Medical Mycology September 2006, **44**, S199–S205

Taylor & Francis
Taylor & Francis Group

Early diagnosis of invasive aspergillosis in infants and children

EMMANUEL ROILIDES
3rd Department of Pediatrics, Aristotle University, Thessaloniki, Greece

Effective management of invasive aspergillosis (IA) requires early and accurate diagnosis. Microscopy and culture of appropriate specimens remain the gold standard of mycological diagnosis. High-resolution computed tomography serially performed constitutes a sensitive mode of diagnosis of pulmonary IA in hematological patients, but similar data is lacking for children. While early diagnosis of IA has been improved with galactomannan assay in adults, its use is problematic in young infants due to specificity inferiority. Galactomannan testing also is characterized by low sensitivity in pediatric patients with primary immunodeficiencies including chronic granulomatous disease and Job's syndrome. Beta-D glucan assay has been studied in adult patients with fungal infections including IA. The high negative predictive value of the assay allows its use for excluding IA; no specific data, however, exist for children. Polymerase chain reaction (PCR) may be a powerful tool for early diagnosis of IA but has not been standardized for routine use yet. No studies address the issue in neonates, whereas in children PCR has not been specifically studied but is probably as good as in adults. A high degree of suspicion in immunodeficient pediatric hosts, suggestive clinical and radiological findings, as well as mycological data by application of multiple diagnostic methods including serology and molecular biology, are expected to enhance the capacity to diagnose IA in young patients.

Keywords invasive aspergillosis, *Aspergillus spp*., pediatrics, early diagnosis

Introduction

Invasive aspergillosis (IA) is an important cause of increased morbidity and mortality in immunocompromised pediatric patients, primarily those with hematological malignancies and those undergoing stem cell transplantation [1,2]. In addition, IA may occur with increased frequency in patients receiving immunosuppressive agents, patients with primary immunodeficiencies, such as chronic granulomatous disease (CGD) [3] and Job's syndrome [4], premature neonates [5] as well as patients infected with human immunodeficiency virus [6].

Correspondence: Emmanuel Roilides, 3rd Department of Pediatrics, Hippokration Hospital, Konstantinoupoleos 49, GR-546 42 Thessaloniki, Greece. Tel: +30 2310 892444; fax: +30 2310 992983; E-mail: roilides@med.auth.gr

Early and accurate diagnosis of IA is critical in both adults and children in order to allow appropriate antifungal therapy to act and to result in mycological destruction and cure of the patient. Late or postmortem diagnosis of IA is related with a mortality rate higher than 80%. By comparison, early diagnosis and initiation of active antifungal therapy is currently followed by a mortality rate of 29% [7].

Improved ability for accurate and early diagnosis of IA has become more than necessary nowadays for the following important reasons: (a) earlier diagnosis is associated with better outcome of IA through prompt initiation of appropriate antifungal therapy; (b) in contrast, more accurate diagnosis with assays possessing high negative predictive value (NPV) would allow less prophylactic and empirical therapy with expensive and potentially toxic new antifungal agents; (c) some antifungal agents have limited or no activity against certain fungal pathogens, i.e., voriconazole against

DOI: 10.1080/13693780600810057

zygomycetes; (d) non-*Aspergillus* filamentous fungi as well as non-*fumigatus* aspergilli, such as *Aspergillus terreus*, with less susceptibility to standard antifungal agents are increasingly recognized as causes of IA nowadays requiring rapid and accurate identification; (e) additionally, exploitation of the clinical efficacy of specific antifungal therapies requires a sensitive and specific early diagnosis of IA [8].

Despite the great benefit resulted from early diagnosis of IA, this is a difficult task due to many factors including difficulty in microscopically detecting and culturing the organism, absence of the organism from an easily accessible site such as blood, and existence of biological properties of the microorganism permitting it to rapidly grow and disseminate in the body even before its detection. In young pediatric patients and particularly in premature neonates accurate diagnosis is even more difficult due to the uniqueness of underlying disease and to non-specificity of the disease presentation.

Clinical suspicion based on appropriate host factors and epidemiological as well as clinical parameters is very important in the early diagnosis of IA. Non-specific laboratory findings, such as white blood cell count, erythrocyte sedimentation rate, C-reactive protein and procalcitonin that assist diagnosis of other infections, are of little help in the diagnosis of IA.

