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MYCOLOGY (J PERFECT, SECTION EDITOR)

What Can the Clinical Mycology Laboratory Do for Clinicians Today and Tomorrow?

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Abstract
Purpose of Review In this review, we discuss the old and new tools available to the clinical mycology laboratory, the "growing pains" in their use, and how they impact patient care and aim to recommend what clinical mycology laboratories need to do to succeed in optimizing patient care.
Recent Findings Through the years, studies have shown the importance of the radiologic, molecular, and non-molecular methods for the diagnosis and follow-up of patients with invasive fungal infections (IFIs) and their impact on patient outcome.
Summary (1) Accurate fungal species identification is essential. (2) Histopathology can be insightful, and the clinical mycology laboratory needs to collaborate with the pathology department for optimization of care. (3) Rapid diagnosis is important and biomarkers need to carefully replace or reduce spiraling antifungal empiricism. (4) Culture techniques need to be carefully integrated with rapid diagnostic methods. (5) IFIs are deadly but a combination of a receptive and progressive mycology laboratory and a small cadre of antifungal agents can save lives. (6) The clinical mycology laboratory needs to be carefully linked to clinical practices, antifungal stewardship, and infection control challenges.

Keywords Clinical mycology · Mycology laboratory

This article is part of the Topical Collection on *Mycology*

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Introduction

Invasive fungal infections (IFIs) continue to burden the significantly increasing population of patients in immunocompromized states [1]. In management of these IFIs, studies have consistently shown that a longer time to initiation of appropriate antifungal therapy in patients with IFIs has been associated with poor patient outcome [2]. Furthermore, selecting the correct antifungal agent and providing appropriate antifungal stewardship with these expensive agents are vital for optimal therapeutic decisions and outcomes in these fragile patients. In order to achieve the best patient outcomes, modern clinical mycology laboratories must have the necessary tools for the accurate and timely diagnosis and follow-up of patients with suspected IFIs and the laboratory must integrate well with clinicians at the bedside. This review aims to summarize the techniques available for the diagnosis and management of patients with IFIs, as well as discuss which of these tools must be present in medical center and hospital laboratories who care for the seriously ill to ensure optimal patient care. Although all acute care hospitals will generally deal at times with some aspect of IFIs, it is important to emphasize that hospitals and/or medical centers vary in their number of patients "at risk" for IFIs and all hospital system hospitals must be aware of their own specific fungal epidemiology.

A Word on Radiology

In medical mycology today and particularly in high-risk patients for mold infections, such as those with hematological malignancies and bone marrow transplants, it is important to note that a primary tool for IFI diagnosis that does not

necessarily utilize the medical mycology laboratory is radiology. For instance, high-resolution computer tomography (CT) may detect nodular infiltrates with or without ground glass attenuation (surrounding halo sign) in a high percentage of high-risk patients early in neutropenia [3]. Furthermore, as a mold infection progresses in the lung, a high-resolution CT pulmonary angiography can detect a vessel occlusion sign or angioinvasion, a hallmark of the invasive mold infection, seen in over 80% of macrodense infiltrates produced by mold infections. This specific radiographic finding can be appreciated and linked to invasive mold infections [4]. Along with these sensitive radiographic tests, the positron emission tomography (PET) scan has now been taken to a new level with the introduction of novel probes for non-invasive detection of *Aspergillus* lung infections. Antibody-guided PET and magnetic resonance (immunoPET/MR) imaging can now allow for increased specificity regarding the identification of lung lesion [5]. Although the immunoPET/MR is in its early stages of development, its principles may be extended routinely for the non-invasive diagnosis of IFIs with further refinements. It is clear that radiographs are an important part of the diagnostic strategy for IFIs and are encouraged to be used by clinicians early in evaluation of high-risk patients. However, it is also obvious that radiology still has significant limitations in identifying specific pathogens as well as measuring their susceptibility to antifungal agents. Therefore, the clinical mycology laboratory remains a pivotal and essential force in helping to accurately diagnose and manage IFIs. In the following discussion, the clinical mycology laboratory will be reviewed to identify what it can and should provide for the clinicians managing immunosuppressed patients at risk for development of an IFI.

