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MINIREVIEW

Updates in Laboratory Diagnostics for Invasive Fungal Infections

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ABSTRACT Appropriate diagnosis of invasive fungal infections (IFIs) is critical due to the high rates of morbidity and mortality, as well as the substantial economic burden, associated with the management of these diseases. The recognition of IFI and differentiation from other infections with similar clinical presentations can be challenging, which can lead to diagnostic error that not only has an impact on individual patient health outcomes but also on antimicrobial drug usage and the growing threat of antimicrobial resistance in bacteria. Therefore, there is a significant need for improved stewardship related to diagnostic testing for and treatment of IFIs. The purpose of this review is to highlight recent advances related to current fungal diagnostics, as well as explore some of the most innovative technology that has emerged with the potential to shift the paradigm of clinical mycology. In general, this review will discuss research related to enhanced fungal culture utilization and identification techniques, expanded applications of fungal antigen testing, and recently developed molecular assays and other novel nonculture fungal diagnostic approaches. Specifically, the application of mass spectrometry, novel glycobiomarker detection, and detection of fungal-specific volatile organic compounds will be reviewed, along with other key updates, to provide the reader with an updated review that extends beyond the basics of IFI laboratory diagnostics. Where appropriate, the reader will be directed to more comprehensive reviews of certain aspects of clinical mycology laboratory testing to provide a broader context for the critical consideration of these updates.

KEYWORDS invasive fungal infection, antigen detection, molecular detection, fungal culture

It is estimated that approximately 3 million individuals suffer from chronic severe fungal infections globally, and almost 1.9 million patients develop an acute invasive fungal infection (IFI) each year (1). Many of these are life-threatening infections, with the mortality associated with all fungal diseases estimated to be greater than 1.6 million deaths per year (1). These infections present not only a significant morbidity and mortality burden but are also associated with a substantial economic burden. In the United States alone, a recent analysis estimated the total direct medical cost of fungal disease hospitalizations to be $4.6 billion; *Candida* infections (26,735 hospitalizations, total cost of $1.4 billion) and *Aspergillus* infections (14,820 hospitalizations, total cost of $1.2 billion) accounted for the most hospitalizations and the highest total costs. Importantly, the authors of this report note that, because many fungal diseases likely go unrecognized, these costs likely underestimate the true economic burden (2). Furthermore, this analysis also did not account for the costs related to unnecessary testing, medical procedures, and inappropriate treatment before fungal diagnosis is established (2).

Not only do the difficulties associated with the diagnosis of IFI likely increase the economic burden associated with these diseases, but the failure to appropriately

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diagnose IFI is a major contributor to the high mortality rates. The rate of premortem diagnosis of IFI is approximately 50%, ranging from only 12% to 60%, depending on the etiology of IFI and the type of underlying disease of the deceased patient (3). The diagnostic error that is reflected in this statistic also has a substantial effect on antimicrobial drug usage and the growing threat of antimicrobial resistance in bacteria (4). Therefore, there is a significant need to improve stewardship related to diagnostic testing for and treatment of IFI, as well as the costs and accessibility of current fungal diagnostics (5).

The current definition for the proven diagnosis of IFI requires a microbiological and/or histopathological diagnostic method per 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), but this definition is aimed at providing clarity and uniformity to improve the quality of clinical studies (6). In reality, the sole use of these more conventional techniques for clinical diagnosis is limited by the relatively low sensitivity, slow turnaround, and invasive nature of the specimens required for this testing. Laboratory techniques that do not require growth of the organism in culture or identification in tissue (e.g., fungal antibody, antigen, and nucleic acid detection) play an important role in clinical decision-making related to IFI. Unfortunately, however, they are still limited in widespread availability, appropriate utilization, and clinical performance (7–9).