Mycological and histological investigations can give a definitive answer to the diagnostic question. For example, microscopic examination of a specimen is a rapid method of diagnosis of fungal infections especially after addition of potassium hydroxide, or staining with highlighting dyes, such as Gomori's methenamine silver stain, fluorescent dyes calcoflour white, blankophor and other stains. Obviously, the sensitivity of the method depends on the degree of infection.

Culturing of appropriate specimens on mycological media such as Sabouraud dextrose agar or potato dextrose agar remains the gold standard of mycological diagnosis of IA in all the patients. The difficulty of obtaining appropriate specimens from small children, especially preterm neonates due to their small size (i.e., small blood specimen volume, inability of performance of BAL, *etc*.), immaturity of tissues as well as critical condition adds to the general problem of achievement of early and accurate diagnosis at this age. In addition, cultures of blood, cerebrospinal fluid (CSF), pleuritic fluid, sputum, *etc*., have either low sensitivity or variably low specificity for diagnosis of IA.

Radiology

Despite the progress in non-culture serological and molecular biology diagnostic methods that has been witnessed during the last few years, radiological evaluation of lung pathology remains a sensitive diagnostic method for invasive pulmonary aspergillosis (IPA). High-resolution computed tomography (HRCT) of the chest serially performed over time constitutes a sensitive modality of early diagnosis of IPA in high-risk hematological patients [9].

Three studies have examined the role of radiology in the diagnosis of IA in pediatric patients. In the most recent study, 20 patients of a mean age 5 years (7 months to 18 years) with IPA seen over a period of 10 years had radiographic findings that included segmental and lobar consolidation, perihilar infiltrates, multiple small nodules, peripheral nodular masses and pleural effusions [9]. In general, pulmonary findings were varied and often non-specific. In contrast to adult disease, the incidence of central cavitation of pulmonary lesions appeared to be low (2 of 8 patients, 25%). Central cavitation of small nodules without air crescent formation was found in younger patients. No evidence of air crescent formation within areas of consolidation on CT existed as compared to approximately 50% with cavitation and 40% with air crescent formation in adult series [9]. In the other two pediatric reports, 22% (6/27) of patients with cavitation on chest x-ray and 43% (6/14) with cavitation on CT was reported respectively [10,11].

However, existence of nodules on HRCT is not enough to distinguish between IPA and pulmonary infections due to moulds other than *Aspergillus* spp., such as *Fusarium* spp. and *Scedosporium* spp., which are more refractory to antifungal therapy. By comparison, a 'halo' sign, an indication of pulmonary haemorrhage, or non-haemorrhagic inflammatory processes, is considered to be more indicative for IPA, although it can be also found in other fungal infections in lower degree, such as those due to Zygomycetes, *Fusarium* and *Scedosporium*, as well as *Pseudomonas* infection and even in neoplastic and inflammatory diseases [12,13].

A 'halo' sign was observed in 13/17 (76.5%) patients with IPA as compared to none among 31 patients with other pulmonary pathologies of fungal and non-fungal etiology in one study [14]. Recently, early HRCT findings were analyzed in immunocompromised adult patients with IPA and were correlated with the outcome of the disease. Eighty-two percent of cases were followed by an early 'halo' sign, but none of the early radiological signs predicted outcome of aspergillosis [15]. In contrast, no patients with IPA exhibited 'halo' sign in the pediatric study [9]. This difference may be due to differences in the underlying pathology of IPA in the two patient populations, such as non-angioinvasive

disease seen in CGD, differences in underlying disease as well as the particular age-dependent immune response of the host.

Galactomannan assay

Diagnosis of IA has benefited from new antigen detection systems, such as the enzyme-linked immunosorbent assay (ELISA) galactomannan (GM, Platelia Aspergillus®) assay. In older patients, numerous studies have shown that GM assay has an overall sensitivity > 85% ranging from 60% to 92.6% and a specificity ranging above 85% [16,17].

Although several GM studies have included children in their cohorts, they have not reported separate pediatric data. In addition, there has been a paucity of studies with predominance of pediatric patients. In one prospective study conducted between 1995 and 1998, 450 adult allogeneic HSCT pts (3883 samples) as well as 347 children with hematological malignancies (2376 samples) were studied [18]. In this study, the first positive GM sample was observed earlier in pediatric patients (median of 36 days) than in adults (median of 74 days post-transplant). The false positive rate in adult patients was 10/406 or 2.5% as compared to 34/338 or 10.1% in children. While the sensitivity and specificity of the assay (using optical density index values >1.5 in at least 2 sequential samples) were 88.6% and 97.5%, respectively, the sensitivity increased to 100% in adult patients, and the specificity dropped to 89.9% in children.