Non-molecular Techniques

Invasive Candidiasis

Culture

Blood cultures remain the gold standard for diagnosis of invasive candidiasis [6••]. However, they have proven to be problematic in terms of delay in initiating targeted therapy since cultures require 24–72 h to grow with an additional 24–48 h for organism identification under routine laboratory conditions, varying with both culture conditions and number of circulating fungal cells [6••, 7]. Furthermore, the sensitivity for blood cultures for invasive candidiasis remains low, and without recent autopsy, studies clinicians continue to rely on the figure that half of invasive candidiasis cases are blood culture negative, a figure that has spawned empirical antifungal therapies and continues to encourage use of biomarker strategies in clinical practice.

β-Glucan Assay

β-D-Glucan is a constituent of the fungal cell wall and can be detected in the serum of patients in the setting of several IFIs such as invasive candidiasis, invasive aspergillosis, *Pneumocystis jirovecii* infection, and invasive fusariosis [8]. The β-D-glucan assay has the advantage of using readily available patient serum to detect β-D-glucan, as well as use of CSF and bronchoalveolar lavage (BAL) fluids [8]. However, this test lacks specificity and is affected by multiple environmental and host factors such as dialysis filters, gauze in wounds, and gamma globulin preparations resulting in high false-positive results [8, 9]. Although serial β-D-glucan measurements have shown increased sensitivity in patients suffering from hematologic malignancies and patients that have undergone allogeneic hematopoietic stem cell transplants, specificity still remains low [8]. As a result, the β-D-glucan assay may not be robust for screening certain patient populations that require preemptive antifungal therapy [10]. However, the β-D-glucan assay can be useful as an adjunct to blood cultures for diagnosis of patients suffering from intra-abdominal infections since blood culture sensitivities by themselves are decreased in this population and even be integrated for better detection of invasive candidiasis with blood PCR technology [6••, 11]. This assay has also been successfully used in the diagnosis of the atypical fungus *P. jirovecii*, but it may be more predictive to rule out pneumocystosis in HIV-infected patients than other risk groups, which require additional clinical factors [12]. In terms of monitoring response to therapy, the β-D-glucan assay may have utility. For instance, decreasing β-D-glucan levels during treatment have been shown to predict a favorable response [8]. Furthermore, this very sensitive test has a significant negative predictive value and could be integrated into a strategy to reduce antifungal agent use with substantial antifungal stewardship traction in protocols to stop antifungal agents after they have been started empirically.

Candida albicans Germ Tube Antibody Assay

The *C. albicans* germ tube antibody (CAGTA) assay uses indirect immunofluorescence to detect antibodies to components of the *C. albicans* germ tubes [13]. The assay is unaffected by *Candida* colonization or previous antifungal use, making it helpful in the critical care setting, but this assay relies on antibodies which can make it less accurate in immunocompromized hosts [14].

Mannan Antigen and Antimannan Antibody Test

Combining both a positive mannan antigen and antimannan antibody test has high negative predictive value [15]. However, it may have limited use in immunocompromized patients, with their inability to reliably form antibodies, as well as patients colonized with *Candida*, who may have preformed antibodies [6••].

Invasive Aspergillosis

Tissue Biopsy and Histopathology

Tissue stains, generally, can be performed with ease and at low cost on several different types of samples, including aspirates, tissue biopsies, CSF, sputum, and BAL fluid [16]. The fungi are stained and identified according to morphology. This classical method lacks specificity since many fungi have similar morphological features, and it cannot be used for accurate speciation of the fungi [16]. However, it does allow for a rapid descriptive diagnosis, which may aid in immediate management of the patient [16]. In fact, with good mycological histopathology, the laboratory can identify adventitial yeast-like forms with hyphae in tissue and this finding may aid in categorizing mold infections immediately away from the diagnosis of *Aspergillus* or mucormycetes [17].

Invasive aspergillosis is diagnosed on histopathology by visualization of fungal hyphae in a tissue sample using Gomori methenamine silver or periodic acid-Schiff (PAS) staining [6••]. This method has the additional advantage of detecting fungal invasion into tissues as well as the level of necrosis, providing clinicians with information on the extent of infection. Histopathology and cultures are usually performed in conjunction with cultures and biomarkers to improve the positive predictive value of the specimens. Hence, tissue staining serves more to confirm a positive culture result or to differentiate between colonization, infection, and contamination [6••] and basic histopathology should be available in all clinical laboratories. Furthermore, immunochemistry techniques can now improve accuracy of diagnosis. For instance, in culture-proven cases of mold infection, the immunohistopathology consistently separated aspergillosis from mucormycosis but was slightly less effective when considering a "probable" case, since it may have been another fungal species not identified by the antibodies [18].

