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Review

Fungal diagnosis: how do we do it and can we do better?

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Abstract

Background:
Morbidity and mortality remain high for patients with invasive fungal infections (IFIs) despite an increasing number of antifungals and other treatments. Many studies indicate that delayed or inaccurate diagnosis and treatment are major causes of poor outcomes in patients with IFIs.

Objective:
The aim of the current paper is to provide a review of traditional and newer approaches to the diagnosis of IFIs, with a particular focus on invasive candidiasis (IC) and aspergillosis (IA). Recent studies from the author's institution are highlighted, along with an advancement in cryptococcal meningitis diagnosis that should improve the care of AIDS and its opportunistic infection in many developing countries.

Findings:
Currently available tools for the diagnosis of IFIs include traditional methods like histopathology, culture, and radiology, and newer antigen- and PCR-based diagnostic assays. Attempts have also been made to predict IFIs based on colonization or other factors, including genetic polymorphisms impacting IFI susceptibility in high-risk patients. Biopsy with histopathologic analysis is often not possible in patients suspected of pulmonary aspergillosis due to increased bleeding risk, and blood cultures for IC, IA, or other IFIs are hindered by poor sensitivity and slow turnaround time which delays diagnosis. Radiology is often used to predict IFI but suffers from inability to differentiate certain pathogens and does not generally provide certainty of IFI diagnosis. Newer antigen-based diagnostics for early diagnosis include the β-glucan assay for IFIs, galactomannan assay for IA, and a recent variation on the traditional cryptococcal antigen (CRAG) test with a Lateral Flow Assay for invasive cryptococcosis. PCR-based diagnostics represent additional tools with high sensitivity for the rapid diagnosis of IFIs, although better standardization of these methods is still required for their routine clinical use.

Conclusion:
Better understanding of the strengths and weaknesses of currently available diagnostic tools, and further devising linked strategies to best implement them either alone or in combination, would greatly improve early and accurate diagnosis of IFIs and improve their successful management.

Introduction

Candidiasis, aspergillosis, and other invasive fungal infections (IFIs) continue to be significant sources of morbidity and mortality in hospitalized patients throughout the world, and major drivers of elevated healthcare costs[1], despite an increasing number of available antifungal agents with varied pharmacokinetic/pharmacodynamic properties and spectra of activity[2,3]. Optimizing antifungal selection and effectiveness (hence improving patient outcomes and reducing healthcare costs) is dependent on early and accurate diagnosis of IFIs, including accurate identification of the particular fungal species,

if possible[4]. This review examines the current state of IFI diagnosis and the integrated strategies for early, accurate diagnosis and precise management of these challenging IFIs.

Importance of early, accurate diagnosis

Several studies have shown IFIs to be associated with extended hospital stays, elevated healthcare costs, and high mortality rates in patients with candidemia[1,5] or invasive aspergillosis (IA)[6–8]. Furthermore, a link between early diagnosis and improved outcomes and lower healthcare costs has been shown in multiple studies of invasive *Candida* infection, particularly candidemia[9–16]. In IA, a 1995 study by von Eiff *et al.* reported a mortality rate of 90% when antifungal therapy was initiated >10 days after onset of pneumonia due to pulmonary aspergillosis, compared with a much lower rate of 41% when antifungal therapy was started ≤10 days from onset[17]. Others have also shown substantial benefits of early IA diagnosis on improved clinical outcomes[18,19]. At Duke University Medical Center from 2004 to 2005 we were using diagnostic tests (the galactomannan antigen and β-glucan tests, among others), along with more conventional tools – such as risk assessment for focused examinations with specialized radiographs including CT, MRI and PET scans – and use of voriconazole, and observed an approximately 90% survival rate of the initial hospitalization for IA. Therefore, although IA is a serious condition associated with substantial morbidity and mortality, it can be controlled and successfully managed with early diagnosis and treatment.