In another study, the false positive rate in a group of neutropenic patients with 797 episodes of fever of unknown origin, including 48 pediatric patients, was 0.9% in adults and 44% in children ($P < 0.0001$) [19]. Overall specificity was 98.2% for adult patients as compared to 47.6% for children ($P < 0.0001$). Thus, the clinical specificity of GM in children appears to be lower than that in adults. Overall positive predictive value was calculated to be 92.1% for adult HSCT recipients and 15.4% for children ($P < 0.0001$). Similarly to the lack of 'halo' sign in pediatric patients observed, this may be due to differences in the particular pathology of IPA in the two patient populations, differences in underlying disease as well as age-dependent immune response of the host to the infection. Children may also receive foods that contain GM in higher proportion than adult patients as discussed below.

Antibiotics, primarily piperacillin-tazobactam, but also amoxicillin alone or combined with clavulanic acid, represent a source of extraneous GM that may compromise clinical specificity of the assay [20–22]. This may be due to the presence of GM in the drugs themselves leading to false-positive results and thus to an inherent difficulty to use the test in patients receiving these antibiotics. This antibiotic-dependent problem occurs in both adults and children and has been well discussed in the recent literature [20–23]. Its molecular basis is not fully determined, but as age-independent problem, it lies beyond the scope of this review and is not further discussed.

An important age-dependent problem of utility of GM assay in pediatrics is the low specificity of the assay observed in neonates and young infants. In an early study, among six premature neonates with prolonged Neonatal Intensive Care Unit stay and birth weight 400–1320 g, a single GM false positive test was observed in 5 of them or 83% as compared to no positive GM results in adult patients with unlikely infection [24]. In another study, not only single samples were found to be false positive, but consecutive samples also were false positive in neonates [25,26].

Two theories have attempted to explain the increased false positivity of GM assay observed in neonates. One of them has suggested that lipoteichoic acid, a constituent of the bacterial wall of *Bifidobacterium bifidum* subspecies *pennsylvanicum* abundantly existing in the intestinal microflora of neonates, mimics the epitope recognized by the EB-A2 in the ELISA GM kit [25,27]. Indeed, *Bifidobacterium* spp. is very common in gut microflora of neonates and young infants up to the age of approximately 2 months. Ninety-one percent of the total microflora of breast-fed neonates is composed of *Bifidobacterium* spp. as compared to 75% of the total microflora of formula-fed neonates and only 3% in adults [25].

A second theory has suggested that GM-positive infant formula used in neonates as well as GM-containing food and even water combined with increased translocation across an immature or damaged intestinal epithelium is responsible for the false positivity observed in the neonates [24]. Indeed, 8/14 milk formulas tested were GM positive as compared to none of breast milk samples. The wide use of *Bifidobacterium* spp. in probiotics administered as supplements of infant formulas implying advantages of a 'breast-fed-like' flora is expected to worsen the problem of non-specific existence of galactomannans in these infants [28,29]. Nevertheless, the complete answer of this difference between pediatric and adult patients remains elusive.

GM testing in children also has the disadvantage of false negative results in certain pediatric patients at high risk for IA such as those with CGD. In particular, while sensitivity of GM assay is higher than 90% in

hematological patients, in children with CGD it appears to be much lower. One study evaluated patients with CGD or Job's syndrome who suffered from IA and found that GM antigenemia was detected in only 4 of 15 (27%) cases of CGD and Job's syndrome as compared to 24 of 30 (80%) cases of all other immunocompromising conditions ($P = 0.0004$) [30]. The reasons of this difference are unclear; it may be due to more localized and less angioinvasive IA disease in CGD patients, to particular sites of infection, i.e., bone infections that are more frequent in these patients than in hematological patients, or to certain immune factors unique in CGD patients. For example, these patients possess intact T and B lymphocytic functions as well as normal numbers of phagocytes, whereas patients with hematological malignancies or HSCT may have profoundly impaired lymphocyte number and/or function as well as non-existing phagocytes.

(1,3)-beta-D glucan assay

Another very promising diagnostic method, detection of (1,3)-beta-D glucan in blood, has been studied in adult patients with fungal infections [31–33]. (1,3)-beta-D glucan is a wall constituent of most fungi with the exception of zygomycetes and *Cryptococcus neoformans* [34].