Because of these limitations, clinical and translational researchers within the field of medical mycology continue to investigate and develop ways to improve fungal diagnostics. There are many excellent reviews that provide in-depth descriptions of the current fungal diagnostics, including discussions of methodology, performance, and utilization; these will be referenced accordingly, as such a broad overview is beyond the scope of this work. The purpose of this review is to feature the most recent and innovative research reported within the field of fungal diagnostics as a means of highlighting the future of IFI detection. The reader's focus will be directed to ongoing efforts to improve the performance characteristics of currently available diagnostic assays, optimize the utilization of these approaches, and develop novel techniques to complement the current armamentarium of fungal diagnostics. Specifically, research related to (i) enhanced fungal culture techniques, (ii) expanded application of fungal antigen testing, and (iii) recently developed molecular assays and other emerging nonculture fungal detection approaches with potential diagnostic utility will be reviewed.

IMPROVING UTILIZATION, INTERPRETATION, AND PERFORMANCE OF CULTURE-BASED FUNGAL DETECTION

Despite its reported lack of sensitivity and lengthy incubation times, the isolation of a fungal pathogen in culture remains the gold standard for the diagnosis of IFI in many situations and plays a critical role in providing *in vitro* susceptibility data. Therefore, within a discussion of the future of fungal diagnostics, this approach still warrants attention. Within the past 5 to 10 years, there have been a number of studies evaluating ways to improve the performance and utilization of fungal culture; two categories of research, surveillance fungal cultures and proteomic-based identification techniques, are described below.

Improved utilization of surveillance fungal cultures. While fungal cultures are often thought of as diagnostic tests for symptomatic patients, a surveillance culture-based approach is sometimes applied in patients at risk for IFI. Historically, studies have demonstrated contrasting utility of this approach (10, 11); more recent findings yield similarly disparate results. For example, in a pilot study performed by Hong et al. (12), researchers found that while 80% of microbiology laboratories do not routinely perform mycology testing on specimens for patients with cystic fibrosis (CF), the inclusion of selective fungal culture media more than doubled the yield of clinically important fungi compared to routine bacterial culture conditions alone. This suggests that current infectious disease diagnostic testing approaches may fail to detect fungal infections in

vulnerable CF patients, highlighting another way in which improved fungal diagnostic test utilization could have a broader impact on antimicrobial stewardship efforts (12).

In contrast, however, Youngster et al. (13) found that, in the context of azole antifungal prophylaxis, the approach of screening pediatric stem cell transplant patients using routine fungal culture of three sites (nares, throat, and stool) performed weekly from the day of admission through discharge of their transplant encounter had unclear to no clinical utility; cultures from nares and throat specimens did not yield results that had an impact on the treatment of patients and stool cultures, which had the highest yield of recovering colonizing fungi (30.3% positivity rate), and did not help distinguish between patients with IFI. Importantly, the authors note that this approach, with its potentially limited clinical utility, is both costly and may lead to the overuse of broad-spectrum antifungals. Therefore, from the perspective of improving IFI diagnostic stewardship, the evaluation of this practice warrants further attention, especially since it is one that is used by 40% of hematopoietic stem cell transplantation (HSCT) centers surveyed as part of this study (13).

Application of MALDI-TOF MS for fungal identification. Regardless of the algorithm used to decide how and when fungal cultures should be used, the identification of potential pathogens is a critical component that determines the performance of this test as a whole. Identification of potential fungal pathogens isolated in culture has historically been performed using macroscopic and microscopic phenotypic observations made by clinical mycologists. This can be a time-consuming and labor-intensive process and is one that relies heavily on the expertise of the clinical mycologists working within individual laboratories. Even with the most highly trained experts, not all clinically relevant molds can be accurately identified using phenotypic methods alone. As a result, many clinical mycology laboratories rely, to some extent, on DNA sequencing as the current gold standard for the identification of fungi. While the more widespread use of sequencing to identify fungal pathogens has improved the overall consistency of and capacity for fungal identification, there are limitations to this approach. For example, accurately identifying and differentiating fungi to the species level using sequencing is still challenging. Incorporating sequencing information from multiple gene targets into an identification algorithm is often necessary for accurately distinguishing between species (14). Furthermore, sequencing-based identification approaches are also limited by the cost of testing, the lack of commercial availability of this technology in all clinical laboratories, and the challenges associated with databases to which sequencing results can be compared for accurate identifications (15). A more in-depth discussion about these databases is beyond the scope of this mini-review but has been well reviewed elsewhere (14).