Difficulties of diagnosis: focus on traditional techniques

Underlying conditions and site of infection associated with IFIs are important in the diagnostic process: they often determine the appearance of the infection and its outcome. For example, while a review of aspergillosis case fatality showed an overall rate of 58%, the rate varied widely based on the underlying disease, ranging from as high as 87% and 86% for patients with a bone marrow transplant and AIDS/HIV, respectively, to 68% and 62% for liver and renal transplant recipients, and 45% for lung and heart transplant recipients[7]. Infection site was also another important variable, with the highest aspergillosis case fatality rate for patients with disseminated or central nervous system infections (88%) and in contrast, the lowest rate for sinusitis (26%)[7]. It is critical for clinicians to keep these important variables in mind when approaching the diagnosis and management of a potential IFI.

Histopathology and culture

One of the major difficulties in accurate diagnosis is obtaining tissue for histopathologic analysis. Invasive techniques, such as a biopsy, may be contraindicated in many neutropenic patients with suspected IA because of elevated bleeding risk due to thrombocytopenia[4,20,21]. Even when obtainable, histopathology alone is not always complete because different fungal species can display similar histopathologic features[4,20] – but under research or 'in house' protocols, the use of specific fluorescent-antibody staining techniques might be used to better identify a specific fungus[22]. However, when used in conjunction with cultures, histopathology can provide the most definitive clue to the causative pathogen. On the other hand depending on certain distinctive morphologic characteristics (septation, branching, advential forms), the pathologist may even be able to make a prediction of the genus for the invading fungus.

With a few exceptions, it is usually possible to differentiate pathogenic yeasts (typically round or oval by histopathology; generally forming smooth, flat colonies and reproducing by budding), molds (composed of tubular structures/hyphae, growth by branching and longitudinal extension)[23], and dimorphic fungi which are yeasts in tissue at 37°C and grow as a mold at room temperature. Detection of a filamentous mold points to infection with *Aspergillus*, *Fusarium*, *Scedosporium*, or molds of the order Mucorales. However, infection with culture backup is required to specifically differentiate between these fungi and identify the unique species and carry out susceptibility testing analyses[24,25]. For instance, important pathogenic *Aspergillus* spp. include *A. fumigatus*, *A. flavus*, *A. terreus*, *A. ustus*, *A. lentulus*, *A. versicolor*, and *A. niger* with different virulence potential and in vitro susceptibility to antifungals[26], so accurately identifying individual fungi are important. Furthermore, with histopathology, the pathogenic mucorales have features that include broad, thin-walled, sparsely septate, ribbon-like hyphae, with open angle (90°) branches[27,28], and in non-sterile sites can be visually distinguished from the uniform, septated hyphae of *Aspergillus*, *Fusarium*, and *Scedosporium*. Finally, certain fungi such as *Fusarium*, *Paecilomyces* and *Acremonium* may produce characteristic advential forms which include yeast-like and hyphal structures observed simultaneously in tissue[29].

Blood cultures are the current 'gold standard' for diagnosis of invasive candidiasis (IC)[4,20], but there are significant difficulties with their use. They are negative in approximately 50% of patients with documented invasive candidiasis candidemia[30–32], and *Aspergillus* spp. and Mucorales fungi are almost never recovered in blood cultures even in disseminated disease[24,33]. Other disadvantages are that invasive techniques may be required to obtain samples from sterile sites[20]; species identification

may take many days[4,34]; and from non-sterile sites it can be difficult to distinguish real infection and disease versus contamination, or colonization[4,20].

Radiology

Radiographic imaging techniques are of value in the diagnosis of IFI, particularly invasive pulmonary fungal infections. Advantages include their noninvasive nature and reasonable predictive value for considering the diagnosis of pulmonary fungal infections[20]. Drawbacks are low specificity and an inability to distinguish among the different fungal species[20]. Chest x-rays can be of limited diagnostic value due to their nonspecificity and an inability to detect early changes consistent with pulmonary mycosis[4]. Conversely, high-resolution computed tomography (CT) in high-risk patients for IFI is capable of detecting early signs consistent with a pulmonary mycosis, but even these early signs ('halo' sign or macronodules) are still not specific for a particular fungal pathogen[4,20].