The presence of (1,3)-beta-D glucan in the walls of other equally important fungi such as *Candida* spp., *Pneumocystis jiroveci, Fusarium* spp. as well as in the walls of other organisms, especially bacteria, limits the significance of the assay in specific diagnosis of IA. Particularly in neonates, the assay is much more promising for the diagnosis of invasive candidiasis, which is the most frequent fungal infection in this patient population [35]. A cut-off of positivity 60 pg/ml has been defined, although cut-off values ranging from 6–80 pg/ml have also been studied [36,37]. By excluding IA in high-risk patients with this assay, expensive and potentially toxic therapy can be delayed or withheld by the physicians, whilst early, preemptive or targeted antifungal therapy can be initiated in those patients who show positive results.

A recent study has compared the utility of GM and D-glucan assays in the diagnosis of invasive candidiasis, IA and other filamentous fungal infections in cancer patients. It has found that while GM is more specific for IA, D-glucan assay may be more sensitive tool for the diagnosis of invasive fungal infections in general [38].

Beta-D glucan assay has been found to possess a specificity of 87.5% and a sensitivity of 84.4%, values that are higher than those of other methods for the diagnosis of invasive fungal infections [39]. Although the same studies have shown that patients with bacterial infections also exhibit D-glucan in high concentrations and thus the positive predictive value for the diagnosis of fungal infection is as low as 59%, it has been shown that the NPV reaches 97% [40]. A recent study has confirmed that beta-D glucan assay has very high NPV (97.8%) and may be most useful for excluding invasive fungal infections including IA in high-risk patients [34]. In this study, while almost all patients with IA who had positive GM assay (31/32 patients) had also positive beta-D glucan assay, beta-D glucan results of patients with negative GM results varied.

In another study, two noninvasive diagnostic tests, beta-D-glucan (Glucatell®) and GM, were used in a twice-weekly screening for the diagnosis of IA in 40 treatment episodes (one hospital visit per patient) in 40 neutropenic adult patients at high risk for IA. Five proven IA cases, three probable IA cases, and three possible IA cases were diagnosed. Diagnostic levels of both beta-D-glucan and GM were detected in 100% of patients with proven IA cases and in 66% of patients with probable IA cases. The sensitivity, specificity, and positive and negative predictive values for beta-D glucan were 87.5, 89.6, 70, and 96.3%, respectively. False-positive reactions occurred at a rate of 10.3%. Both tests anticipated the clinical diagnosis, computed tomography abnormalities, and the initiation of antifungal therapy in most patients, but beta-D-glucan tended to become positive earlier than GM. A combination of the two tests improved the specificity and positive predictive value of each test to 100% without affecting the sensitivity and negative predictive values. This study showed that beta-D-glucan and GM assays are useful tests for the diagnosis of IA in high-risk hematological patients, but a combination of the two tests is more useful to identify false-positive reactions by each test [32].

In conclusion, a negative D-glucan test can rule out IA; if positive, one should perform more tests. The strength of the assay relies on its combination with other tests. False positive results may be obtained from surgical gauze, immunoglobulin products and hemodialysis with cellulose filters. In addition, fungal glucan may be found in dust-containing air environment.

There are no data reported that address the clinical sensitivity of the (1,3)-beta-D glucan assay specifically for infections due to *Aspergillus* spp. in pediatric patients.

Antibody and *Aspergillus* metabolite detection

Specific antibodies against *Aspergillus* may not be useful in immunocompromised patients with disability to produce antibodies, but they may be very useful in patients who possess intact capacity to produce antibodies such as children suffering from CGD or relatively immunocompetent or non-neutropenic patients with chronic or less invasive form of aspergillosis. This usefulness has been illustrated in the case of pulmonary aspergillosis in a patient with CGD [41].

Various metabolites of *Aspergillus* spp. such as D-mannitol or gliotoxin may be used as markers of IA. However, the studies on this issue are very limited, methodology is complicated and specific data for pediatric patients do not exist [42–44].

Nucleic acid detection

Detection of genetic material of *Aspergillus* spp. by polymerase chain reaction (PCR) is a powerful tool for the diagnosis of IA. However, the assay has not been standardized yet, and thus guidelines for the use of PCR to aid the diagnosis of IA in high-risk patients in hematology or other fields of medicine have not been formulated [45]. The studies that have been performed up to date evaluating the value of PCR in comparison to GM and other tests have included few pediatric patients [45–48].

