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Have novel serum markers supplanted tissue diagnosis for invasive fungal infections in acute leukemia and transplantation?

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Abstract

Invasive fungal infections (IFIs) are difficult to diagnose, especially early in the course of infection when antifungal therapy is most effective. There are two commercially available biomarker assays useful for detection of the IFIs most commonly seen in patients with hematologic malignancies, the galactomannan and beta glucan assays. The former is specific for aspergillosis, the latter positive for not only *Aspergillus* and *Candida* species, but several other clinically relevant fungal pathogens as well. Both have good assay performance characteristics, provide rapid test results, are widely available, can be assayed non-invasively, and are positive early in the course of infection, often before onset of signs and symptoms of infection. Adoption of these assays into clinical practice has led to reduced need to perform invasive procedures to obtain deep tissue to establish the diagnosis of invasive fungal infections. Improved survival rates from aspergillosis are, in part, due to earlier detection of infection and earlier therapy.

Keywords

aspergillosis; *Aspergillus*; beta glucan; *Candida*; galactomannan; invasive fungal infection (IFI); serum markers; transplantation

Introduction

Historically, invasive fungal infections (IFIs) have been difficult to diagnose. Cultures and histology of infected tissue or blood often are falsely negative or are positive only late in the course of infection, making effective therapy suboptimal. Moreover, invasive diagnostic procedures are fraught with considerable risks.

With the adoption of routine azole prophylaxis, invasive *Candida* infections are today infrequent. The major concern today is mold infection. The principal mold pathogen is

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Aspergillus, accounting for approximately 90% of mold infections with rates of 2% – 28% and 4% – 24% in acute myeloid leukemia (AML) and allogeneic hematopoietic cell transplantation (HCT), respectively. Pneumonia is the most common presentation of invasive aspergillosis (IA). IA accounts for approximately half of all nodular pneumonias in patients with AML and HCT [1].

Chest CT scans are sensitive means to detect pneumonia in AML and HCT patients. Findings most characteristic of IA are circumscribed nodules with or without halos, cavities, and air-crescent signs [Greene, 2007 1444/id]. Lately, other types of nodular infiltrates have been recognized as well [3,4]. Although the presence of such imaging findings should strongly suggest IA, other fungal and bacterial pathogens and some non-infectious conditions can be causative. Further, sometimes infections have multiple pathogens. Thus, mycological confirmation is crucial to ensure appropriate therapy.

Bronchoscopy with bronchoalveolar lavage (BAL) is commonly preferred over tissue biopsy to investigate pneumonia in AML and HCT patients because of the frequency of thrombocytopenia, making percutaneous or open lung biopsies too risky. However, culture and cytology are often negative even with documented IA.

Biomarker assays of fungal molecules have been developed to assist in the diagnosis of IA. These offer the advantage of ease of performance, safety avoiding invasive diagnostic procedures, and earlier diagnosis. The galactomannan and beta glucan assays have been commercialized globally. Adoption of these biomarker assays has revolutionized our approach to the diagnosis of IA. Indeed, survival rates from IA have improved over the past decade in patients with AML and HCT [5,6], in part due to improved diagnosis earlier in the course of invasive infection allowing earlier therapy.

Galactomannan assay

Galactomannan (GM) is a cell wall constituent that is released during hyphal growth in infected tissue. It can be detected in the blood during IA, but not in uninfected patients, including those with *Aspergillus* colonization. Especially attractive is the fact that it can be detected early, often up to a week earlier than the onset of clinical manifestations of IA [7]. GM is relatively specific for IA, but is also found in *Penicillium* infections (Table 1), which are rare pathogens in AML and HCT therapy in the US and Europe. The utility of the galactomannan assay has been best studied in patients with hematologic malignancies and HCT recipients. To optimize early detection and because positivity can vary over time, the assay is generally performed twice weekly during the interval of greatest risk. This risk period would roughly correspond to the full duration of neutropenia for AML therapy and the first 100 days after HCT.