To help fill the need for more rapid and accessible identification of fungal pathogens, clinical microbiologists have begun applying matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification techniques to yeast and molds isolated from clinical specimens; many excellent reviews of this technology and its application in the field of clinical microbiology have been published previously (16, 17). MALDI-TOF MS has proven to be quite effective for the identification of many yeasts, including *Candida*, *Cryptococcus*, *Rhodotorula*, and *Saccharomyces* species (18). There have been challenges, however, with the implementation of MALDI-TOF MS for filamentous fungi identification, including the lack of a standardized process for culturing and extracting protein for MALDI-TOF MS analysis (19). Furthermore, a continuous extension of spectral libraries will be required to improve the reliability of this technique (20). Work to standardize the processes and expand these databases are underway (21); optimization of this technology will likely improve the clinical microbiology laboratory's capacity for fungal identification, especially for common species or typical strains of filamentous fungi (19), which is a particularly acute need, especially in the context of declining numbers of clinical mycologists who are trained in classical mold identification, which has resulted in a critical gap in expertise (22).

EXPANDED APPLICATIONS OF AND TECHNIQUES FOR FUNGAL ANTIGEN DETECTION

Beyond fungal cultures, one of the most commonly used techniques to aid in the diagnosis of IFI is the detection of fungal-specific antigen clinical samples. This approach often offers more rapid results to aid with diagnostic decision-making, but clinical performance of these tests tends to vary widely based on etiologic agent, specimen type, host characteristics, and testing algorithm used. Therefore, despite the important contribution antigen-based testing has made to the field of fungal diagnostics, there remains significant room for improvement.

Expanded testing for (1,3)-β-D-glucan. Specifically, detection of the fungal cell wall component (1,3)-β-D-glucan (BDG) is commonly used as both a screening tool and a single-use diagnostic for patients with suspected IFI (23). BDG is a pan-fungal antigen found in many organisms, including *Candida* species, *Pneumocystis jiroveci*, and multiple filamentous fungi, such as *Aspergillus* spp., *Fusarium* spp., and *Acremonium* spp.; notable exceptions are *Cryptococcus* spp, *Mucorales*, and the yeast phase of *Blastomyces dermatitidis*. There is currently one FDA-approved assay for the detection of BDG in clinical samples (Fungitell assay; Associates of Cape Code Inc., Falmouth, MA). This assay is only approved for use with serum specimens, but other specimen types have been used and potentially validated for off-label use by individual laboratories. For example, in a retrospective observational study, the diagnostic accuracy of cerebrospinal fluid (CSF) BDG measurements for fungal meningitis among patients exposed to contaminated methylprednisolone acetate was evaluated. Using the manufacturer-recommended cutoff for serum levels ≥80 pg/ml, the researchers found that detection of BDG in CSF performed with 96% and 95% sensitivity and specificity, respectively, for proven meningitis and 84% and 95% sensitivity and specificity, respectively, for probable or proven meningitis. By lowering the threshold value used to interpret the results to 66 pg/ml, optimal sensitivity and specificity could be achieved for proven meningitis at 100% and 94%, respectively (24). These findings were consistent with previously published reports documenting the clinical utility of BDG detection in CSF samples during the multistate outbreak of fungal meningitis (25). Furthermore, in comparing CSF BDG levels to that of serum, researchers found that patients with probable or definitive central nervous system (CNS) fungal infection had significantly higher CSF BDG levels and lower serum-CSF BDG ratios than patients with nonfungal CNS disease, suggesting a potential role for this biomarker in evaluating this disease (26).