High-resolution CT is commonly used to aid in the diagnosis of suspected pulmonary IA. It can also be used serially to monitor changes in the pulmonary infection over time. A distinctive pattern of changes has been observed in pulmonary IA patients even with treatment and is characterized by initial lesional growth with associated rise in the number and size of lesions, followed by a plateau in size and then gradual reduction[35]. In fact, development of cavitary pulmonary lesions has been shown to be a strong predictor of both longer time until radiographic remission and improved survival in IA patients. Initial or maximal lesion number and/or size do not predict outcomes[35]. The paradox of longer time until radiographic remission and better outcome may be explained by the physiologic connection between formation of pulmonary cavities and the white blood cell recovery in neutropenic patients and thus reflects the impact of the inflammatory reconstitution observed in the radiographs.

When trying to determine the etiology of a pulmonary IFI and its radiographic changes, clinicians may consider suggestive radiographic clues such as the presence of multiple (≥10) nodules and pleural effusion (but not other radiographic findings) on initial CT scans that have been reported to be significant independent predictors of pulmonary zygomycosis versus pulmonary aspergillosis in neutropenic patients[36]. Although not distinguishing between pulmonary aspergillosis and zygomycosis, the halo sign is another IFI radiographic feature that has been associated with significantly better treatment response and greater survival when present on the initial CT scans from IA patients[37]. However, the halo sign caused by hemorrhage occurs less frequently in non-neutropenic patients with IA, such as those receiving corticosteroid therapy[4]. In addition, sinusitis is a concomitant clinical presentation for pulmonary zygomycosis and its presence is an additional factor favoring a diagnosis of pulmonary zygomycosis[38,39].

Predicting IFIs based on colonization or other factors

Several studies have examined whether the presence and degree of *Candida* colonization can be used to predict invasive *Candida* infections. Some studies identified colonization of non-sterile body sites as a relevant risk factor[40,41]; others have not[42]. In general, recommending guidelines using fungal colonization as a risk factor for disease are impractical, largely because of quality control issues. For example, how many cultures or body sites should be examined? What are the criteria for positive versus negative cultures? Can the expense of initial or repeat cultures be justified?

Ostrosky-Zeichner *et al.* utilized retrospective data to develop a clinical prediction rule for the early detection of invasive candidiasis (IC) in the intensive care unit (ICU) that was based on combinations of known clinical risk factors other than colonization[43]. The investigators were able to validate a risk prediction rule, demonstrating an IC rate of 9.9% among patients meeting the rule. The prediction rule captured 34% of the IC cases in the ICU, and was characterized by 4.36 relative risk, 0.34 sensitivity, 0.90 specificity, and positive and negative predictive values of 0.01 and 0.97, respectively. Despite the relatively low percentage of IC patients captured by the rule, it was a first step in identifying patients at high risk for IC who might be best served by early empiric antifungal therapy.

Since only a subset of patients with similar underlying disease risk profiles for candidiasis, aspergillosis, or other IFIs actually develop the disease, it seems that differences in host genetic susceptibility to IFI may be an important risk factor. For instance, studies suggest that genetic variations within the plasminogen pathway[44] and Toll-like receptor 4 polymorphisms[45] may predispose high-risk bone marrow transplant patients to develop IA. Other studies on susceptibility to candidemia have focused on genetic variations in the Dectin-1/CARD9 recognition pathway[46], cytokine gene polymorphisms[47,48], Toll-like receptor 1 polymorphisms[47], and *CASPASE-12* alleles[49]. It is likely that with further studies and prospective evaluation clinicians will be able to risk-stratify patients for particular IFIs based on an examination of their genetic profile, and then can adjust antifungal management strategies accordingly.

Antigen-based diagnostics for cryptococcosis and endemic mycoses

Antigen-based assays have demonstrated generally high sensitivity and specificity when used for diagnosis of

various endemic mycoses, including histoplasmosis (82–95% and 98%)[50], blastomycosis (89–100% and 98%)[51], coccidioidomycosis (56% and 99%)[52], paracoccidioidomycosis (98–100% and 100%)[53], and penicilliosis (72–90% and 100%)[48], as well as for the opportunistic mycosis cryptococcosis (99% sensitivity, 97% specificity)[54]. Cryptococcosis is of particular note because an inexpensive 'point-of-care' cryptococcal antigen (CRAG) test through lateral flow assay technology (LFA) now exists that can be used on samples from various body compartments (CSF, blood, urine) to provide simple, cheap and rapid diagnosis in developing and developed countries[55].

