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Diagnosis of invasive fungal infections in immunocompromised children

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Abstract

Early recognition and rapid initiation of effective treatment is a prerequisite for successful management of children with invasive fungal infections. The increasing diversity of fungal pathogens in high-risk patients, the differences in the antifungal spectra of available agents and the increasing rates of resistance call for identification of the infecting isolate at the species level and for information on drug resistance, in order to provide state-of-the-art patient care. Microscopy and culture of appropriate specimens remain the reference standard for mycological diagnosis, despite difficulties in obtaining appropriate and/or sufficient specimens, long durations of culture and false-negative results. Modern imaging studies and detection of circulating fungal cell wall components and DNA in blood and other body fluids or in affected tissues may improve the laboratory diagnosis of invasive mycoses.

Keywords: Children, diagnosis, fungal infections, imaging, non-culture methods, review
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Introduction

Early mycological diagnosis and antifungal therapy are crucial for surviving invasive fungal infections (IFIs) in patients of all ages. In a prospective study in adult patients with pulmonary aspergillosis, timely implementation of antifungal therapy based on positive bronchoscopy and high-resolution computed tomography (CT) scan results had a significant impact on survival. Starting antimycotics within the first 10 days after the onset of pneumonia resulted in a fatality rate of 41%, as opposed to 90% when treatment was begun later ($p < 0.01$) [1]. Early initiation of amphotericin was also associated with improved outcome in adult patients with sinopulmonary mucormycosis [2]. Similarly, in patients with candidaemia, a delay in antifungal treatment of greater than 12 h after clinical suspicion (i. e. obtaining a blood culture) was associated with a 20% increase in mortality [3], with further significant increases on each of the following 3 days [4]. However, detection of IFIs by conventional culture and microscopic methods remains time-consuming and insensitive. Berenguer *et al.* [5] found that blood cultures were frequently negative in patients who had autopsy-proven deeply invasive candidiasis. Difficulty in collecting appropriate tissue specimens for culture or histology, particularly in neutropenic and thrombocytopenic patients, represents a further limitation in the conventional diagnosis of IFI.

Clinical Evaluation

A diagnosis of IFI in an immunocompromised child begins with an assessment of risk. Such an assessment includes evaluation of the probability that a patient's clinical manifestations are caused by an IFI. Factors such as primary neoplastic disease, quantitative and qualitative defects in neutrophils, monocytes, T-cells, B-cells, antibodies and cytokines, mucosal integrity and immunosuppressive treatment are critical in this evaluation of risk. A careful history and meticulous physical examination complement bedside assessment, while also providing a guide to rational selection of diagnostic imaging modalities and laboratory studies.

Diagnostic Imaging Studies

Invasive aspergillosis (IA) and other mould infections

Whereas conventional chest radiographs lack sensitivity in neutropenic patients, serial high-resolution CT in neutropenic adults has been shown to positively impact on the early diagnosis and outcome of invasive pulmonary aspergillosis (IPA) [6]. The characteristic 'halo' sign is an early stage of the disease; it is indicative of perifocal haemorrhage, coagulative necrosis and oedema surrounding a dense fungal nodule [7,8], and is associated with improved prognosis [9]. Pulmonary nodules with a 'halo' sign have also been found in non-*Aspergillus* fungal pneumonia caused by *Fusarium* spp., *Scedosporium* spp. or Zygomycetes, in bacterial pneumonia caused by *Pseudomonas aeruginosa*, and in neoplastic and non-infectious inflammatory diseases [10,11], rendering this radiographic feature less specific for IPA.

There is growing recognition that CT features of pulmonary lesions characteristic for adult IPA are less frequent in children [12,13]. Further signs, such as central cavitation of pulmonary lesions and the 'air-crescent' sign, appearing later in the course of IPA in approximately 40% in adult series, were not or less commonly observed in children [12,14]. Instead, focal bronchopneumonia is more frequently identified in paediatric patients with IPA.

Routine serial CT imaging in children carries a considerable risk of radiation exposure that is proportionally greater than that of adults [15] and may lead to additional infections following environmental exposures during patient transportation and contact.