No studies have addressed the issue of PCR-based detection of *Aspergillus* spp. nucleic acid specifically in neonates and young children, whereas in older pediatric patients PCR is probably as good as in adult patients. Challier *et al*. tested 207 serum samples from 41 immunocompromised patients (21 adults and 20 children of age <1 to 18 years) with PCR and GM [49]. Patients with proven or probable IA had positive results with at least one of the two methods. The PCR method seems to be more specific, and a combination of the two could improve the diagnosis of IA. While there was no discrepancy between adults and children in PCR results, many children with possible IA cases according to the European Organization for Research and Treatment of Cancer/Mycosis Study Group criteria had false-positive GM assay results. Prospective studies in well defined pediatric patients at various risks for development of IA are needed for the study of specific or panfungal PCR assays [50].

The combination of serial (i.e., twice a week) measurement of GM as well as repeated performance of PCR may enable earlier diagnosis of IA allowing prompt initiation of specific therapy and thus leading to improved outcome. Kawazu *et al*. prospectively evaluated the diagnostic potential of three noninvasive tests for IA that were used in a weekly screening strategy: GM, a real-time PCR assay for *Aspergillus* DNA and beta-D-glucan assay. There were 149 consecutive treatment episodes analyzed in 96 patients with hematological disorders who were at high risk for IA, and there were 9 proven IA cases, 2 probable IA cases, and 13 possible invasive fugal infections diagnosed. Among these weekly screening tests for IA, GM test was the most sensitive at predicting the diagnosis of IA in high-risk patients with hematological disorders, using a reduced optical density index cutoff of 0.6 [33]. This strategy, however, has not been tested in children. Another study with predominantly adult patients showed that there may be a role for PCR after a positive initial GM test to guide initiation of antifungal therapy in hematological patients [51].

Conclusions

What is the ideal strategy for accurate diagnosis of IA at an early stage in pediatric patients? Several studies have compared combinations of any of HRCT findings from lungs, GM serology, D-glucan results or PCR findings in adult patients without definite conclusions [46,48,50–52]. At present, this question remains incompletely answered, although intense research work is being carried out to define this. A high degree of suspicion in an immunodeficient or immature pediatric host, suggestive clinical and radiological findings as well as mycological data by the application of multiple diagnostic methods (visual detection of the fungus, its growth on appropriate culture media, detection of its nucleic acids and/or antigens) are the most important modalities for increasing physicians' capacity to diagnose IA in young patients. Since there is a continuous move of fungi from the environment (e.g., air, surfaces) to colonization state (e.g., gut, trachea), to infection and to end-organ dissemination, every test needs to be thoughtfully interpreted with regard to the particular host and host's immune response. Earlier and more accurate diagnosis of IA in neonatal and pediatric hosts is expected to result in better outcome of this devastating infection in patients at risk for IA.

References

1 Abbasi S, Shenep JL, Hughes WT, Flynn PM. Aspergillosis in children with cancer: a 34-year experience. *Clin Infect Dis* 1999; **29**: 1210–1219.

2 Steinbach WJ. Pediatric aspergillosis. Disease and treatment differences in children. *Pediatr Infect Dis J* 2005; **24**: 358–364.