Culture

The sensitivity of cultures for diagnosing invasive aspergillosis is low and varies with the patient population being tested and at times may require unacceptable patient risk to obtain tissue for culture [19]. The positive predictive value of cultures in patients receiving a hematopoietic cell transplant was the highest at 72% and the lowest in HIV patients, at 14% [20]. When analyzing sputum cultures, a positive result for an *Aspergillus* spp. most commonly reflects colonization in immunocompetent patients, but in the immunocompromized host, a positive result more likely represents invasive disease [21]. In addition to the variation in interpretation of the cultures based on at-risk populations, the slower identification of growth of *Aspergillus* spp. may delay initiation of appropriate antifungal agents and thus further compromise patient outcome [22]. However, a positive culture does allow the laboratory to identify the species of *Aspergillus*, which may have important clinical implications. And for certain therapeutic decisions regarding antifungal agents, the culture can be used for in vitro susceptibility testing.

β-D-Glucan Assay

The β-D-glucan assay can be used in combination with cultures for diagnosis of invasive aspergillosis. However, there are some patient populations where this may not be the case. For instance, with patients on dialysis (as discussed with candida) as well as those with concurrent Gram-negative bacterial infections, there may be false-positive results [23, 24]. Specifically, bacterial β-D-glucan of *Pseudomonas aeruginosa* can cross-react with the assay, resulting in a false-positive result for patients with bacteremia but without fungemia or IFI [25].

Galactomannan Assay

Galactomannan (GM) is found in the cell walls of *Aspergillus* spp., and the GM assay has a relatively high sensitivity and specificity for detecting invasive disease in certain at-risk populations [26]. It can be performed on patient serum, CSF, BAL, or pleural fluid. However, GM can also be detected in *Histoplasma capsulatum* and *Fusarium* spp. infections, decreasing the specificity of the test and broadening potential detection of invading fungal genus/species, and furthermore, it does not allow for *Aspergillus* speciation [27, 28]. The host population as well as the species of *Aspergillus* itself also affects the assay. Patients with hematological malignancies or recipients of hematopoietic cell transplantation have a high test sensitivity, while patients who have received only steroids having low sensitivities [29, 30]. Furthermore, previous antibiotic therapy has been known to affect the test, but the effect of antibiotic preparations has been reported to be of less concern today [31]. The prior use of antifungal agents decreases the sensitivity of the assay with the exception of caspofungin, which actually increases sensitivity compared to others [29, 32]. The sensitivity of the GM assay was highest when performed on BAL fluid as compared to sera and increases with sequential testing in combination with cultures [33–38]. Although false-positive results may reflect colonization in some patient populations, specificity remains high and minimally affected [38]. The GM assay can also be used to monitor response to antifungal therapy since it commonly remains positive with elevated titers in treated patients who fail therapy, with a study suggesting that the GM assay may even help predict all cause mortality [26, 39].

Lateral-Flow Devices

Quick and simple to use lateral-flow devices (LFDs) can detect a glucoprotein antigen of *Aspergillus fumigatus* in serum or BAL fluid with high specificity [40]. It has been shown to be more

accurate than standard serology and has a higher sensitivity and specificity than the GM assay [41–43]. The interpretation of the result of LFDs at present is subjective and appears more useful for screening high-risk patients rather than direct confirmation of invasive disease [40, 44]. However, in the arena of reduced health resources and/or rapid screening for treatment algorithms, LFD technology may become very attractive to the clinician if it is further validated for patient care.

Electronic Noses

Electronic noses or E-noses use exhaled volatile organic compounds from patients with invasive aspergillosis as a creative and sensitive biomarker for diagnosis [45, 46]. These strategies have been shown to have fairly high in accuracy and are user friendly, quick, and potentially cheap once the equipment is acquired. This interesting technology will be helped by further clinical validation followed by marketing of reliable, cost-effective equipment to medical centers.