Chronic disseminated or hepatosplenic candidiasis

Whereas open liver biopsy with mycological and histological evaluations remains the reference standard diagnostic procedure for hepatosplenic candidiasis, magnetic resonance imaging (MRI) has the highest diagnostic value, detecting hyperintense fungal lesions on T2-weighted images with a sensitivity of 100% and a specificity of 96% [16]. However, MRI requires sedation in small children, and may often be unavailable or too expensive as compared with other methods. Biphasic liver CT scan as an alternative shows lesions with typical central contrast enhancement and a peripheral double ring in the arterial phase in adults [17]. As radiation exposure should be considered, particularly in children, high-resolution ultrasonography is a reasonable initial alternative for screening, despite a lower level of sensitivity than those of CT and MRI [18–21]. As the lesions of disseminated candidiasis are more readily observed when associated with inflammation, imaging modalities are best performed in oncology patients and haematopoietic stem cell transplant recipients after recovery from neutropenia.

Conventional Laboratory Methods for Diagnosis of IFIs

Microscopy by direct examination, wet mount, cytology and histology are practical and relatively inexpensive methods for detection of IFIs. Culture techniques, including for blood cultures continue to improve, but nonetheless remain limited for the early detection of mycoses.

Some studies suggest that approximately 60–80% of candidaemia patients can be diagnosed with contemporary blood culture systems [22,23], albeit with a delay that significantly affects survival [3,4]. Among moulds, *Fusarium* spp., *Scedosporium* spp. and *Aspergillus terreus* may cause fungaemia that is detectable by blood culture. Other moulds are less likely to be detectable as a cause of fungaemia [24].

Detection of Fungal Cell Wall Markers

Carbohydrate molecules of the fungal cell wall have been intensively studied in the last two decades as markers for IFI. Glucans are almost universal fungal cell wall components, whereas mannans are mainly found in yeasts, and galactomannan (GM) is rather specific for *Aspergillus* spp. [25].

On the basis of their performance in adults, two standardized methods for detection of fungal biomarkers in serum have been approved by the US Food and Drug Administration and have been included in the consensus criteria for diagnosis of IFI [26]: the GM antigen enzyme immunoassay (EIA) (Platelia Aspergillus; BioRad, Marnes-la-Coquette, France) and two assays detecting (1 $\rightarrow$ 3)-β-D-glucan (BDG) (Fungitell; Associates of Cape Cod, Falmouth, MA, USA; Fungitec G; Seikagaku Corp., Tokyo, Japan).

Galactomannan (GM)

GM is a heteropolysaccharide found within the cell wall of *Aspergillus* spp. During hyphal growth and cell wall turnover, GM is released into the extracellular fluid. The GM antigen ELISA allows for the detection of *Aspergillus* spp. in serum [27]. A recent meta-analysis revealed various sensitivity rates, from 30% to 100%, and similarly wide-ranging specificities, from 38% to 98% [28]. By means of this method, GM can also be detected in urine, bronchoalveolar lavage (BAL) fluid, cerebrospinal fluid (CSF) and other specimens [29,30].

Relative to adults, there is also a comparably wide range of sensitivity of GM EIA applied to serum samples of children

with IA. Hayden *et al.* studied the expression of GM antigen by EIA in 56 paediatric oncology patients (aged 3 months to 18 years), of whom 17 had European Organization for Research and Treatment of Cancer-defined proven or probable IA. At least one serum sample was positive for 11 of 17 paediatric oncology patients (65.7% sensitivity, 95% CI 38.3–85.7) with IA. Among the seven evaluable GM-positive patients with IA, the GM EIA produced a positive result before clinical or radiographic evidence of infection in six cases, with a lead time to diagnosis ranging from 1 day to 34 days (median: 10 days) [31]. On the other hand, a relatively low sensitivity was reported in paediatric cancer patients by Herbrecht *et al.* [32], as well as recently by Castagnola *et al.* [33]. Moreover, non-neutropenic children with chronic granulomatous disease or Job's syndrome (Walsh *et al.*, 40th IDSA meeting, 2002, Abstract 345) also had low expression of GM, possibly because of more localized and less angio-invasive disease as well as the unique immunological factors mentioned previously [34].