3 Winkelstein JA, Marino MC, Johnston RB, *et al*. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine (Baltimore)* 2000; **79**: 155–169.
4 Almyroudis NG, Holland SM, Segal BH. Invasive aspergillosis in primary immunodeficiencies. *Med Mycol* 2005; **43**: S247–S259.
5 Groll AH, Jaeger G, Allendorf A, *et al*. Invasive pulmonary aspergillosis in a critically ill neonate: case report and review of invasive aspergillosis during the first 3 months of life. *Clin Infect Dis* 1998; **27**: 437–452.
6 Shetty D, Giri N, Gonzalez CE, Pizzo PA, Walsh TJ. Invasive aspergillosis in human immunodeficiency virus-infected children. *Pediatr Infect Dis J* 1997; **16**: 216–221.
7 Herbrecht R, Denning DW, Patterson TF, *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–415.
8 Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect Dis* 2005; **5**: 609–622.
9 Thomas KE, Owens CM, Veys PA, Novelli V, Costoli V. The radiological spectrum of invasive aspergillosis in children: a 10-year review. *Pediatr Radiol* 2003; **33**: 453–460.
10 Allan BT, Patton D, Ramsey NK, Day DL. Pulmonary fungal infections after bone marrow transplantation. *Pediatr Radiol* 1988; **18**: 118–122.
11 Taccone A, Occhi M, Garaventa A, Manfredini L, Viscoli C. CT of invasive pulmonary aspergillosis in children with cancer. *Pediatr Radiol* 1993; **23**: 177–180.
12 Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* 2000; **355**: 423–424.
13 Lee YR, Choi YW, Lee KJ, *et al*. CT halo sign: the spectrum of pulmonary diseases. *Br J Radiol* 2005; **78**: 862–865.
14 Kami M, Kishi Y, Hamaki T, *et al*. The value of the chest computed tomography halo sign in the diagnosis of invasive pulmonary aspergillosis. An autopsy-based retrospective study of 48 patients. *Mycoses* 2002; **45**: 287–294.
15 Horger M, Hebart H, Einsele H, *et al*. Initial CT manifestations of invasive pulmonary aspergillosis in 45 non-HIV immunocompromised patients: association with patient outcome? *Eur J Radiol* 2005; **55**: 437–444.
16 Yeo SF, Wong B. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin Microbiol Rev* 2002; **15**: 465–484.
17 Florent M, Katsahian S, Vekhoff A, *et al*. Prospective evaluation of a polymerase chain reaction-ELISA targeted to *Aspergillus fumigatus* and *Aspergillus flavus* for the early diagnosis of invasive aspergillosis in patients with hematological malignancies. *J Infect Dis* 2006; **193**: 741–747.
18 Sulahian A, Boutboul F, Ribaud P, *et al*. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* 2001; **91**: 311–318.
19 Herbrecht R, Letscher-Bru V, Oprea C, *et al*. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002; **20**: 1898–1906.
20 Viscoli C, Machetti M, Cappellano P, *et al*. False-positive galactomannan platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004; **38**: 913–916.
21 Sulahian A, Touratier S, Ribaud P. False positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* 2003; **349**: 2366–2367.
22 Aubry A, Porcher R, Bottero J, *et al*. Occurrence and kinetics of false-positive *Aspergillus* galactomannan test results following treatment with {beta}-lactam antibiotics in patients with hematological disorders. *J Clin Microbiol* 2006; **44**: 389–394.
23 Walsh TJ, Shoham S, Petraitiene R, *et al*. Detection of galactomannan antigenemia in patients receiving piperacillin-tazobactam and correlations between *in vitro*, *in vivo*, and clinical properties of the drug-antigen interaction. *J Clin Microbiol* 2004; **42**: 4744–4748.
24 Siemann M, Koch-Dorfler M, Gaude M. False-positive results in premature infants with the Platelia *Aspergillus* sandwich enzyme-linked immunosorbent assay. *Mycoses* 1998; **41**: 373–377.
25 Mennink-Kersten MASH, Klont RR, Warris A, Op den Camp HJM, Verweij PE. Bifidobacterium lipoteichoic acid and false ELISA reactivity in *Aspergillus* antigen. detection. *Lancet* 2004; **363**: 325–327.
26 Warris A, Abrahamsen TG, Breuker IM, *et al*. Fungal antigenemia in premature neonates due to the presence of galactomannan and mannan in milk formulas [J-848]. In: *41st Annual Interscience Confeence Antimicrobial Agents Chemotherapy*. Chicago, IL: American Society of Microbiology, 2001.
27 Monique A, Mennink-Kersten SH, Ruegebrink D, *et al*. Bifidobacterial lipoglycan as a new cause for false-positive Platelia *Aspergillus* enzyme-linked immunosorbent assay reactivity. *J Clin Microbiol* 2005; **43**: 3925–3931.
28 Talwalkar A, Kailasapathy K. The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium* spp. *Curr Issues Intest Microbiol* 2004; **5**: 1–8.
29 Phillips M, Kailasapathy K, Tran L. Viability of commercial probiotic cultures (*L. acidophilus, Bifidobacterium sp., L. casei, L. paracasei* and *L. rhamnosus*) in cheddar cheese. *Int J Food Microbiol* 2006; **108**: 276–280.
30 Walsh TJ, Schaufele RL, Sein T, *et al*. Reduced expression of galactomannan antigenemia in patients with invasive aspergillosis and chronic granulomatous disease or Job's syndrome (abstract 345). In: *40th Annual Meeting of Infectious Disease Society of America*; Chicago IL: Infectious Disease Society of America, 2002.
31 Obayashi T, Yoshida M, Mori T, *et al*. Plasma (1 ≥3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995; **345**: 17–20.
32 Pazos C, Ponton J, Del Palacio A. Contribution of (1 ≥3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005; **43**: 299–305.
33 Kawazu M, Kanda Y, Nannya Y, *et al*. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1 ≥3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004; **42**: 2733–2741.
34 Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1 ≥3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* 2005; **43**: 5957–5962.
35 Roilides E, Farmaki E, Evdoridou J, *et al*. Neonatal candidiasis: analysis of epidemiology, drug susceptibility, and molecular typing of causative isolates. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 745–750.
36 Odabasi Z, Mattiuzzi G, Estey E, *et al*. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogen-