In 2018, a second commercial assay to detect BDG in plasma samples, the Wako β-glucan test (GT) (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) was introduced to the European market (27). Studies have shown that there is a strong correlation of BDG levels detected in serum compared to plasma using the GT assay (28). When serum samples were used to compare the performance of the GT assay with the Fungitell assay in a retrospective study, the data suggested that the Fungitell assay is superior to the GT with respect to sensitivity in patients with candidemia (86.7 versus 42.5%, respectively) and *P. jiroveci* pneumonia (PJP) (100% versus 88.9%, respectively) when the manufacturer-recommended cutoff value for the GT was used. Specificity of the GT, however, exceeded that of the Fungitell assay for candidemia (98% versus 85.0%, respectively) (27). Interestingly, this study also highlights differences in the workflow of the two assays. For both assays, the mean time to results is approximately 120 min to complete; for every additional sample, 2 min must be added to the GT workflow, and the analysis of 15 batched samples requires 150 min. In contrast, no additional time is required for the analysis of Fungitell results, but due to the cost of reagents and controls, testing the maximum possible number of samples ($n = 21$) in duplicate in a batched manner is the most economical (27). These variations in workflow and capacity for individual test runs may be important considerations in the effort to make nonculture fungal diagnostics more widely available.

***Aspergillus* galactomannan.** Another cell wall biomarker for IFI is galactomannan (GM). GM is produced by a variety of fungi, including *Aspergillus* spp., *Penicillium* spp., *Paecilomyces* spp., *Purpureocillium licacinum*, and *Histoplasma* spp. There is currently one FDA-approved assay for the detection of *Aspergillus* GM (Platelia *Aspergillus* enzyme immunoassay (EIA); Bio-Rad, Marnes-la-Coquette, France) using both serum and bronchoalveolar lavage (BAL) specimens; additional specimen types, such as CSF, have also been used in clinical practice. There are many good reviews discussing the clinical performance and recommended utilization of this fungal diagnostic (29, 30), and serum GM testing for invasive aspergillosis (IA) diagnostics should now be considered standard, as indicated by the strong recommendations for use in patients with severe immunocompromising conditions presenting with unexplained lung infiltrates suspicious for invasive pulmonary aspergillosis (IPA) by multiple expert bodies (31, 32).

Expanding on the work that has provided evidence supporting these recommendations, which are largely based on neutropenic and allogeneic HSCT recipients, is a recent evaluation of the diagnostic performance of GM testing in patients with nonneutropenic IPA. Using an optimized optical density index cutoff threshold of 0.7, researchers found that detection of GM in BAL fluid was 73% sensitive and 89% specific for the diagnosis of IPA, suggesting its potential value in this patient population as well (33). In this same study, serum GM testing was shown to have a lower sensitivity for IPA than BAL testing (38% versus 76%), with statistically insignificant differences in specificity (87% and 81% for serum and BAL fluid, respectively) (33). This is similar to the findings of a previous study, which evaluated the clinical performance of serum GM testing in nonhematological patients and documented a sensitivity of 23.1% (95% confidence interval [CI], 6.1 to 54.0%) and a specificity of 76.1% (98% CI, 72.9 to 79.0%) (34).

Antigen detection for other fungal pathogens. For the diagnosis of many other IFIs, there have been a number of recent noteworthy advances in the field. For example, the available techniques for cryptococcal antigen (CrAg) detection, which is a mainstay for diagnosing cryptococcal infections, have expanded to include a point-of-care immunochromatographic dipstick testing that has demonstrated promising results with equal or superior sensitivity and specificity in CSF, plasma, and serum samples compared to other commercially available CrAg tests (35, 36). This has significant implications for patient care, especially in settings where early antigen detection in CSF and blood in high-prevalence settings may allow for the more rapid implementation of preemptive strategies required to reduce cryptococcal-related mortality (37).

Histoplasmosis is another IFI for which antigen testing is commonly used for diagnosis. Multiple testing platforms exist for *Histoplasma* antigen detection; a comparison of the MiraVista *Histoplasma* antigen quantitative enzyme immunoassay (EIA) (MiraVista Diagnostics, Indianapolis, IN) and an analyte-specific reagent manufactured by IMMY (Norman, OK) for the detection of *Histoplasma* GM using an EIA showed 90% overall agreement (38). This study was performed specifically on urine specimens; a recently published review of paired urine and serum antigen tests completed on the same patient within 1 week demonstrated that there is a 98% agreement between the tests of the two specimen types (39). In this study, there were six urine antigen tests that were considered false positives based on an electronic medical record review that included other relevant laboratory findings and clinical impression; there was also one true-positive and one false-negative urine antigen test included within this discrepant result review (39). The appropriate interpretation and follow-up of potential false-positive urine antigen results, especially in the context of test results below the limit of quantification, is one that has been critically discussed within the field (40, 41).