Several factors limiting the specificity of the GM EIA also have been identified. Whereas immunoreactivity with other organisms, such *Penicillium* spp., *Paecilomyces* spp., *Blastomyces dermatitidis*, *Nigrospora* and *Trichothecium* is uncommon, false-positive results can be caused more frequently by contaminating GM in certain β-lactam antibiotics, particularly piperacillin–tazobactam [35,36] and amoxycillin with or without clavulanate [37], dietary GM in pasta, cereals and formula milk, and immunoreactivity of the EB-A2 antibody used in the method involving lipoteichoic acids of *Bifidobacterium* spp., which are commonly found in the infant gut microflora [38]. In contrast, two paediatric studies, analysing twice-weekly screening periods in 119 and 64 high-risk patients, respectively, revealed favourable specificity of the GM EIA, particularly when patients receiving piperacillin–tazobactam were excluded from the analysis [33,39].

Another recent paediatric study also suggests utility of the GM EIA when applied to BAL fluid of patients with suspected PA [40]. This observation of the diagnostic utility of GM in BAL fluid is compatible with what has been observed in adults and in laboratory animals with IPA [41,42].

(1 → 3)-B-D-glucan (BDG)

BDG is present in the cell wall of most pathogenic fungi. BDG detection is not species-specific or genus-specific, but is sensitive for a broad range of fungal pathogens, including *Aspergillus* spp., other moulds, *Candida* spp. and *Pneumocystis*. As the Mucorales (*Mucor* spp., *Rhizopus* spp. and *Lictheimia* (*Absidia*) spp.), *Cryptococcus neoformans* and *Blastomyces dermatitidis* contain relatively small amounts of cell wall BDG, infections caused by these organisms may not be reliably detected by BDG assays. In the two available assays, BDG acts as a specific trigger of the coagulation cascade occurring in the endolymph of the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*) through activation of factor G (after removal of the endotoxin-sensitive factor C) [43–45].

In clinical studies examining the diagnostic value of the available BDG assays in adult patients, sensitivities of 55–100%, specificities of 71–93%, positive predictive values (PPVs) of 40–89% and negative predictive values (NPVs) of 73–100% have been reported, with the use of various cut-off values for positivity ranging from 6 to 120 pg/mL [44,46–53].

For the Fungitec assay (developed in Japan), which utilizes the endolymph of *T. tridentatus*, a cut-off level of 20 pg/mL was defined for plasma samples. An early analysis of 202 febrile episodes, including 41 episodes of IFI, revealed 90% sensitivity and 100% specificity for this test [44]. Later, a sensitivity of 67% and a specificity of 84% were observed in 33 patients with IA [46]. Recently, this BDG assay was prospectively re-evaluated with twice-weekly screening during 190 neutropenic episodes in 95 patients treated for acute leukaemia with a high incidence of IFI (nine proven, 23 probable and 30 possible cases). If two consecutive positive results were considered to be diagnostic, a threshold of 7 pg/mL resulted in the best diagnostic performance, with 63% sensitivity, 96% specificity, 79% PPV and 91% NPV in patients with proven or probable IFI [53].

The Fungitell (formerly Glucatell) assay (USA) utilizes the endolymph of *L. polyphemus*. A 60 pg/mL cut-off value was recommended, on the basis of a study in serum samples of 30 adult patients with candidaemia and 30 healthy controls. The false-positive rate was 4.3%. The Fungitec G assay—analysed in parallel—was found to be 2.5 times less reactive than the Glucatell assay, but performed equally when the respective lower threshold was used [47].

In a multicentre clinical evaluation with 60 and 80 pg/mL cut-off values, the Fungitell assay detected proven and probable IA in eight of ten patients, proven candidiasis (n = 107) in 81.3% and 77.6%, and fusariosis in all three patients. Lower overall sensitivities (69.9% and 64.4%, with corresponding specificities of 87.1% and 92.4%) were observed among all IFI cases, because, expectedly, only three of 12 patients with *Cryptococcus* infection and none of the three patients with zygomycoses were BDG-positive. Among patients on fluconazole prophylaxis, a slightly, but non-significantly, lower sensitivity was observed than in subjects who had proven IFI not receiving antifungal therapy [48].