ous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004; **39**: 199–205.
37 Ostrosky-Zeichner L, Alexander BD, Kett DH, *et al*. Multicenter clinical evaluation of the (1 ≥3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* 2005; **41**: 654–659.
38 Hachem RY, Boctour M, Daugherty T, *et al*. The utility of galactomannan enzyme immunoassay and (1,3) B-D glucan in the diagnosis of invasive fungal infections in cancer patients (M-161). In: *45th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC, 2005: 415.
39 Miyazaki T, Kohno S, Mitsutake K, *et al*. Plasma (13)-β-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *Microbiology* 1995; **33**: 3115–3118.
40 Digby J, Kalbfleisch J, Glenn A, *et al*. Serum glucan levels are not specific for presence of fungal infections in intensive care unit patients. *Clin Diagn Lab Immunol* 2003; **10**: 882–885.
41 Sambatakou H, Guiver M, Denning D. Pulmonary aspergillosis in a patient with chronic granulomatous disease: confirmation by polymerase chain reaction and serological tests, and successful treatment with voriconazole. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 681–685.
42 Strandberg GW. D-mannitol metabolism by *Aspergillus* candidus. *J Bacteriol* 1969; **97**: 1305–1309.
43 Francis P, Lee JW, Hoffman A, *et al*. Efficacy of unilamellar liposomal amphotericin B in treatment of pulmonary aspergillosis in persistently granulocytopenic rabbits: the potential role of bronchoalveolar lavage D-mannitol and galactomannan as markers of infection. *J Infect Dis* 1994; **169**: 356–368.
44 Lewis RE, Wiederhold NP, Chi J, *et al*. Detection of gliotoxin in experimental and human aspergillosis. *Infect Immun* 2005; **73**: 635–637.
45 Halliday C, Hoile R, Sorrell T, *et al*. Role of prospective screening of blood for invasive aspergillosis by polymerase chain reaction in febrile neutropenic recipients of haematopoietic stem cell transplants and patients with acute leukaemia. *Br J Haematol* 2006; **132**: 478–486.
46 Scotter JM, Campbell P, Anderson TP, *et al*. Comparison of PCR-ELISA and galactomannan detection for the diagnosis of invasive aspergillosis. *Pathology* 2005; **37**: 246–253.
47 Lass-Florl C, Gunsilius E, Gastl G, *et al*. Clinical evaluation of *Aspergillus*-PCR for detection of invasive aspergillosis in immunosuppressed patients. *Mycoses ; 48 Suppl* 2005; **1**: 12–17.
48 Buchheidt D, Hummel M, Schleiermacher D, *et al*. Prospective clinical evaluation of a LightCycler-mediated polymerase chain reaction assay, a nested-PCR assay and a galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. *Br J Haematol* 2004; **125**: 196–202.
49 Challier S, Boyer S, Abachin E, Berche P. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* 2004; **42**: 844–846.
50 Bialek R, Moshous D, Casanova JL, Blanche S, Hennequin C. Aspergillus antigen and PCR assays in bone marrow transplanted children. *Eur J Med Res* 2002; **7**: 177–180.
51 Millon L, Piarroux R, Deconinck E, *et al*. Use of real-time PCR to process the first galactomannan-positive serum sample in diagnosing invasive aspergillosis. *J Clin Microbiol* 2005; **43**: 5097–5101.
52 Kami M, Tanaka Y, Kanda Y, *et al*. Computed tomographic scan of the chest, latex agglutination test and plasma (1 ≥3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica* 2000; **85**: 745–752.