A meta-analysis of 27 studies of GM testing demonstrated good sensitivity and specificity in leukemia and HCT patients (Table 2), but the sensitivity was suboptimal in solid organ transplant (SOT) recipients [8]. The sensitivity and specificity performance characteristics of the GM assay are based on data derived from patients undergoing serial testing. The value of a single test result drawn at the time of suspected IA has been less well studied but the sensitivity is much less.

There are limitations of the GM assay. False positives have been reported in the past in patients receiving concomitant piperacillin tazobactam and amoxicillin-clavulanate. Recent studies suggest false positivity is no longer an issue with several manufacturers but may remain an issue with some [9,10]. *Penicillium* and *Paecilomyces* may also give rise to positivity and there can be cross-reactivity with certain other fungi, including *Histoplasma*

and *Blastomyces* [11]. Dietary sources of GM can cause false positives and patients with aspiration pneumonia may have false positives [12]. False positives have been reported in patients with autoimmune liver disease who undergo liver transplant. Lipoteichoic acid from *Bifidobacterium bifidum*, mainly in neonates, can cause false positive test results. False negative test results can also occur. The presence of *Aspergillus* antibody can lead to false negative test results. The use of anti-mold antifungal drugs, increasingly used, can be associated with invasive infection at low levels of serum galactomannan [13]. Tracheobronchitis, most commonly seen in lung transplant recipients, can occur with negative test results. Abscesses in patients with chronic granulomatous disease occur without positive test results.

Recent studies have studied the utility of GM testing in bronchoalveolar lavage (BAL) specimens. Bronchoscopies have historically had poor yields for IA. Adding a transbronchial biopsy to the procedure has added little to the diagnostic yield but increases the risk of bleed or pneumothorax substantially. However, a number of studies have shown that testing of BAL fluid for galactomannan can increase the yield from 22% – 30% to 45% – 54% [14,15]. A meta-analysis of 13 studies has shown that testing of the BAL sample has a sensitivity of 0.90 and sensitivity of 0.94 in the diagnosis of IA [16]. The BAL GM is an important diagnostic adjunct to the bronchoscopy. An important caveat, use of Plasmalyte as the BAL diluent has been associated with false positive test results. In our experience with routine testing of BAL for GM, we have found a majority of the nodular pneumonias in patients with AML and undergoing HCT are due to IA either alone or along with copathogens. In our center, one third of patients undergoing bronchoscopy for suspected IA had the diagnosis confirmed by the BAL GM while all other tests, including serum GM, were negative [15].

The galactomannan assay can also be used both for prognosis and to monitor response to therapy. Among those with positive GM test at start of therapy, those with very high levels of GM have poorer prognosis than those with lower levels of positivity [17]. Studies of serial testing of GM after start of therapy show that those in whom initially positive GM tests become negative over the first 2 weeks of therapy have a high likelihood of survival, while those who remain GM positive rarely survive IA [18,19]. This is often quite useful since radiologic and clinical manifestations may worsen at the time of neutrophil recovery, not because of lack of antifungal response but rather due to immune reconstitution.

Beta D glucan assay

Beta D glucan is present in the cell wall of most fungi, including *Candida*, *Aspergillus*, *Fusarium*, *Schedosporium*, *Trichosporin*, and *Pneumocystis jirovecii*. Thus, the glucan assay can detect most of the relevant fungal pathogens encountered in patients with AML and undergoing HCT. Important pathogens not detected are the agents of mucormycosis and *Cryptococcus*. Most of the initial clinical studies with this assay were performed in patients with *Candida fungemia*, where the test distinguished infected from non-infected patients. Subsequently, more data have been developed in the AML and HCT setting (Table 2). A recent meta-analysis of experience in more than 1700 patients with hematologic malignancies found an excellent specificity, but the sensitivity was less promising [20].