It is worth noting possible causes of false-positive BDG assays results. Small amounts of glucans affecting test

specificity have been found in cellulose used in surgical gauze, haemodialysis membranes and for filtration of blood products such as immunoglobulin, albumin and coagulation factors [25]. A case report described false-positive BDG results in six consecutive patients treated with amoxycillin–clavulanic acid [54]; however, *in vitro* studies suggest that serum BDG levels can be only minimally influenced by infusion of most contaminated antibiotics [55].

Comparative studies between GM and BDG

A BDG assay (Glucatell) and the GM EIA (Platelia Aspergillus) were compared in 40 neutropenic adults with the use of a twice-weekly screening schedule for diagnosis of IA. In order to improve the specificity of the tests, rather high thresholds (120 pg/mL for the BDG assay and 1.5 for the GM EIA) were chosen. Five proven, three probable and three possible IA cases were diagnosed. Among this low number of cases, the sensitivity, specificity, PPV and NPV were identical for both the BDG and the GM assays (87.5%, 89.6%, 70% and 96.3%, respectively), with a false-positive rate of 10.3% each, albeit represented by different patients. The sensitivities of both tests were 100% in patients with proven IA and 66% in patients with probable IA. The use of both assays in combination further improved the specificity and PPV to 100%, without affecting the sensitivity and NPV [49].

Another comparative study confirmed an NPV of 97.8% for the BDG assay, suggesting particular utility for excluding IFIs in high-risk patients. Almost all (31/32) study patients with IA who had positive GM assay results were also positive in the BDG assay, whereas the BDG results of GM-negative patients were repeatedly positive. In contrast, the specificity was only 77%, owing to a considerable number of false-positive results, mainly in patients with (mostly Gram-positive) bacteraemia [50]. Whereas, in this study, 56% (14/25) of bacteraemic patients were BDG-positive, a recent study found elevated BDG values in only two of 93 patients with documented bacteraemia without concomitant IFI; 'false'-positive BDG findings occurred only in patients with fungal colonization and/or mucositis who received empirical antifungal treatment, suggesting an occult fungal cause [53].

A recent prospective trial comparing GM and BDG for early diagnosis of invasive mould infections or candidaemia included 82 adult patients with haematological malignancies. Sensitivities of the BDG assay were consistently higher than that of the GM EIA among all patient groups (47–64%), with a specificity above 90% [56]. The lower performance of GM detection may have been caused by concurrent antifungal treatment. Interestingly, the GM assay revealed a significantly lower sensitivity for IA caused by *Aspergillus fumigatus* (13%) than for non-fumigatus aspergillosis (49%), possibly reflecting variable amounts of GM release among different *Aspergillus* species.

There are currently inadequate data on the expression of BDG and the diagnostic value of the BDG assays for IFIs in paediatric patients. BDG detection may be a promising tool for the diagnosis of invasive candidiasis in neonates and in paediatric intensive-care patients [57]. Among the IFIs that are especially distinctive in paediatric patients is haematogenous *Candida* meningoencephalitis [58,59]. As this infection is difficult to diagnose and carries a high burden of morbidity, CSF BDG may be a sensitive means of detection of this life-threatening and debilitating infection [60,61]. However, further validation of the BDG assays, including the definition of cut-off values in children [62], requires further study.

Mannan and anti-mannan antibodies

Like GM in moulds, circulating mannan, a characteristic cell wall component of yeasts, has been evaluated as a diagnostic marker for invasive candidiasis or candidaemia in adult patients with neutropenia [63] and after haematopoietic stem cell transplantion [64], with variable results. A preliminary study in neonates has given promising results, particularly for exclusion of candidiasis, considering the high NPV of 98% [65].

Detection of Fungal DNA

Molecular biology may provide powerful tools for the early diagnosis of IFI. PCR has yielded the most promising data, with high sensitivity and specificity. However, fungal PCR differs from the molecular detection of other eukaryotic and prokaryotic microorganisms with regard to several features: low amounts and more complicated isolation of DNA (particularly regarding the durable fungal cell wall), requiring special extraction protocols; lack of standardized nucleic acid detection procedures; and considerable susceptibility to airborne and waterborne contamination, making the interpretation of results difficult.

Highly conserved DNA sequences within the multicopy genes encoding 28S or 18S rRNA have been utilized as 'panfungal' primers, and primers spanning the 5.8S rRNA, ITS-1 and ITS-2 regions have been used for the identification of specific fungal pathogens [66–68]. Sequences within the 28S rRNA region have been recently utilized for the development of quantitative PCR assays for the detection of Mucorales [69].