Use of this novel point-of-care LFA test to diagnose HIV-associated cryptococcal meningitis was recently described[55]. It is similar to prior CRAG assays in that it also provides immunodiagnosis through detection of cryptococcal capsule polysaccharide glucuronoxylomannan (GXM). However, the new CRAG test is a lateral flow assay that uses a dipstick to detect GXM in serum, plasma, urine, or cerebrospinal fluid (CSF), whereas prior tests detected GXM by either latex agglutination (LA) or sandwich enzyme-linked immunosorbent assay (ELISA)[56,57]. These immunoassays, unlike the LFA dipstick test, are both expensive and with extra technology needs and thus not practical in many African settings, where HIV and cryptococcal meningitis is endemic and a frequent cause of death[58]. The LA or ELISA tests often entail sending blood or CSF samples to a central laboratory with trained personnel, which delays diagnosis and treatment. In contrast, the novel LFA dipstick CRAG test is easy to use at the point of care without highly specialized equipment or training, and may utilize CSF, serum plasma, whole blood or urine specimens. In fact, similar results were shown for plasma, serum, and urine with the novel CRAG assay, and there was high correlations between LFA and a quantitative sandwich ELISA in serum (0.93), plasma (0.94), and urine (0.94)[55]. It is hoped that this new point-of-care option will enable earlier diagnosis and improved management strategies for better outcomes, particularly in developing countries with more limited treatment options and resources. Currently, in some African centers, an estimated 70% of patients with AIDS-related cryptococcosis present after HIV diagnosis, and 20%–30% present after initiation of antiretroviral therapy[59,60]. Assuming a 5% incidence of CRAG positivity in HIV-infected patients (a recent study reported more than 7% HIV cryptococcal antigen prevalence in Kenya[61]) and a per LFA dipstick test cost of $2.50, the estimated cost per life saved is $46. Even in resource-limited areas, this test would be expected to have a major impact on patient outcomes[62,63], particularly as studies have shown improved survival with fluconazole treatment of serum CRAG-positive patients[62].

Newer diagnostics for candidiasis

β-glucan (BG) assay

BG is a major cell wall component of most fungal pathogens (except *Mucor* and *Cryptococcus*), and is released into the blood during IFI. Its presence in blood serves as a pan-fungal marker for most IFIs. Various assays have been developed to detect bloodborne BG (Table 1)[64]. These assays now represent standardized tests for the early diagnosis (or exclusion) of a wide range of IFIs. However, because a more specific test is available for IA (the galactomannan assay), the BG test tends to be more commonly used for diagnosis of IC than IA.

Various studies have documented high specificity (64–90%) and sensitivity (73–100%) for detection of candidemia with proper use of different BG assays and cutoffs, and very high negative predictive value (73–97%)[65–69]. A recent meta-analysis of many studies examining the BG assay for the diagnosis of a wide range of IFIs reported pooled results of 72% sensitivity and 85% specificity[70]. Another meta-analysis of several studies utilizing the assay for IFI diagnosis in a specific high-risk group, hematologic malignancy patients, reported similar performance among the four different BG assays examined, and a better diagnostic performance with two consecutive positive tests

Table 1. Comparison of β-D-glucan assay kits (reproduced from Yoshida *et al.* 2010)[64].