As with the GM assay, there are false positive test results due to a variety of conditions. Dialysis with cellulose membranes, concomitant use of amoxicillin-clavulanate, bacteremia due to several Gram positive organisms, especially *Streptococcus pneumoniae*, gauze in surgical dressings, use of products manufactured using cellulose depth filters, such as some albumin products, coagulation factors, and plasma protein fractions, contamination by extensive manipulation, and exposure to laundry detergent all have been reported to be factors associated with false positive test results.

Molecular testing

A variety of investigators have evaluated molecular assays, primarily PCR assays for *Aspergillus*-specific DNA sequences or consensus sequences for a variety of fungal pathogens. A number of PCR assays have been developed, most commonly tested in single center studies and a few multicenter studies. A meta-analysis suggests sensitivity of 0.75 to 0.87 and specificity of 0.87 to 0.75 depending on whether one or two positive tests are required, respectively [21]. While promising, the technology has been plagued by a variety of methodological issues. Widespread environmental contamination of collection vessels, test reagents and test devices can cause false positive test results. There are difficulties in DNA extraction. There are a number of methodological variations, some of which have been shown to be crucial in test performance. Attempts to standardize these procedures are underway in Europe where much of this testing has taken place [22]. Use of PCR testing of BAL has also been studied and appears promising [23]. As yet, no PCR assay has been commercialized for patients with AML and undergoing HCT.

What have we learned?

Today, most clinicians have abandoned pursuit of the diagnosis of IFI by invasive biopsies of deep tissues to obtain a tissue diagnosis. This shift in practice has been documented by an abundance of observational studies and analyses of the IFIs in clinical trials of antifungal therapy where the evidence for documentation in most cases is not based on tissue histology or culture but rather reliance on less invasive diagnostic tools. The reason for this shift in practice has been a justifiable attempt to avoid the risks of the invasive biopsies themselves. For a time, many clinicians relied on empiric antifungal therapy in neutropenic patients with persistent fever not responding to antibiotics, and resorted to biopsies only in patients not responding to empiric antifungal therapy. Although empiric therapy provided some protection from IFIs, this strategy was suboptimal for several reasons. First, some patients not needing antifungal therapy are subjected to it. Second, others who are infected with IFIs are given inadequate courses of therapy and will not be given antifungal coverage during subsequent courses of chemotherapy subjecting them to the sizable risk of recurrence if they truly had IA and are not given secondary prophylaxis [24]. Third, delay in bronchoscopy for evaluation of pneumonia has been associated with lower diagnostic yields and poorer treatment outcomes compared to early bronchoscopy [25]. Fourth, many patients with IA have concomitant infections from viruses, bacteria, and occasionally other fungi; in our experience, mixed infections are seen in approximately one fourth of patients with IA.

Because of the high risk of achieving suboptimal treatment outcomes if one chooses the wrong therapy for a patient with IFI, getting a microbiologic diagnosis is strongly urged [1]. For pneumonia, bronchoscopy with either or both BAL and transbronchial biopsy historically had poor yields for fungal pathogens. However, today, testing of BAL with GM or one of the other fungal biomarkers has changed the prospects and dramatically improved the yield of positively making a diagnosis of IFI and also detecting other copathogens and can exclude other possible etiologies. Early diagnosis is key to optimizing antifungal treatment success.

Because the biomarkers have occasional false positive and negative test results and there remains mucormycosis, for which there currently is no biomarker, there still will be some situations in which tissue must be obtained. Patients who are not responding to antifungal therapy prompted by a positive biomarker test should be reassessed to exclude additional pathogens or a drug-resistant species. Fortunately, these situations have dramatically decreased in frequency.

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Table 1

Clinically relevant fungal pathogens detected by biomarker assays.

Galactomannan assay	Beta-D-glucan assay
Aspergillus	Most fungal species except for Cryptococcus and the agents of Mucormycosis
Penicillium	

Table 2

Performance characteristics of the galactomannan and beta glucan assays in meta-analyses.

Galactomannan ⁸				
Patient group	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Leukemia	70	92		
HCT	82	86		
SOT	22	94		
Beta glucan ²⁰				
Hematologic malignancies	50	99	84	95