As with the biomarkers already discussed, twice-weekly screening of high-risk patients [70–72] and rapid, specific detection of fungal pathogens in tissue specimens [73] have

emerged as the most feasible clinical applications of PCR in IFI diagnosis to date. However, the fact that several millilitres of whole blood are required per sample remains a quantitative problem in small children. Although nested PCR is an option for the detection of even small amounts of DNA [74], it suffers from a higher probability of contamination. However, the sensitivity and specificity were 80% and 81%, respectively, when 291 blood, CSF or BAL samples from 71 paediatric and adolescent patients with two proven and three probable IA cases were examined by means of a nested PCR assay [75]. An earlier study using a panfungal PCR assay in blood samples of paediatric cancer patients with 28 cases of proven or probable IFI in 91 febrile neutropenic episodes had yielded a sensitivity, specificity, PPV and NPV of 75%, 92%, 84% and 87%, respectively [76].

Following classical, rather time-consuming approaches [74,77], semi-automated DNA extraction [78] and real-time PCR methods [46,79–81] have been developed, enabling the rapid detection of a relevant array of fungal pathogens within a few hours. In-house-developed PCR protocols with limited availability and novel technologies such as fluorescence *in situ* hybridization have been studied [82,83] (Lass-Floerl *et al.*, 18th ECCMID, 2008, Poster P734) (Willinger B, personal communication). However, further studies on standardization and validation are needed [84].

Outlook

As bedside evaluation continues to be augmented by expanding technologies of diagnostic imaging and novel systems for detection of fungal growth, carbohydrates, proteins, lipids, metabolites and nucleic acids, the capacity for diagnosis of IFIs in children will continue to improve. Future studies conducted in children are critical for understanding these infections in paediatric patients.

Transparency Declaration

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References

1. von Eiff M, Roos N, Schulten R, Hesse M, Zuehlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration* 1995; 62: 341–347.
2. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008; 47: 503–509.
3. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. *Antimicrob Agents Chemother* 2005; 49: 3640–3645.
4. Garey KW, Rege M, Pai MP *et al.* Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. *Clin Infect Dis* 2006; 43: 25–31.
5. Berenguer J, Buck M, Witebsky F, Stock F, Pizzo PA, Walsh TJ. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis: disseminated versus single organ infection. *Diagn Microbiol Infect Dis* 1993; 17: 103–109.
6. Caillot D, Casanovas O, Bernard A *et al.* Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* 1997; 15: 139–147.
7. Caillot D, Couaillier J-F, Bernard A *et al.* Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; 19: 253–259.
8. Hruban RH, Meziane MA, Zerhouni EA, Wheeler PS, Dumler JS, Hutchins GM. Radiologic–pathologic correlation of the CT halo sign in invasive pulmonary aspergillosis. *J Comput Assist Tomogr* 1987; 11: 534–536.
9. Greene RE, Schlamm HT, Oestmann JW *et al.* Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis* 2007; 44: 373–379.
10. Denning DW. Early diagnosis of invasive aspergillosis. *Lancet* 2000; 355: 423–424.
11. Lee YR, Choi YW, Lee KJ *et al.* CT halo sign: the spectrum of pulmonary diseases. *Br J Radiol* 2005; 78: 862–865.
12. 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.
13. Burgos A, Zaoutis TE, Dvorak CC *et al.* Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008; 121: e1286–e1294.
14. 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.
15. Brenner DJ, Hall EJ. Computed tomography—an increasing source of radiation exposure. *N Engl J Med* 2009; 357: 2277–2284.
16. Masood A, Sallah S. Chronic disseminated candidiasis in patients with acute leukemia: emphasis on diagnostic definition and treatment. *Leuk Res* 2005; 29: 493–501.
17. Rudolph J, Rodenwaldt J, Ruhnke M, Hamm B, Kopka L. Unusual enhancement pattern of liver lesions in hepatosplenic candidiasis. *Acta Radiol* 2004; 45: 499–503.
18. Pastakia B, Shawker TH, Thaler M, O'Leary T, Pizzo PA. Hepatosplenic candidiasis: wheels within wheels. *Radiology* 1988; 166: 417–421.
19. Görg C, Weide R, Schwerk WB, Köppler H, Havemann K. Ultrasound evaluation of hepatic and splenic microabscesses in the immunocompromised patient: sonographic patterns, differential diagnosis, and follow-up. *J Clin Ultrasound* 1994; 22: 525–529.
20. Sallah S, Semelka R, Kelekis N, Worawattanakul S, Sallah W. Diagnosis and monitoring response to treatment of hepatosplenic candidiasis in patients with acute leukemia using magnetic resonance imaging. *Acta Haematol* 1998; 100: 77–81.
21. Groll AH, Walsh TJ. Antifungal chemotherapy: advances and perspectives. *Swiss Med Wkly* 2002; 132: 303–311.