Kit	G-test[a]	Fungitec G test-MK	β-glucan test WAKO	Fungitell
Manufacturer	Seikagaku Corporation	Seikagaku Corporation	Wako Pure Chemical Indust.	Associates of Cape Cod Inc.
Lysate	*Tachypleus tridentatus*	*Tachypleus tridentatus*	*Limulus polyphemus*	*Limulus polyphemus*
Pretreatment	Perchloric acid	Alkaline	Dilution-heating	Alkaline
Method	Endpoint chromogenic	Kinetic chromogenic	Kinetic turbidimetric	Kinetic chromogenic
Standard glucan	Pachyman (*Poria cocos*)	Pachyman (*Poria cocos*)	Curdlan (*Alcaligenes faecalis*)	Pachyman (*Poria cocos*)
Detection range (pg/mL)	2–60	3.9–500	6–600	31.25–500
Cutoff value (pg)	20	20	11	60–80
Approval year	1995	1996	1996	2004

[a]This test was removed from the market and replaced by the kinetic formulation (Fungitec G test-MK).
Availability and approval of the kits varies in different countries.

versus a single positive test[71]. One study in hematologic patients showed time to candidemia diagnosis was significantly shorter with BG assay than with clinical, microbiologic, radiologic, and/or histopathologic criteria[69]. Similarly, another study of surgical ICU patients after 3 days in the unit showed that the BG assay detected IC 4–8 days before the clinical diagnosis[69]. Taken together, these results indicate that the BG assay is a useful tool with high sensitivity, specificity, and excellent negative predictive value for the early diagnosis of IC.

Given its ability to detect IFIs early in the disease process, investigators have begun to study and find support for the notion that the BG assay may be used to select patients at high risk for IC who would benefit from early empiric or pre-emptive antifungal therapy[72,73]. One recent study also reported a tendency for BG levels to decrease in successfully treated IC patients and, furthermore, increase in unsuccessfully treated patients[74], suggesting that the BG assay may be useful for monitoring outcomes in IFI patients treated with antifungal agents. However, this study also noted different BG level trends during treatment with different antifungal agents, and particularly a trend for initial increased levels in patients successfully treated with polyenes – possibly related to their fungicidal effects and release of BG with follow-up antigen measurements. More research is urgently needed in this treatment monitoring area.

Overall, advantages of the BG assay include noninvasiveness, possibility of early diagnosis, high sensitivity and specificity for IFIs in general (ability to screen for IFIs), and high negative predictive value (ability to eliminate an IFI diagnosis)[4,20,34]. Disadvantages include nonspecificity for particular fungal pathogens ('panfungal' detection), proneness to false-positive results due to a large number of factors (cellulose-based dialysates, certain antibiotics, drugs containing glucan, surgical gauze, presence of serious bacterial infections, administration of immunoglobulin or albumin preparations contaminated with fungal components, and environmental fungi [dust]), and general 'user unfriendliness' of the assay in the clinical laboratory that often leads institutions to send samples out to a reference laboratory, thereby increasing turnaround time and removing the potential benefits of early diagnosis[4,20,34,64]. However, with its outstanding negative predictive value, the clinician could consider a strategy in high-risk patients of starting an empiric antifungal agent after obtaining blood for a BG assay, and if it returns negative along with negative blood cultures they could stop the empirical antifungal agent. This strategy would help with antifungal use stewardship since presently many patients receive prolonged echinocandins with their potential costs and resistance development complications. Finally, the BG test cannot be reliably used for diagnosis of mucormycosis or cryptococcosis.

Nucleic acid based diagnostics for invasive candidiasis

Molecular or nucleic acid (NA) based methods are a rapidly developing field in fungal diagnostics, particularly for IC or IA. Like the BG assay, NA-based approaches are noninvasive and promise more rapid diagnosis than traditional approaches[4,20]. Depending on the primers used, NA diagnostics can detect fungal pathogens generally or more specifically, including rapid identification of particular fungal pathogenic species with suitable primers and assays like real-time PCR[4,20,75].

