Early diagnosis of fungal infection in immunocompromised patients

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Techniques for the diagnosis of invasive fungal infection, including antigen testing, nucleic acid detection and radiological imaging, have improved greatly in recent years. They have the potential to impact on patient management through replacing empirical antifungal strategies with targeted and pre-emptive therapy. Factors that influence performance of these diagnostic tests include underlying disease, the prevalence of fungal infection in particular populations and prophylactic antifungal drug strategies. Understanding these factors is necessary for rational use of antifungal agents and optimal management and prevention of fungal infection in immunosuppressed patients.

Keywords: Candida spp., Aspergillus spp., antigen detection, PCR

Introduction

Invasive fungal infection is a major cause of morbidity and mortality in immunocompromised patients. The precise prevalence of disease is not known but population-based surveillance estimates it at 12–17 per 100 000 population.1,2 Candidiasis and aspergillosis remain the most significant problems in the UK. Invasive Candida infections are most commonly seen in critically ill patients in intensive care units (ICUs) and very low birth weight infants. Reported mortality in patients with candidaemia ranges from 36% to 63%, although mortality in ICU patients has decreased in recent years,3 possibly due to more prompt initiation of antifungal therapy.4 Diagnosis remains reliant on traditional culture methods that have improved through the introduction of larger volume lytic-automated culture systems. Even without documented candidaemia, it is possible to identify groups of patients within the ICU who benefit from prophylaxis or pre-emptive therapy5 but widespread antifungal prophylaxis is not recommended and is likely to drive antifungal resistance and pathogen shifts.

Aspergillosis is the most significant fungal infection in immunocompromised patients, particularly those with haematological malignancy and patients undergoing haematopoietic stem cell transplantation (HSCT). Despite the increasing incidence, mortality from invasive aspergillosis (IA) has decreased over recent years6 due partly to the improvements in diagnosis and more effective antifungal drugs. Epidemiological surveillance demonstrates that invasive disease following HSCT is now often associated with ongoing immunosuppression beyond the classic neutropenic period, associated with graft-versus-host disease. Critically ill patients within the ICU may also be at risk of IA with corticosteroid usage being an underestimated risk factor in certain patient groups such as those with chronic pulmonary disease.7 The pathogenesis of disease differs between patient groups and is dependent on the host response and infective fungal load, which can influence the performance of diagnostic tests.

Antifungal drug usage can be divided into four strategies for prevention and treatment. These represent a continuum from prophylaxis (administration of drug to high-risk groups without evidence of disease), through empirical (administration of antifungals to neutropenic patients with persistent refractory fever) and pre-emptive (using clinical, radiological and laboratory markers to determine the likelihood of disease) to treatment of established fungal infection.8 Accurate diagnosis of invasive infection, particularly aspergillosis, remains problematic in patients with haematological malignancy, in whom signs and symptoms are non-specific and often develop late in the course of infection. Mortality from aspergillosis is >70% if diagnosis is delayed.9 Treatment is prolonged and associated with drug toxicities, such that the remission-induction treatment of the underlying disease is delayed, leading to higher relapse rates and increased mortality. This has resulted in the practice of empirical antifungal treatment in at-risk patients with refractory fever. Although considered as ‘standard practice’, there is little evidence to suggest that this is a ‘gold standard’ or that it confers a survival benefit and reduces invasive fungal infection10 compared with patients not on antifungal prophylaxis.11 The costs and toxicities associated with this practice are considerable, as antifungal drugs not only increase the length of stay, but drug-related adverse events are associated with further rises in morbidity and mortality.

