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Invasive Mycoses: Diagnostic Challenges

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ABSTRACT

Despite the availability of newer antifungal drugs, outcomes for patients with invasive fungal infections (IFIs) continue to be poor, in large part due to delayed diagnosis and initiation of appropriate antifungal therapy. Standard histopathologic diagnostic techniques are often untenable in at-risk patients, and culture-based diagnostics typically are too insensitive or nonspecific, or provide results after too long a delay for optimal IFI management. Newer surrogate markers of IFIs with improved sensitivity and specificity are needed to enable earlier diagnosis and, ideally, to provide prognostic information and/or permit therapeutic monitoring. Surrogate assays should also be accessible and easy to implement in the hospital. Several nonculture-based assays of newer surrogates are making their way into the medical setting or are currently under investigation. These new or up-and-coming surrogates include antigens/antibodies (mannan and antimannan antibodies) or fungal metabolites (D-arabinitol) for detection of invasive candidiasis, the *Aspergillus* cell wall component galactomannan used to detect invasive aspergillosis, or the fungal cell wall component and panfungal marker β-glucan. In addition, progress continues with use of polymerase chain reaction– or other nucleic acid– or molecular-based assays for diagnosis of either specific or generic IFIs, although the various methods must be better standardized before any of these approaches can be more fully implemented into the medical setting. Investigators are also beginning to explore the possibility of combining newer surrogate markers with each other or with more standard diagnostic approaches to improve sensitivity, specificity, and capacity for earlier diagnosis, at a time when fungal burden is still relatively low and more responsive to antifungal therapy.
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KEYWORDS: β-Glucan; D-arabinitol; Galactomannan; Mannan; Non–culture-based diagnostics; PCR assays

The incidence of invasive fungal infections (IFIs) is on the rise, largely due to an increasing pool of immunocompromised or severely ill patients at elevated risk for IFIs.[1] IFIs are associated with significant morbidity and mortality, and are increasingly caused by fungal pathogens or subspecies with diminished susceptibility or resistance to many standard antifungal agents.[1] Poor outcome in patients with IFIs can often be related to delayed treatment with an effective antifungal agent or combination of agents due to limitations of standard diagnostic techniques. Diagnosis of IFIs is extremely challenging, because current diagnostic methods are not sufficiently sensitive or specific, and results are often available too late to be clinically useful.[2-4] Newer diagnostic markers and techniques are available and continue to evolve, but many clinicians are unfamiliar with these approaches. Early diagnosis and/or treatment have been shown to improve patient outcomes.[5-8] Hence, there is a clear need to educate clinicians about different techniques available to diagnose and manage patients with IFIs. This article provides a general overview of the importance of early diagnosis, the need for surrogates in medical mycology, and the relative advantages and disadvantages of standard histopathologic and culture-based approaches to IFI diagnosis and newer nonculture-based diagnostic techniques.

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CASE STUDY: PATIENT IN INTENSIVE CARE

A 39-year-old Hispanic woman was admitted to the surgical intensive care unit (ICU) after a major trauma related to a motor vehicle accident. The patient was very ill, had a central line inserted, and was receiving parenteral nutrition owing to a gut injury. On day 10 of ICU admission, she presented with new-onset fever (102.2°F/39°C) and was initiated on broad-spectrum antibiotics. Blood, urine, and sputum samples were collected. After 5 days of therapy, the blood and urine culture results became available and were negative. The sputum culture was growing yeast. The lines were changed and the patient continued to be febrile. Empiric fluconazole therapy was started on day 14 of ICU admission. On day 17, the patient developed hypotension and did not respond to intravenous (IV) fluids and pressors, and subsequently died. Autopsy cultures showed *Candida glabrata* in her blood, liver, kidneys, and spleen.