Studies have demonstrated high sensitivity, specificity, and positive and negative predictive values for *Candida* spp. in blood with real-time PCR or other NA-based diagnostics[75,76]. The very high sensitivity afforded by PCR-based approaches is particularly appealing and surprising given a report that >50% of initial positive blood cultures for *Candida* species had ≤1 CFU/mL[77]. A recent study reported real-time PCR was even more sensitive than BG assay in diagnosing candidemia, and deep-seated candidiasis with comparable specificity[78]. In addition, both tests were significantly more sensitive than traditional blood cultures in detecting deep-seated candidiasis. In the future, diagnosis of deep-seated *Candida* infections may best be accomplished by PCR, BG assay and blood cultures as a combined testing strategy. For future studies using the host side to read out an underlying IFI may be possible. For example, peripheral blood (monocyte) transcriptional profiling has recently been suggested as an additional molecular tool for the early diagnosis of IC in an animal model[79].

Drawbacks of PCR and other NA-based diagnostics are a proneness to false-positive results (an inability to distinguish colonization or contamination from real infection or disease), non-standardization and lack of commercial availability, and (especially for PCR-based assays) inconvenience for routine use due to high cost and labor-intensive nature[4,20,80]. To date, a variety of NA-based approaches have been studied, using a wide range of primers and amplification, DNA extraction, and measurement approaches, and yielding varied sensitivity and specificity. Because of this lack of standardization, NA-based diagnostics are best considered research or investigational tools at this time[4]. Regarding the inconvenience of PCR technology, a recent study at Duke reported generally similar sensitivity between an innovative digital microfluidic real-time PCR and conventional real-time PCR platform for the detection of culture-proven candidemia[80]. This new system has the potential for full automation, point of care use and rapid turnaround, and thus may help to increase PCR test convenience.

Newer diagnostics for aspergillosis

Galactomannan assay

One increasingly common approach to IA diagnosis involves use of an enzyme-linked immunoassay (Platelia Aspergillus EIA; Bio-Rad Laboratories Inc.) to detect galactomannan, a cell wall component of *Aspergillus* spp., in blood or bronchoalveolar lavage (BAL) fluid[4,20]. Similar to the BG assay, advantages of the galactomannan assay include its noninvasive nature and ability to diagnose IA more rapidly than traditional tools. Unlike BG, galactomannan is more selective for *Aspergillus* spp. and possibly a few other molds. Sensitivity and risk of false-negative results vary depending on the cutoff (optical density index, ODI) selected for positivity, with an ODI of 0.5 appearing to be optimal[4]. A meta-analysis of studies using the assay to diagnose IA based on galactomannan detection in blood reported an overall sensitivity and specificity of 71% and 89%, respectively, for proven cases of IA[81]. The study also reported better results in patients with hematologic malignancy than in solid organ transplant recipients. Negative predictive values for proven IA were higher than positive predictive values and varied by prevalence (ranging from 98–92% and 25–62%, respectively, when prevalence ranged from 5–20%).

High assay sensitivity (76–88%) and specificity (87–100%) for IA diagnosis have also been reported for galactomannan in BAL[82–84], with particular studies reporting higher sensitivity in BAL than serum[82,84] and similar sensitivity and specificity in BAL compared with quantitative PCR[84]. A hematology–oncology patient who gets a BAL and is being considered for IA should probably have the galactomannan test performed on the BAL since many of these patients cannot be biopsied and cultures may be insensitive.

There is some support for the idea that the galactomannan test can be used to select high-risk patients for pre-emptive antifungal therapy[85,86]. Pre-emptive therapy has been reported to reduce the rate of antifungal use and lower antifungal drug costs compared with empiric therapy, with similar clinical outcomes, in neutropenic patients at high risk for IFIs for whom positive galactomannan assay results were used as part of the selection process[85,86]. Other studies suggest serial measurements of galactomannan may also have some value in monitoring treatment response or progression of disease[87–89], although this monitoring strategy is still generally considered experimental. Given the strong correlation between galactomannan values and IA progress, the galactomannan test may also play an important role in distinguishing immune reconstitution inflammatory syndrome from continuing or worsening IA through fungal growth[90], which has critically important treatment implications[91].