P. jirovecii

Histopathology

Pneumocystis pneumonia is a severe lung disease in at-risk patients, caused by *P. jirovecii*. Traditional diagnostic methods rely on histopathology and staining to visualize the cysts/trophozoites from a sample of sputum, BAL fluid, or lung tissue [47]. Histopathology and staining have very high specificities with BAL fluid being the best specimen for diagnosis, although more invasive to obtain and not the most cost-efficient of specimens [47]. However, if lower tract pulmonary specimens can be obtained, the most robust test for diagnosis today is likely a PCR examination of pulmonary secretions [48].

β-D-Glucan Assay

Serum β-D-glucan assay is a suitable test for screening at-risk patients as it has high sensitivities and specificities for diagnosis of *P. pneumonia* and correlates well with fungal load [49, 50]. It is particularly effective when adequate pulmonary samples cannot be obtained, and results can be reported in a timely manner. The assay is also able to distinguish between colonization and infection. In terms of monitoring treatment, the β-D-glucan assay lags behind clinical improvement and so may not be as useful for following up of an immediate response to treatment [51].

Cryptococcus Species

Histopathology (India Ink)

For the diagnosis of cryptococcal meningitis, India ink staining of the CSF will result in visualization of the round encapsulated yeasts in around 50–75% of patients depending on the state of the host's immunosuppression [52]. This simple method necessitates a lumbar puncture for a specimen that may be difficult to obtain in certain patients.

Cerebrospinal Fluid Culture

CSF cultures of patients with suspected cryptococcal meningitis require 72–168 h to show adequate growth for diagnosis [52]. The culture can result in a delay to diagnosis but will be an important feature in monitoring disease and will generally not delay a therapeutic decision. Persistent disease has been judged to be 4 weeks of positive CSF cultures despite receiving recommended treatments. CSF cultures are always very helpful in understanding differences between persistence of infection vs. immune reconstitution inflammatory syndrome (IRIS). An area with substantial research experience is serial quantitative CSF yeast counts [53]. This simple technique has been shown to correlate well with burden of yeasts and provides prognosis for outcome, with the rapidity of change being correlated with outcome during treatment [54, 55]. Unfortunately, despite the substantial experience and real potential to help in management, this test has not yet been integrated into guidelines or routine clinical practice strategies.

Latex Agglutination and Enzyme Immunoassay

Cryptococcal antigen testing is the most accurate fungal serological diagnostic method for screening either serum or CSF of patients at risk in all of medical mycology [52]. This test has the added advantage of being able to use either or both serum and CSF while retaining good sensitivity. Clinicians must respond to a positive serum test in a high-risk patient even when it is difficult to obtain a CSF sample or the CSF is not diagnostic of infection [52]. Although it is recommended that all patients considered to have meningitis receive a CSF evaluation, whether or not the serum antigen is positive, there are, however, occasional false-positive or false-negative tests.

Lateral-Flow Immunoassay

Lateral-flow immunoassay has the advantage of being highly accurate, easy to use, and cost-efficient and can be used on CSF, serum, and urine (with less sensitivity) as an aid for the diagnosis of cryptococcal disease [56]. The simplicity and low cost of this test have made it the standard, both in resource-available and resource-limited clinical care centers.

Despite the widespread use of non-molecular techniques for diagnosing IFIs, the time and expertise needed for these tests as well as variations in sensitivity and specificity of the biomarkers with increased demand for rapid and accurate fungal diagnostics may make them unsustainable in clinical labs

on many medical center sites. Molecular techniques potentially provide an immediate solution.

Molecular Techniques

A Word About Polymerase Chain Reaction

Despite the use of PCR for DNA amplification in detection of several bacterial and viral infectious agents, PCR has still not yet been routinely adopted for the diagnosis of fungal infections. One reason is that PCR does not quantify the amount of fungal DNA in the specimen and hence cannot differentiate between colonization and infection [6••]. However, this has been overcome using real-time PCR (rtPCR) to quantify the amount of amplified fungal DNA for diagnosis [6••]. Other obstacles standing in the way of using PCR regularly for the diagnosis of fungal infections include choice of specimen, method of DNA extraction and isolation, selection of primers, competition with host DNA for amplification, contamination of surfaces and collection tubes due to ubiquitous nature of fungi resulting in false positives, and standardization of results/levels to differentiate colonization from infection [6••, 57]. In other words, PCR needs standardization of procedures and validation of results in clinical practice.