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Early diagnosis

Antigen detection

Antigen testing and radiology are included within consensus definitions for diagnosing fungal infection issued jointly by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG)\textsuperscript{12} and may be considered part of the expected standard of care for haematology patients at risk of fungal infection.\textsuperscript{13} Commercially available ELISA kits for the detection of mannann and galactomannan, respectively, are available for Candida and Aspergillus antigens and demonstrate good specificity but variable sensitivity.\textsuperscript{14} Most data exist for Aspergillus galactomannan antigen testing using the Platelia ELISA format. Although many studies have shown that the use of this cell wall biomarker has good specificity for diagnosis of IA, sensitivity is variable ranging from 17\% to 100\%.\textsuperscript{15,16} A major reason for this has been the cut-off used to determine positivity, which uses the ratio between a weak positive control and the test specimen. Until recently, a serum ratio of 1.5 was recommended by the manufacturers. More recent studies have used a cut-off ratio of 0.5–1.0, and the US Food and Drug Administration (FDA) approved a value of 0.5. This greatly increases the sensitivity, albeit at some loss of specificity.\textsuperscript{17} The revised EORTC/MSG\textsuperscript{18} have held back from defining a cut-off, although the body of expert opinion recommends a single value of 0.7 or above or multiple values of 0.5 or greater.

Another factor determining the performance of Aspergillus antigen testing is the prevalence of the disease in a particular population. This is well demonstrated in a recent meta-analysis of 27 studies employing galactomannan antigen detection in the diagnosis of IA.\textsuperscript{19} The positive predictive value of the test rose from 31\% in a population with a prevalence of 5\%, to 69\% when the prevalence was 20\%. Consequently, the use of Aspergillus antigen testing as a screening test is unlikely to be beneficial or cost-effective if the pre-test probability of the disease is low, and should be reserved for high-risk populations including allogeneic stem cell transplant patients, acute myeloid leukaemia patients and patients undergoing aggressive chemotherapeutic regimens for relapsed disease. Serial testing of patients is required to achieve acceptable sensitivity and false-positive results may occur, especially in patients treated with piperacillin/tazobactam.\textsuperscript{20}

Few data are available for the utility of β-1,3-glucan testing but this too may have value in identifying early infection and appears highly sensitive.\textsuperscript{21} Experience in high-risk populations remains limited. Three commercial assays are available and the negative predictive value appears high. However, false-positive results have been reported in patients with bacterial infections and through interfering substances such as haemoglobin and albumin.\textsuperscript{22} Specificity appears to be particularly poor in critically ill patients in the ICU and further studies are required. In December 2006, the FDA approved the Fungitell\textsuperscript{TM} assay although the 60 pg/mL cut-off currently recommended may require some modification to overcome the specificity limitations.

Molecular tests

Molecular amplification methods (PCR and NASBA) have the potential to improve diagnosis but the lack of standardization and absence of any evaluated commercial systems means PCR testing is not included within the EORTC/MSG criteria.\textsuperscript{23} Performance variables include specimen type, molecular target, amplification platforms and detection of amplicon,\textsuperscript{24} and until some form of international consensus is agreed\textsuperscript{25,26} inclusion of nucleic acid amplification methods cannot be recommended as a standard.

Recently a UK PCR consensus group has optimized all aspects of molecular testing and developed a semi-automated extraction method and standardized real-time PCR assay that can be used in routine diagnostic laboratories.\textsuperscript{27} Preliminary evaluation has confirmed the usefulness of this approach both clinically\textsuperscript{27} and in comparison with antigen detection.\textsuperscript{28,29}

Antigen detection and PCR have also been performed on other specimens such as bronchoalveolar lavage (BAL) fluid.\textsuperscript{14} Although more prone to contamination, the negative predictive value of the assay remains high and these specimens may have some diagnostic utility.

It is important to distinguish between the use of these non-invasive tests as a screening tool in high risk patient groups (such as acute myeloid leukaemia and allogeneic transplant patients) and as targeted investigatory tools in other compromised groups such as corticosteroid-treated patients and critically ill patients including neonates. There are limited data on evaluation of these assays in these populations.

Imaging

The radiographic appearances of IA are often non-specific and occur late in the course of disease. Segmental or subsegmental consolidation, patchy infiltrates, nodules (single or multiple), nodular infiltrates and cavitation can occur but they have limited usefulness in the early diagnosis of invasive disease. Chest computed tomography (CT) can detect invasive disease at an earlier stage of infection. The classic halo sign and macronodules are considered early indicators of invasive disease progressing to consolidation (often with pleural involvement), infarcts and cavitary disease with time. Treatment with antifungal drugs based on these early CT findings has been associated with improved survival.\textsuperscript{30}