22. Meyer M-H, Letscher-Bru V, Jaulhac B *et al.* Comparison of mycosis IC/F and plus aerobic/F media for diagnosis of fungemia by the Bactec 9240 system. *J Clin Microbiol* 2004; 42: 773–777.
23. Terlecka JA, du Cros PA, Orla Morrissey C, Spelman D. Rapid differentiation of Candida albicans from non-albicans species by germ tube test directly from BacTAlert blood culture bottles. *Mycoses* 2007; 50: 48–51.
24. Simoneau E, Kelly M, Labbe AC, Roy J, Laverdiere M. What is the clinical significance of positive blood cultures with *Aspergillus* sp. in hematopoietic stem cell transplant recipients? A 23 year experience. *Bone Marrow Transplant* 2005; 35: 303–306.
25. Mennink-Kersten M, Verweij PE. Non-culture-based diagnostics for opportunistic fungi. *Infect Dis Clin North Am* 2006; 20: 711–727.
26. DePauw B, Walsh TJ, Donelly JP *et al.* Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; 46: 1813–1821.
27. Maertens J, Verhaegen J, Lagrou K *et al.* Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; 97: 1604–1610.
28. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using galactomannan assay: a metaanalysis. *Clin Infect Dis* 2006; 42: 1417–1427.
29. Viscoli C, Machetti M, Gazzola P *et al.* Aspergillus galactomannan antigen in the cerebrospinal fluid of bone marrow transplant recipients with probable cerebral aspergillosis. *J Clin Microbiol* 2002; 40: 1496–1499.
30. Klont RR, Menninck-Kersten MA, Verweij PE. Utility of Aspergillus antigen detection in specimens other than serum specimens. *Clin Infect Dis* 2004; 39: 1467–1474.
31. Hayden R, Pounds S, Knapp K *et al.* Galactomannan antigenemia in pediatric oncology patients with invasive aspergillosis. *Pediatr Infect Dis J* 2008; 27: 815–819.
32. 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.
33. Castagnola E, Furfaro E, Caviglia I *et al.* Performance of the galactomannan antigen detection test in the diagnosis of invasive aspergillosis in children with cancer or undergoing haemopoietic stem cell transplantation. *Clin Microbiol Infect* 2010; 16: 1197–1203.
34. Roilides E. Early diagnosis of invasive aspergillosis in children. *Med Mycol* 2006; 44: S199–S205.
35. 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.
36. 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.
37. 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.
38. Mennink-Kersten MA, Ruegebrink D, Klont RR *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.
39. Steinbach WJ, Addison RM, McLaughlin L *et al.* Prospective Aspergillus galactomannan antigen testing in pediatric hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2007; 26: 558–564.
40. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009; 28: 283–286.
41. Musher B, Fredricks D, Leisenring W, Balajee SA, Smith C, Marr KA. Aspergillus galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* 2004; 42: 5517–5522.
42. Francesconi A, Kasai M, Petraitiene R *et al.* Characterization and comparison of galactomannan enzyme immunoassay and quantitative real-time PCR assay for detection of Aspergillus fumigatus in bronchoalveolar lavage fluid from experimental invasive pulmonary aspergillosis. *J Clin Microbiol* 2006; 44: 2475–2480.
43. Miyazaki T, Kohno S, Mitsutake K, Maesaki S, Tanaka K, Hara K. (1 → 3)-β-D glucan in culture fluid of fungi activates factor G, a limulus coagulation factor. *J Clin Lab Anal* 1995; 9: 334–339.
44. 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.
45. Girouard G, Lachance C, Pelletier R. Observations of (1,3)-beta-D-glucan detection as a diagnostic tool in endemic mycoses caused by *Histoplasma* or *Blastomyces*. *J Med Mycol* 2007; 56: 1001–1002.
46. Kami M, Fukui T, Ogawa S *et al.* Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* 2001; 33: 1504–1512.
47. 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 myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004; 39: 199–205.
48. 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.
49. 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.
50. 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.
51. Persat F, Ranque S, Derouin F, Michel-Nguyen A, Picot S, Sulahian A. Contribution of the (1,3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* 2008; 46: 1009–1013.
52. Ellis M, Al-Ramadi B, Finkelman M *et al.* Assessment of the clinical utility of serial beta D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* 2008; 57 (Pt 3): 287–295.
53. Senn L, Robinson JO, Schmidt S *et al.* 1,3-β-D-glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. *Clin Infect Dis*2008; 46: 878–885.
54. Mennink-Kersten MA, Warris A, Verweij PE. Detection of circulating 1,3-beta-D glucan in patients receiving intravenous amoxicillin–clavulanic acid. *N Engl J Med* 2006; 354: 2834–2835.
55. Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR. Reactivity of (1 → 3)-β-D glucan assay with commonly used intravenous antimicrobials. *Antimicrob Agents Chemother* 2006; 50: 3450–3453.
56. Hachem RY, Kontoyiannis DP, Chemaly RF, Jiang Y, Reitzel R, Raad I. Utility of galactomannan enzyme immunoassay and (1,3) beta-D-glucan in diagnosis of invasive fungal infections: low sensitivity for Aspergillus fumigatus infection in hematologic malignancy patients. *J Clin Microbiol* 2009; 47: 129–133.
57. 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.