Invasive Candidiasis

Polymerase Chain Reaction

PCR testing for invasive candidiasis has been shown to detect infection in high-risk patients with negative blood cultures, who were later identified as being truly positive for *Candida* spp. [58]. However, the lack of standardization of the PCR method has prevented the development of specific cutoff points to define true negatives and positives for diagnosis of invasive candidiasis [6••]. Similar to *Aspergillus* spp., *Candida* spp. DNA is found circulating in free form and so multiple specimens can be used for detection. Some studies have shown that rtPCR was able to accurately detect and identify *Candida* spp. in under 2 h [59]. This rapid and accurate method for detection and diagnosis is what new molecular techniques should aim to achieve. Furthermore, the PCR assay is able to detect certain strains/mutations with specific antifungal resistance, allowing a short time to not only initiation of antifungal therapy but also identification of appropriate therapy [6••].

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) uses fluorescent probes and microscopy to detect parts of microbial genomes from human samples. This method has been evaluated and marketed for detecting *Candida* spp. from blood culture bottles and shown to have reasonably high accuracy [60]. The probes can also be engineered to detect genes for antifungal resistance potentially allowing cost savings on antifungal choices and initiation of targeted therapy in as little as 5 h [60, 61•]. However, FISH is limited to the identification of five species, and at times, this could be problematic as more species of yeasts frequently infect severely immunosuppressed hosts.

Matrix-Assisted Laser Desorption/Ionization Time of Flight

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) uses mass spectrometry to identify species and strains with unique proteins from a database of known patterns [62]. Compared to conventional methods, MALDI-TOF has shown promising performance in detecting *Candida* from positive cultures both cost-effectively and in a matter of minutes [63]. It has also been shown to have utility in antifungal stewardship, allowing initiation of targeted therapy in a time-efficient manner [64].

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy has been recently used to identify *Candida* spp. from blood cultures using a database of the magnetic resonance spectra of known organisms. More recently, a combination of PCR and NMR spectroscopy to detect *Candida* spp. directly from blood samples without the need for culture is emerging. PCR amplification of known *Candida* spp. DNA sequences is performed on whole blood followed by hybridization to nanoparticles. The nanoparticles elicit T2 magnetic resonance and are detected using NMR spectroscopy [65]. Results have shown that using this technique of combining PCR and NMR spectroscopy can accurately identify five species of *Candida* in an average of 2 h with little sample preparation time [66].

Host Response

A recent technique has been developed for diagnosing candidemia by utilizing the host immune response. Host blood cell RNA gene expression varies depending on the pathogen eliciting the immune response. By detecting this pattern or signatures of gene expressions in response to *Candida* spp., this technique can differentiate a host with fungemia compared to one with bacteremia or no infection [67]. This technique has been studied for candidemia in mice with promising results that may be translated to use in humans with further studies [67].

Invasive Aspergillosis

Polymerase Chain Reaction

The sensitivity and specificity of PCR for detecting invasive aspergillosis vary greatly between studies, and this is due to

the reasons stated previously [6••]. The lack of standardization of the technique between studies proves to be a recurrent factor in the variation of the test results. However, due to the fact that *Aspergillus* DNA circulates as free DNA, the use of serum instead of whole blood for the detection of infection gives the advantage of reducing the processing required to extract and isolate the DNA [68]. This allows for a reduced risk of contamination [6••]. Incorporating PCR with non-molecular techniques, however, could be useful in creating diagnostic strategies with high sensitivity and specificity. For example, combining PCR with a GM assay on a serum specimen in a test requiring only one of the two to turn positive for the diagnosis produces highly sensitive and specific results [69]. PCR can also be performed using BAL fluid with sensitivities similar to the GM assay [70]. Furthermore, some studies have questioned the performance of PCR testing with concurrent antifungal treatment. However, the accuracy of PCR was not affected by antifungal therapy unless two or more antifungal agents were being used [71].

In terms of patient outcome and management of antifungal therapy, PCR also has the potential for clinical utility. Some have suggested that consecutively positive results with PCR are associated with higher mortality rates in patients with suspected invasive aspergillosis [72]. There have also been some inconclusive results regarding the utility of PCR in antifungal stewardship, suggesting that using consecutive PCR results for diagnosis may lead to a decrease in empiric antifungal agent use [73].