Clinical utility of antigen testing in pediatric patient populations. Much of the work related to the use of antigen detection for the diagnosis of IFI has been completed in adult patient populations, thus limiting the applicability of the findings to children. To help evaluate the current state of knowledge related to this unique patient population, a meta-analysis was recently completed to evaluate the use of fungal biomarker

and PCR-based testing for the diagnosis of IFI in pediatric cancer and HSCT patients. Based on the 25 studies that were included within this analysis, the authors found that the sensitivity, specificity, and positive predictive values of GM-, BDG-, and PCR-based assays were highly variable between studies and not necessarily robust in either the screening setting or as a diagnostic test in symptomatic patients (42). Due to the high heterogenicity of the studies included within this analysis, however, as well as the paucity of data for certain applications of these diagnostics, these findings are somewhat limited. To confirm the performance and clinical utility of these nonculture diagnostic methods in pediatric patient populations, additional studies are required. Until their utility can be further clarified, routine BDG testing to guide clinical decisions in children has not been recommended, while prospective monitoring of GM levels twice weekly in high-risk pediatric populations is considered reasonable to aid in an earlier diagnosis of IA (42, 43).

Combining molecular and antigen testing results. Another method for improving the clinical utility and performance of current fungal diagnostics is by interpreting the results of two individual tests used in conjunction. The approach that has been applied most frequently, albeit still relatively rarely compared to standalone testing approaches, is the combination of GM testing and *Aspergillus* PCR diagnostics that use a variety of gene targets (e.g., mitochondrial DNA [44, 45], 28S rRNA [46], 18S rRNA [47], and internal transcribed spacer [ITS] regions of ribosome DNA [48]) to screen for IA in high-risk patient populations. In a meta-analysis of 13 studies that combined these assays in some way, Arvanitis et al. (49) found that using at least one GM- or PCR-positive result to define a positive case achieved the highest sensitivity (99%) of the approaches tested, which was significantly higher than any single test, while combining GM- and PCR- positive results for the same patient was not significantly better than at least two positive GM or PCR results. Importantly, from this meta-analysis, which included 1,670 patients, the authors found that combining the results of GM and PCR weekly screening tests has a negative predictive value of 100% in high-risk patients and that screening with GM and PCR can identify cases of IA earlier than traditional approaches (49). These performance characteristics help guide treatment and are critical to appropriate antifungal stewardship decisions.

A similar approach using a combined interpretation of fungal antigen and PCR detection was recently evaluated for the first time to determine its performance for *P. jiroveci* pneumonia (PJP) diagnosis in a large cancer patient population. In this retrospective cohort study, a laboratory-developed qualitative real-time PCR assay targeting the cyclin-dependent protein kinase *cdc2* gene of *P. jiroveci* was used for bronchoscopy samples in conjunction with the serum Fungitell BDG assay to test specimens collected from HSCT patients being evaluated due to clinical suspicion for PJP. Using this approach, researchers found that serum BDG has a high negative predictive value (95.2%) when the manufacturer-recommended cutoff threshold of less than 80 pg/ml is used in patients being evaluated for PJP. Additionally, in patients with positive-BAL *P. jiroveci* PCR results, a positive serum BDG assay is 100% specific with a 100% positive predictive value for definite/probable infection when a threshold greater than 200 pg/ml is used (50). These findings demonstrate how serum BDG could be used to lower or exclude PJP from a differential diagnosis in non-HIV-immunocompromised cancer patients, which is a relatively novel finding since the majority of prior studies were performed in HIV-infected patients (51), and that a combined testing approach could assist with the interpretation and application of the results from newer fungal diagnostics to treatment decisions in oncology patients at risk for PJP (50).

INTRODUCING NOVEL APPROACHES FOR FUNGAL DIAGNOSTICS AND MONITORING

Beyond the important research that has been conducted in an effort to improve the utilization and performance of currently available fungal diagnostics, there have also been significant contributions to the field recently in the form of novel approaches and

innovative technology aimed at rapidly and accurately identifying IFIs. Some of the most compelling work is highlighted in the following section of this review.