OVERVIEW OF FUNGAL DIAGNOSTICS: STANDARD TECHNIQUES

Difficulties in Fungal Diagnostics

The case study above illustrates the difficulties in fungal diagnostics and some of the limitations of current standard technologies. Clinical symptoms of IFIs are often nonspecific and therefore generally of little use by themselves when trying to make a timely diagnosis. Histopathologic identification of fungal pathogens in tissue samples and fungal growth using culture-based techniques are the usual means used to diagnose IFIs caused by common fungal pathogens such as *Candida* and *Aspergillus* species.[9] Unfortunately, patient populations at highest risk for IFIs are also those at high risk for complications associated with invasive biopsies, limiting the utility of histopathology.[10,11] For example, biopsy is usually not an option for patients with neutropenia with a suspected IFI such as aspergillosis, because these patients are also likely to have thrombocytopenia and be at risk for bleeding complications.[11] In addition, although microscopic examination of tissue specimens allows for rapid detection and a generic diagnosis of fungal infection, sensitivity and specificity are limited, and culture-based techniques are typically required for identification of the fungal genus or particular pathogen.[9,12,13]

Blood culture is currently considered the "gold standard" for diagnosis of invasive candidiasis, particularly when coupled with clinical symptoms. However, blood cultures are negative for *Candida* in roughly 50% and 30% of patients with biopsy-proven disseminated and single-organ candidiasis, respectively,[14] meaning blood samples will miss *Candida* infection in ≥50% of patients with documented disease. In addition, it typically takes 24 to 72 hours for identification of *Candida* to the species level in culture. Hence, waiting for culture results before making a clinical decision means a delay in diagnosis and initiation of appropriate antifungal therapy.[9] Finally, positive cultures of nonsterile tissue specimens do not distinguish between *Candida* colonization versus disseminated disease, thus complicating interpretation.

Diagnosis of invasive aspergillosis is even more difficult and generally is based on a constellation of patient risk factors (immune status), clinical signs/symptoms, radiologic manifestations, histologic data (when available), and microbiologic evidence, including culture results and, more recently, detection of fungal wall components like galactomannan in serum or other bodily fluids (discussed in further detail below).[15-18] Negative blood cultures are the general rule for invasive aspergillosis, and hence not useful for its diagnosis, even in cases of widely disseminated disease.[16,18] By themselves, clinical signs and symptoms of invasive aspergillosis are generally vague. Chest x-rays are typically too nonspecific to be useful, and, furthermore, changes consistent with invasive pulmonary aspergillosis tend to occur late in the disease course, limiting their use for early diagnosis.[16,19] A high-resolution computed tomography (CT) scan of the chest can provide early signs consistent with pulmonary aspergillosis (e.g., the "halo sign" and macronodules), but these also are not specific for *Aspergillus* infection.[15,18-21] In addition, the halo sign appears to occur less frequently in patients without neutropenia who have pulmonary aspergillosis than in those with neutropenia, particularly when they are receiving corticosteroid therapy.[15,19,22]

Histopathologic analysis is often untenable in patients with suspected aspergillosis, because (as mentioned) patients with neutropenia also tend to have thrombocytopenia, limiting use of biopsy or other invasive techniques. Even when a histopathologic analysis can be performed, typically it is not possible to distinguish *Aspergillus* species from other filamentous fungi,[3,15] and a failure to identify fungi in a pathology specimen does not necessarily mean absence of aspergillosis (or another IFI).[16] Cultures of bronchoalveolar lavage (BAL) fluid, sputum, or other relevant tissue or fluid compartments can be useful in the diagnosis of invasive (pulmonary) aspergillosis and identification of a particular pathogen that can be used to guide subsequent antifungal therapy, but they have the disadvantages of limited sensitivity and a relatively prolonged time for results.[3,18,23] Culture results are best interpreted in the context of risk factors,[15] because most *Aspergillus* culture isolates from nonsterile sites represent contamination or colonization rather than disease,[24] and the positive predictive value for invasive aspergillosis increases with rising immunosuppression.[17,24] *Aspergillus* species also are slow-growing fungi, meaning it may take several days to weeks for positive culture results.[3,25] In addition, culturing techniques typically require specialized expertise for recovery and species determination.[3,9]

Hsu et al.[26] recently identified characteristics of an ideal fungal detection or diagnostic platform, including early detection, good sensitivity, ability to obtain species-level discrimination, detection of a broad range of fungal pathogens (multiplex capacity), reliability, quantitativity (ability to distinguish between disease and colonization), and nonin-