Many studies on PCR with molds have been performed with *Aspergillus*, but recent work has encouraged blood PCR to detect Mucorales DNA. For instance, in a retrospective study, it was found that in burn patients, Mucorales DNA was detected on average 11 days before there was a standard method diagnosis [74]. In prior studies, it has been identified that early identification of mucormycosis leads to better outcome [75]. Furthermore, PCR quantitation of blood Mucor DNA loads may be useful to follow in making treatment decisions on lengths of therapy [76].

Finally, the combination of PCR, in situ hybridization, and fluorescence has been shown to be potentially useful with tissue samples. From in situ hybridization and fluorescence to actually see fungus in tissue to the PCR of parafilm-embedded tissue and sequencing amplicon to identify fungus when culture cannot be performed are available. With proper safeguards and careful techniques, these strategies can be extremely helpful [77].

Nucleic Acid Sequence-Based Amplification

Nucleic acid sequence-based amplification (NASBA) amplifies mRNA giving it the advantage of potentially detecting active infection rather than colonization [78]. NASBA has been evaluated alone and in combination with rtPCR for diagnosis and screening for invasive aspergillosis in high-risk patients with results showing high concordance in comparisons of PCR and GM assays, and thus, combination diagnostics may have a promising future [79].

Matrix-Assisted Laser Desorption/Ionization Time of Flight

MALDI-TOF is also highly accurate for detection and identification of *Aspergillus* spp. and can improve on the morphological criteria for the clinical laboratory [80]. There have also been studies on antifungal susceptibility testing using MALDI-TOF to detect strain responses to drugs for rapid detection of drug resistance, allowing potentially earlier initiation of appropriate antifungal agents [81•, 82].

Antifungal Drug Monitoring

After accurate mycological diagnosis and the initiation of appropriate antifungal therapy, drug pharmacokinetics then contribute to the outcome of treatment. Several antifungal drugs display variability in drug levels once administered. This variability may be attributed to inconsistencies in absorption, metabolism, elimination, or interaction with concomitant medications [83]. Monitoring drug concentrations in the blood is a laboratory tool that can be used to ensure that drug levels are within a therapeutic range. The three main methods to measure blood drug concentrations are bioassay, high-performance liquid chromatography (HPLC) with ultraviolet (UV) fluorescent detection, and liquid chromatography-mass spectrometry [84]. In order to utilize these tools clinically, they must be accurate, rapid, and cost-effective [83]. The bioassay is the most cost-effective and simple to perform but will have cross-reactivity with other antifungal agents when drugs are used together and therefore may not be accurate [84]. HPLC with UV fluorescent detection is widely available and can run several drug concentrations in the same assay. However, it may have interactions with several unknown substances and requires a significant amount of time to perform [84]. Liquid chromatography-mass spectrometry is the most sensitive and specific of the tests but is the most expensive and is generally an unavailable tool to clinical microbiology laboratories [84].

The drug in question must fulfill two major requirements to warrant monitoring: First, there is an unpredictable dose-exposure relationship, and second, the relationship between blood drug concentrations and response to treatment must be validated [83]. In terms of antifungal drugs, there are four that fulfill these requirements: flucytosine (5-FC), itraconazole, voriconazole, and posaconazole [84]. Used to treat a wide range of IFIs, studies have shown that 5-FC, itraconazole, voriconazole, and posaconazole blood concentrations vary between patient populations and even within the same patients depending on several factors such as underlying medical conditions, interacting medications, and specific patient

metabolism [85–88]. It has also been shown that patients with a drug concentration within the therapeutic range have better overall outcomes than those with lower levels below a certain threshold [83]. As such, it is important to consider therapeutic drug monitoring (TDM) of these antifungal agents when dose adjustments are made or when interacting drugs are started or stopped, when there is uncertainty with compliance or concerns with GI absorption, and if there are any clinical or laboratory manifestations of toxicity [83, 84, 89, 90]. More specifically to each antifungal agent, a blood level should be measured after initiation of therapy and reaching steady state, so within 72 h for 5-FC, after 5–7 days for itraconazole, and within 7 days for voriconazole and posaconazole. In terms of using these drugs for IFI prophylaxis, the same indications for TDM measuring drug concentrations apply but with a lower trough level required. Similar to treatment studies, patients with drug levels within an appropriate range for effective prophylaxis had fewer breakthrough fungal infections [83].

