Presterl E, Jacobi V, Just-Nübling G, Bialek R. Comparison of histopathological analysis, culture, and polymerase chain reaction assays to detect invasive mold infections from biopsy specimens. *Clin Infect Dis* 2007;**44**:1078–83.

54. Garber G. An overview of fungal infections. *Drugs* 2001;**61**(Suppl. 1):1–12.
55. Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis* 2003;**3**:685–702.
56. Radentz WH. Opportunistic fungal infections in immunocompromised hosts. *J Am Acad Dermatol* 1989;**20**:989–1003.
57. Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, Sobel JD, Dismukes WE. Practice guidelines for the management of cryptococcal disease. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:710–8.
58. Franquet T, Muller NL, Gimenez A, Guembe P, de La Torre J, Bague S. Spectrum of pulmonary aspergillosis: histologic, clinical, and radiologic findings. *Radiographics* 2001;**21**:825–37.
59. Messina G, Quartarone E, Console G, Cuzzola M, Iacopino O, Martino M, Irrera G, Morabito F, Iacopino P. Systemic aspergillosis in a patient with non-Hodgkin's lymphoma developing acute graft-versus-host disease after autologous peripheral blood stem cell transplantation. *Haematologica* 2002;**87**:ECR22.
60. Watsky KL, Eisen RN, Bolognia JL. Unilateral cutaneous emboli of *Aspergillus*. *Arch Dermatol* 1990;**126**:1214–7.
61. Dannaoui E, Meletiadis J, Mouton JW, Meis JFGM, Verweij PE. In vitro susceptibilities of zygomycetes to conventional and new antifungals. *J Antimicrob Chemother* 2003;**51**:45–52.
62. Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin Infect Dis* 2004;**39**:743–6.
63. Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. *N Engl J Med* 2004;**350**:950–2.
64. Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, Bartelt LA, Kilborn SB, Hoth PL, Diekema DJ, Pfaller MA. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. *Clin Infect Dis* 2004;**39**:584–7.
65. Trifilio SM, Bennett CL, Yarnold PR, *et al.* Breakthrough zygomycosis after voriconazole administration among patients with hematologic malignancies who receive hematopoietic stem-cell transplants or intensive chemotherapy. *Bone Marrow Transplant* 2007;**39**:425–9.
66. Boutati EI, Anaissie EJ. *Fusarium*, a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. *Blood* 1997;**90**:999–1008.
67. Panackal AA, Marr KA. *Scedosporium/Pseudallescheria* infections. *Semin Respir Crit Care Med* 2004;**25**:171–81.
68. Hughes WT, Armstrong D, Bodey GP, *et al.* 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 2002;**34**:730–51.
69. Walsh TJ, Anaissie EJ, Denning DW, *et al.* Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
70. Pappas PG, Kauffman CA, Andes D, *et al.* Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
71. Ullmann AJ, Lipton JH, Vesole DH, *et al.* Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007;**356**:335–47.
72. Cornely OA, Maertens J, Winston DJ, *et al.* Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;**356**:348–59.
73. Wingard JR, Carter SL, Walsh TJ, *et al.* Results of a randomized, double-blind trial of fluconazole (FLU) vs. voriconazole (VORI) for the prevention of invasive fungal infections (IFI) in 600 allogeneic blood and marrow transplant (BMT) patients. Presented at the 19th American Society of Hematology Annual Meeting. December 8–11, 2007, Atlanta, GA.
74. Garey KW, Rege M, Pai MP, Mingo DE, Suda KJ, Turpin RS, Bearden DT. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. *Clin Infect Dis* 2006;**43**:25–31.
75. Greene RE, Schlamm HT, Oestmann JW, *et al.* Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis* 2007;**44**:373–9.
76. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. *Antimicrob Agents Chemother* 2005;**49**:3640–5.
77. Parkins MD, Sabuda DM, Elsayed S, Laupland KB. Adequacy of empirical antifungal therapy and effect on outcome among patients with invasive *Candida* species infections. *J Antimicrob Chemother* 2007;**60**:613–8.
78. von Eiff M, Roos N, Schulten R, von Eiff M, Roos N, Schulten R, Hesse M, Zühlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration* 1995;**62**:341–7.
79. Walsh TJ, Finberg RW, Arndt C, *et al.* Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med* 1999;**340**:764–71.
80. Walsh TJ, Pappas P, Winston DJ, *et al.* Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002;**346**:225–34. Erratum in: *N Engl J Med* 2007;**356**:760.
81. Oshima K, Kanda Y, Asano-Mori Y, *et al.* Presumptive treatment strategy for aspergillosis in allogeneic haemato-

- poietic stem cell transplant recipients. *J Antimicrob Chemother* 2007;**60**:350–5.
82. Hebart H, Klingspor L, Klingebiel T, *et al.* A prospective randomized controlled trial comparing PCR-based and empirical treatment with liposomal amphotericin B in patients after allo-SCT. *Bone Marrow Transplant* 2009;**43**:553.
 83. Lin MT, Lu HC, Chen WL. Improving efficacy of antifungal therapy by polymerase chain reaction-based strategy among febrile patients with neutropenia and cancer. *Clin Infect Dis* 2001;**33**:1621–7.
 84. Cordonnier C, Pautas C, Maury S, *et al.* Empirical versus preemptive antifungal therapy for high-risk, febrile, neutropenic patients: a randomized, controlled trial. *Clin Infect Dis* 2009;**48**:1042–51.
 85. Segal BH, Almyroudis NG, Battiwalla M, Herbrecht R, Perfect JR, Walsh TJ, Wingard JR. Prevention and early treatment of invasive fungal infection in patients with cancer and neutropenia and in stem cell transplant recipients in the era of newer broad-spectrum antifungal agents and diagnostic adjuncts. *Clin Infect Dis* 2007;**44**:402–9.