58. Benjamin DK Jr, Stoll BJ, Fanaroff AA *et al.*, National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. *Pediatrics* 2006; 117: 84–92.
59. Cohen-Wolkowiez M, Smith PB, Mangum B *et al.* Neonatal Candida meningitis: significance of cerebrospinal fluid parameters and blood cultures. *J Perinatol* 2007; 27: 97–100.
60. Petraitiene R, Petraitis V, Hope WW *et al.* Cerebrospinal fluid and plasma (1 → 3)-beta D-glucan as surrogate markers for detection and monitoring of therapeutic response in experimental hematogenous Candida meningoencephalitis. *Antimicrob Agents Chemother* 2008; 52: 4121–4129.
61. Hope WW, Mickiene D, Petraitis V *et al.* The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous Candida meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008; 197: 163–171.
62. Smith PB, Benjamin DJ, Alexander BD, Johnson MD, Finkelman MA, Steinbach WJ. Quantification of 1,3-β-D-glucan levels in children: preliminary data for diagnostic use of the β-glucan assay in a pediatric setting. *Vacc Immunol* 2007; 14: 924–925.
63. Ellis M, Al-Ramadi B, Bernsen R, Kristensen J, Alizadeh H, Hedstrom U. Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive *Candida* infections in patients with neutropenic fever. *J Med Microbiol*2009; 58 (Pt 5): 606–615.
64. Lunel FM, Mennink-Kersten MA, Ruegebrink D *et al.* Value of Candida serum markers in patients with invasive candidiasis after myeloablative chemotherapy. *Diagn Microbiol Infect Dis* 2009; 64: 408–415.
65. Oliveri S, Trovato L, Betta P, Romeo MG, Nicoletti G. Experience with the Platelia Candida ELISA for the diagnosis of invasive candidosis in neonatal patients. *Clin Microbiol Infect* 2008; 14: 391–393.
66. Einsele H, Loeffler J. Contribution of new diagnostic approaches to antifungal treatment plans in high-risk haematology patients. *Clin Microbiol Infect Dis* 2008; 14: 37–45.
67. O'Sullivan CE, Kasai M, Francesconi A *et al.* Development and validation of a quantitative real-time PCR assay using fluorescence resonance energy transfer (FRET) technology for detection of *Aspergillus fumigatus* in experimental invasive pulmonary aspergillosis. *J Clin Microbiol* 2003; 41: 5676–5682.
68. Kasai M, Francesconi A, Petraitiene R *et al.* Use of quantitative real-time PCR to study the kinetics of extracellular DNA released from *Candida albicans*, with implications for diagnosis of invasive candidiasis. *J Clin Microbiol* 2006; 44: 143–150.
69. Kasai M, Harrington SM, Francesconi A *et al.* Detection of molecular biomarkers for *Rhizopus* spp., *Mucor* spp., and *Cunninghamella* spp. by quantitative PCR and melt curve analysis in plasma, bronchoalveolar lavage, and lung tissue in experimental pulmonary zygomycosis. *J Clin Microbiol* 2008; 46: 3690–3702.
70. Hebart H, Loeffler J, Meisner C *et al.* Early detection of Aspergillus infection after allogeneic stem cell transplantation by polymerase chain reaction screening. *J Infect Dis* 2000; 181: 1713–1719.