Drug Susceptibility Testing

Treatment success rates for invasive mycosis in some instances can be unacceptably low and results attributed commonly to clinical and not direct antifungal resistance [91]. Although reference methods and guidelines for performance of antifungal susceptibility testing have been published, the challenge is in how to interpret these direct in vitro susceptibility tests to help measure resistance. Measuring the minimum inhibitory concentration (MIC) of antifungals to individual fungal species is a widely established method of assessing direct antifungal resistance [92]. However, MIC values and breakpoints do not always correlate with patient response to antifungal therapy. This is particularly highlighted by the "90-60 rule," which predicts that 90% of infections caused by susceptible organisms and 60% of infections caused by resistant organisms will respond to treatment [92]. MIC has also been questioned as to whether it is the most appropriate measure of fungal resistance. However, despite the inconsistencies in antifungal susceptibility testing, it does have clinical utility in certain instances such as mucosal or invasive candidiasis not responding to usual or initial antifungal therapy, respectively, invasive disease caused by fungal species with high rates of acquired resistance, invasive disease caused by unusual fungal species lacking known susceptibility patterns, and isolated *Candida glabrata* infections [90, 92–95]. For all other clinical encounters, the importance of susceptibility testing is yet to be established and clinical break points are less certain.

Combined Testing

An important theme with fungal diagnostics has begun to emerge, and it is important to recognize it. It appears that from single institutions to multicenter studies to meta-analyses, a single test may not be as robust as multiple biomarkers used together with routine cultures [96••]. For instance, a combined monitoring strategy based on both serum GM and *Aspergillus* DNA was associated with earlier diagnosis and lower incidence of invasive *Aspergillus* in hematological patients on antimold prophylaxis [97]. Furthermore, in pediatric cancer patients, a comprehensive meta-analysis found that all biomarkers individually had variable sensitivity, specificity, and positive predictive values but high negative predictive values. Its final recommendation was to focus on usefulness of a combination of fungal biomarkers in these high-risk populations [98]. Finally, several biomarkers will help in the diagnosis of invasive candidiasis. For example, *Candida* blood PCR and, to a lesser extent, serum β-D-glucan tests enhanced the ability of blood cultures to diagnose invasive candidiasis, especially intra-abdominal candidiasis [11]. Another complicated diagnostic issue in highly immunosuppressed patients is the concept of mixed or co-infections with multiple pathogens. This situation may not be appreciated with biomarkers and thus makes it essential to have cultures remain as a primary force in any diagnostic strategy. In fact, in severely immunosuppressed patients with invasive pulmonary aspergillosis, it has been estimated that half of the patients were co-infected with other respiratory pathogens [99].

Conclusion

There are a series of basic needs for the clinician by the clinical mycology laboratory today. In any hospital or medical center taking care of acute and/or immunosuppressed patients, the mycology laboratory must have or be in the process of acquiring the following skill sets: (1) Accurate fungal species identification is essential. This ability is now being switched from classical mycological descriptions and biochemistries to MALDI-TOF and PCR/sequencing. It has implications in treatment at the bedside and in infection control. (2) Histopathology can be insightful, and the clinical mycology laboratory needs to collaborate with the pathology department for optimization of care. (3) Rapid diagnosis is important, and therefore, the mycology labs need to work with clinicians to understand and implement effective biomarkers tailored to the local host populations and management strategies. Biomarkers need to carefully replace or reduce spiraling antifungal empiricism. (4) Culture techniques need to be carefully integrated with rapid diagnostic methods. Clinicians have validated clinical practice outcomes on viable fungi (cultures), but this must be combined with more efficient diagnostic methods. Clinicians need to embrace new technology. (5) IFIs are deadly but a combination of a receptive and progressive mycology laboratory and a small cadre of antifungal agents can save lives. (6) The clinical mycology laboratory

needs to be carefully linked to clinical practices, antifungal stewardship, and infection control challenges.

In general, it is a dynamic time for the clinical mycology laboratory with many tools to use and validate. What can the clinical mycology lab do? Used wisely, it has the ability to save the lives of our most fragile patients.

Compliance with Ethical Standards

Conflict of Interest Dr. Perfect reports grants, personal fees, and other from Astellas, Pfizer, Merck, and Amplyx and personal fees and other from ARON, F-2G, Cidara, Scynexis, Viamet, Teva, and Matinas, outside the submitted work.

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