Targeted molecular direct detection. For *Candida*, direct detection assays have been applied for diagnosis of both superficial infections (52) and invasive candidiasis (IC) (53). Recently, the FDA approved the first direct detection *Candida* nucleic acid test (T2Candida; T2 Biosystems, Inc., Lexington, MA). This assay combines targeted PCR with T2 magnetic resonance to allow for the detection of the five most common *Candida* species directly from blood specimens with an overall specificity of 99.4% (95% CI, 99.1 to 99.6%) and overall sensitivity of 91.1% (95% CI, 86.9 to 94.2%) compared with blood culture (54). These findings were largely validated in a study using samples from patients with documented candidemia, with an 89% clinical sensitivity compared to the corresponding positive companion blood culture (55). This study also demonstrated that the T2Candida assay may be better equipped to detect nonviable, growth-inhibited, or latent *Candida* compared to blood culture, which may be particularly relevant in the context of antifungal therapy. The findings of this study, when combined with the original clinical trial findings, indicate that while the negative predictive value remains consistently high (98 to 99.9%) regardless of prevalence, the positive predictive value of the T2Candida varies widely, depending on the expected prevalence of the represented patient population (15 to 92%) (55). Similarly, the cost-effectiveness of using T2Candida to guide antifungal therapy compared to empirical therapeutic approaches is also highly dependent on the prevalence of candidemia, as well as on the cost of antifungal therapy and T2Candida test reagents (56).

In addition to the direct molecular detection of the top five most prevalent *Candida* species, there have also been recent advances in the techniques available to detect the emerging multidrug-resistant fungal pathogen *Candida auris*. A research use-only (RUO) *C. auris*-specific assay was developed to be used on the same T2Dx platform as the *Candida* species assay described, using either blood, skin swabs, or hospital environmental samples. Additionally, both a TaqMan quantitative PCR (qPCR) and an SYBR green qPCR assay have been developed and evaluated for the detection of *C. auris* in skin swabs. All of these assays have demonstrated high performance standards (57–59).

Syndromic multiplex molecular panels also exist and continue to be developed and introduced to the field, which include fungal targets in addition to bacterial, viral, and/or parasitic pathogens. These include panels use for positive blood cultures (60, 61), lower respiratory tract infections (62), and meningitis/encephalitis (63). These types of molecular panels have many advantages, including rapid turnaround times and good analytical performance characteristics; appropriate utilization and interpretation of multiplex panels, however, remains an important discussion point within the field (64, 65).

Pan-fungal approaches. Broad-range molecular detection assays exist to aid in the diagnosis of IFI. These can be valuable diagnostic tools, especially in the context of culture-negative infections or when a specimen has not been collected for culture, but a discussion of these approaches is beyond the scope of this mini-review. More in-depth reviews of this technology, as well as recent advances, can be found in a number of excellent publications (15, 66–69).

Detection of novel biomarkers for invasive aspergillosis. Approaching the challenge of identifying IFI from a novel perspective, the detection of volatile organic compounds (VOCs) in breath that result from metabolism of *Aspergillus*, has been recently used to distinguish invasive disease from other pneumonic processes (70). After researchers used a biologically guided approach to biomarker identification, breath samples from 64 patients with suspected IFI were tested. Researchers found that detection of any of the elements of a specific 4-metabolite signature distinguished the breath of patients with IA compared to patients without IA with 94% (95% CI, 81 to 98%) sensitivity and 93% (95% CI, 9 to 98%) specificity against the reference standard of proven and probable IA by EORTC/MSG consensus criteria (70). While other research

efforts have demonstrated that *in vitro* VOC profiles of *Aspergillus* isolates cultured from clinical specimens cannot always be detected in the breath from the same patients (71), Koo and colleagues provide an interesting proof of concept supporting the potential use of direct detection of exogenous fungal metabolites for the diagnosis of IA (70).