71. Lass-Flörl C, Aigner J, Gunsilius E *et al.* Screening for Aspergillus spp. using polymerase chain reaction of whole blood samples from patients with haematological malignancies. *Br J Haematol* 2001; 113: 180–184.
72. Hebart H, Klingspor L, Klingebiel T *et al.* A prospective randomized controlled trial comparing PCR-based and empirical treatment with liposomal amphotericin B in patients after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2009; 43: 553–561.
73. Lass-Floerl C, Resch G, Nachbaur D *et al.* The value of computed tomography-guided percutaneous lung biopsy for diagnosis of invasive fungal infection immunocompromised patients. *Clin Infect Dis* 2007; 45: e101–e104.
74. Buchheidt D, Baust C, Skladny H *et al.* Detection of Aspergillus species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis* 2001; 33: 428–435.
75. Hummel M, Spiess B, Roder J *et al.* Detection of Aspergillus DNA by a nested PCR assay is able to improve the diagnosis of invasive aspergillosis in paediatric patients. *J Med Microbiol* 2009; 58 (Pt 10): 1291–1297.
76. El-Mahallawy HA, Shaker HH, Ali Helmy H, Mostafa T, Razak Abo-Sedah A. Evaluation of pan-fungal PCR assay and Aspergillus antigen detection in the diagnosis of invasive fungal infections in high risk paediatric cancer patients. *Med Mycol* 2006; 44: 733–739.
77. Loeffler J, Hebart H, Sepe S, Schumacher U, Klingebiel T, Einsele H. Detection of PCR amplified fungal DNA by using a PCR-ELISA system. *Med Mycol* 1998; 36: 275–279.
78. Loeffler J, Schmidt K, Hebart H, Schumacher U, Einsele H. Automated extraction of medically important yeast species and filamentous fungi by using the MagNa Pure LC system. *J Clin Microbiol* 2002; 40: 2240–2243.
79. Loeffler J, Henke N, Hebart H *et al.* Quantification of fungal DNA by using Fluorescence Resonance Energy Transfer and the Light Cycler system. *J Clin Microbiol* 2000; 38: 586–590.
80. Schabereiter-Gurtner C, Selitsch B, Rotter ML, Hirschl AM, Willinger B. Development of novel real-time PCR assays for detection and differentiation of eleven medically important *Aspergillus* and *Candida* species in clinical specimens. *J Clin Microbiol* 2007; 45: 906–914.
81. Basková L, Landlinger C, Preuner S, Lion T. The Pan-AC assay: a single-reaction real time PCR test for quantitative detection of a broad range of Aspergillus and Candida species. *J Med Microbiol* 2007; 56 (Pt 9): 1167–1173.
82. Wallet F, Nseir S, Baumann L *et al.* Preliminary clinical study using a multiplex real-time PCR test for the detection of bacterial and fungal DNA directly in blood. *Clin Microbiol Infect* 2010; 16: 774–779.
83. Shepard JR, Addison RM, Alexander BD *et al.* Multicenter evaluation of the *Candida albicans*/*Candida glabrata* peptide nucleic acid fluorescent in situ hybridization method for simultaneous dual-color identification of *C. albicans* and *C. glabrata* directly from blood culture bottles. *J Clin Microbiol* 2008; 46: 50–55.
84. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect Dis* 2009; 9: 89–96.