The halo sign appears to be less sensitive in non-neutropenic corticosteroid-treated patients than in neutropenic patients,\textsuperscript{7} possibly reflecting the different inflammatory response with decreased fungal load and increased inflammatory cellular trafficking in the non-neutropenic lung. This may also affect the performance of antigen testing accounting for variations in sensitivity between different patient groups.\textsuperscript{19}

Clinical impact

Regular surveillance testing is required and, if combined with clinical and radiological information, can allow a move away from empirical therapy towards a targeted approach. Maertens \textit{et al.} used daily serum galactomannan testing combined with early CT scanning in 88 neutropenic patients during 117 febrile episodes. Antifungal treatment was given only if two positive consecutive serum galactomannan results were confirmed on CT-directed BAL or if CT findings suggested invasive fungal infection. Although this was only a feasibility study, the approach halved the potential empirical use of amphotericin B
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without any detectable increase in mortality or excess fungal-related death.\textsuperscript{31}

Early and frequent CT scanning has been used to direct BAL galactomannan antigen detection and has shown good sensitivity prior to instigation of empirical therapy but impact on antifungal usage was not explored.\textsuperscript{32} A randomized study of a PCR-directed versus empirical antifungal approach in more than 400 HSCT patients failed to demonstrate a reduction in antifungal drug use.\textsuperscript{33}

More recently, we have been evaluating twice weekly antigen detection and PCR surveillance in a high-risk population receiving itraconazole prophylaxis combined with early CT investigation of suspected disease. Antifungal therapy is used only in patients with two or more positive diagnostic tests or clinical or radiological evidence of invasive fungal infection. Empirical therapy is withheld in patients lacking positive results unless prophylaxis is considered inadequate through intolerance of itraconazole or subtherapeutic drug levels. The sensitivity and specificity of combined testing are high and considerable savings in antifungal drug expenditure have outweighed the laboratory costs. However, more studies are needed to fully evaluate clinical impacts and long-term survival.

Conclusions

Significant advances in early diagnosis, most notably through the application of non-invasive techniques including antigen testing, nucleic acid detection and radiological detection of fungal infection, have the potential to impact on empirical strategies. When incorporated into care pathways, these techniques may be used to guide pre-emptive therapy and reduce unnecessary empirical antifungal use. Benefits from this approach include not only reduced drug acquisition costs but also reduced morbidity and mortality from drug-related adverse events and decreased hospital length of stay. The ultimate goal will be a reduction in funga-related death and improved overall survival.

Optimal patient management will require identification of high-risk groups in whom the prevalence of fungal infection is known to be 'high' (e.g. 5% to 10% or more) coupled with targeted surveillance. Other lower risk groups should be targeted for diagnostic testing based on clinical signs.

It is possible, however, that newer prophylactic agents, such as posaconazole, could reduce disease prevalence to levels where the performance of surveillance diagnostic tests becomes suboptimal. The recent study by Cornely \textit{et al.}\textsuperscript{34} compared posaconazole prophylaxis with itraconazole and fluconazole in patients undergoing chemotherapy for acute leukaemia or myelodysplastic syndromes. The incidence of fungal infection (proven or probable) in the posaconazole arm was reduced to 2%. It would be difficult to justify costly diagnostic surveillance interventions at this level of disease. However, these new drugs are expensive and cost-effectiveness studies of prophylaxis versus diagnostic surveillance are needed to establish the true benefits of these different approaches.

Other surrogate markers for invasive fungal infection include the detection of secondary metabolites, proteomic approaches and the use of novel immunolabelling approaches to positron emission tomography. Many appear to be promising diagnostic tools but indiscriminate use of expensive technologies in low-risk patient groups is unlikely to bring about benefits in patient management or prove to be cost-effective. Understanding risk stratification, disease prevalence and pathogenesis and factors affecting test performance are key to the rational use of tools for early diagnosis.

Transparency declarations

R. A. B. has served on UK advisory boards for a variety of antifungal agents, including voriconazole and anidulafungin (Pfizer), caspofungin (MSD), AmBisome (Gilead), posaconazole (Schering Plough) and micafungin (Astellas), and has received sponsorship to attend international meetings and honoraria for educational lectures from these companies.

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