In a similar theme of identifying novel biomarkers for IA, researchers have recently taken advantage of mass spectrometry, not only for the identification of fungi isolated in culture but also in the detection of fungal molecules circulating in patients' sera. In a 2015 report, Sendid et al. (72) described a method of the detection and relative quantification of a pan-fungal serum disaccharide (DS), which was then shown to perform comparably to commercially available BDG and GM detection assays used for the diagnosis of IFI (73). In a retrospective collaborative study of 95 patients, including IFI cases ($n = 52$) and both uninfected neutropenic controls ($n = 10$) and bacteremic patients ($n = 20$), researchers found that this methodology could be used to detect invasive candidiasis (IC) with 51% sensitivity and 87% specificity and IA with 64% sensitivity and 95% specificity compared to mannan plus BDG or galactomannan plus BDG antigen detection for IC and IA, respectively (74). The authors acknowledge that, while these findings are promising, much more work is required to confirm and further explore the potential role for MS-DS as an additional fungal diagnostic tool that may improve antifungal stewardship based on increasing the diagnostic and prognostic significance of glycobiomarkers (74).

As a third approach using a novel detection technique for the diagnosis of IA, researchers have used a novel galactofuranose-specific anti-*Aspergillus fumigatus* monoclonal antibody in a lateral flow immunodiagnostic device in dipstick format to demonstrate that testing urine specimens can be sensitive and specific for the diagnosis of IA. They did observe some cross-reactivity in patients with histoplasmosis, similar to other immunoassays that detect the shared β-galactofuranose epitopes. Specificity for the overall cohort of patients was 92% (95% CI, 74 to 99%). Sensitivity of the assay varied depending on the patient population; for example, in the overall cohort of patients with proven/probable IA, the sensitivity was 80% (95% CI, 61.4 to 92.3%), but it was 89.5% (95% CI, 66.7 to 98.7%) in patients with hematological malignancy, bone marrow transplant, or neutropenia ($n = 50$) and 63.6% (95% CI, 30.8 to 89.1%) in noncancer patients (75). This is consistent with the varying performance of the Platelia GM immunoassay, which was found to range from 61% to 82% sensitivity and 81% to 93% specificity in a Cochrane Library review of 54 studies published prior to 2014 (76). Importantly, the combination of the minimal required sample preparation and the visual interpretation within 30 min of testing (75), along with the relative ease of urine specimen collection, make this new assay particularly promising in the field of IA diagnostics.

Application of flow cytometry for cryptococcal meningitis prognosis and monitoring. Beyond initial diagnosis, novel fungal testing approaches can help guide appropriate antifungal usage by facilitating the monitoring of patients with proven IFI. For example, CSF fungal burden for patients with cryptococcal meningitis is an important determinant of mortality and may be used to guide therapy. Historically, a quantitative culture has been used to make this assessment. The cryptococcal counts measured by flow cytometry correlated strongly with both quantitative culture (Pearson's $r = 0.91$; $P < 0.0001$) and CrAg titer (Spearman's rho $= 0.75$; $P < 0.0001$), suggesting that this technique could be used to provide rapid and accurate measurements of fungal burden in patients with HIV-associated cryptococcal meningitis (77).

SUMMARY

Compared to its counterpart of clinical bacteriology, which has been revolutionized by increasing complex technology (78), the field clinical mycology has been somewhat slower to change. The gold standard for the laboratory diagnosis of IFIs is still largely culture based, with a heavy dependence on phenotypic or genotypic identification techniques. The use of serological and fungal antigen detection assays, along with the introduction of targeted and pan-fungal molecular techniques, have made nonculture

diagnostics an essential component of the evaluation for suspected IFI because they offer more rapid, and often more sensitive, results. None of these tests, however, have proven sufficient to replace the gold standard as standalone tests and should all be used in combination with assessments of the host and radiographic features to optimally manage at-risk patients. Because of the limitations that remain with the current armamentarium of fungal diagnostics, which make the diagnosis of IFI challenging, there is a significant need to improve stewardship related to diagnostic testing for and treatment of IFI, as well as the costs and accessibility of current fungal diagnostics (5). Clinical researchers continue to strive to meet this need, experimenting with innovative applications of current technology and developing novel detection techniques. These efforts should continue to be supported to help address the challenges related to the appropriate diagnosis and management of the devastating diseases caused by invasive fungal pathogens.

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