A major drawback of the galactomannan test is a propensity for false-positive and sometimes false-negative results[4,20]. False-positive results have been associated with use of β-lactam antibiotics or plasmalyte infusional solutions, colonization with *Bifidobacterium*, or presence of histoplasmosis, blastomycosis, or *penicillinosis*[4,20]. False-negative results may occur from antifungal (anti-mold) therapy, low fungal burden, or an infection that has been walled off in the tissue. Given that the galactomannan assay, BG assay, and PCR assay all exhibit good and often similar but complementary positive predictive values for IA[91,92], optimal early diagnosis may eventually involve using these tests in combination.

Nucleic acid based diagnostics for invasive aspergillosis

PCR-based assays for detection of *Aspergillus*-related NAs are rapidly developing as sensitive tools for the early diagnosis of IA, although many challenges remain, including need for a complex laboratory infrastructure/equipment, and lack of consensus concerning amplification and extraction protocols, targets, and best approaches to measurement[93,94]. Similar to PCR-based diagnostics for IC, a wide range of targets, primers, and amplification, extraction, and measurement approaches have been studied to date for IA diagnosis, resulting in varied outcomes. Nonetheless, progress continues to be made in this area towards uniformity.

A 2009 meta-analysis noted similar mean diagnostic odds ratios and sensitivity for proven/probable IA whether using a single or two consecutive positive blood samples to define positivity, but significantly lower specificity when only a single positive test was used[95].These findings suggest a single PCR-negative result is sufficient to exclude an IA diagnosis, but two consecutive positive tests are required to confirm one. Moreover, progress has been made toward standardization of PCR methodology for *Aspergillus* diagnosis. The European *Aspergillus* PCR Initiative (EAPCRI) Working Group was founded in 2006 with the stated goals to 'develop a standard for *Aspergillus* PCR methodology and to validate this in clinical trials so that PCR could be incorporated into future consensus definitions for diagnosing invasive fungal disease'[96]. Two recent studies from the EAPCRI Working Group demonstrate progress in this direction[97–99], although more work is needed.

In addition to blood, PCR can also be used to detect *Aspergillus* in BAL, respiratory tract biopsy, and sputum samples. Lass-Flörl *et al.* reported a sensitivity of 100% and specificity of 86% when using an *Aspergillus* PCR on CT-guided lung biopsy specimens compared with respective values of 88% and 94% with the galactomannan assay[100]. Another study demonstrated significantly

superior detection of invasive mold infections from histologically positive samples with PCR assays targeting *Aspergillus* and Zygomycetes DNA, respectively, than with culture, indicating potential utility of PCR in patients with suspected mold infection but negative cultures[101]. With respect to use of PCR with BAL samples, a 2007 systematic review reported overall sensitivity and specificity values for IA diagnosis of 79% and 94%[102], while a more recent meta-analysis and systematic review reported values of 91% and 92%, respectively[103]. Of note, recent studies suggest the ability of PCR assays to detect *Aspergillus* spp. in BAL or (particularly) blood samples is diminished when samples are collected after starting mold-active antifungal therapy[104,105].

Conclusions

Outcomes for patients with IFIs are best achieved when rapid and accurate diagnosis enables early treatment with a suitable antifungal regimen. Clinicians now have a variety of diagnostic tools, some better standardized and well integrated into institutional settings than others, and each with its own advantages and disadvantages. Traditional methods commonly have limited sensitivity or specificity, and often require invasive procedures that are not suitable for particular patients. Newer diagnostics based on detection of fungal cell wall components (e.g., β-glucan and galactomannan assays) represent noninvasive approaches for earlier diagnosis of IFIs than afforded by more traditional methods. PCR-based diagnostics represent another noninvasive approach with high sensitivity for the early diagnosis of IFIs, but more work in standardization is needed before they can be considered for general clinical use.

Clinicians need to better understand the newer available diagnostic tools, facilitate incorporation of these tools into their respective healthcare institutions, develop strategies to best utilize them and, where appropriate, combine them with each other and/or traditional diagnostics to achieve rapid, accurate diagnosis. There is also a need for continued research to simplify more complex diagnostic methods, to make them more user-friendly, less expensive, and more easily implemented as point-of-care approaches to early diagnosis. Genetic polymorphisms that impact IFI susceptibility represents another area of research likely to influence future focus for IFI diagnosis and care.

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