

## Review

# Invasive aspergillosis in pediatric patients

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### William J. Steinbach

Duke University Medical Center, Durham, NC, USA

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#### Address for correspondence:

William J. Steinbach, MD, Associate Professor of Pediatrics, Molecular Genetics & Microbiology, Division of Pediatric Infectious Diseases, Duke University Medical Center, Box 3499 DUMC, Durham, NC 27710, USA.

Tel.: +1 919 681 1504; Fax: +1 919 613 5175; stein022@mc.duke.edu

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### Abstract

This case-based review examines the growing literature on critical issues related to the epidemiology, diagnosis, and treatment of pediatric invasive aspergillosis. Immunocompromised children are at heightened risk for invasive aspergillosis. Children at highest risk include those with new-onset or relapsed hematologic malignancy and recipients of allogeneic stem cell transplants. Additional risk factors in stem cell transplant recipients include impaired lymphocyte engraftment and graft-versus-host disease. Pediatric invasive aspergillosis is associated with a high mortality rate (generally >50%) and requires prompt diagnosis and treatment to prevent dissemination and death. Tools available for diagnosis include radiologic examinations (primarily computed tomography), the galactomannan assay, bronchoalveolar lavage, and tissue biopsy. Age-related differences in computed tomography and galactomannan assay results have been suggested. Recommended primary therapy for pediatric invasive aspergillosis is voriconazole (7 mg/kg IV q12 hours). Currently approved alternative therapies include liposomal amphotericin B, amphotericin B lipid complex, and caspofungin. Posaconazole and itraconazole are also possibilities, but there is no established pediatric dose for posaconazole, and itraconazole dosing is difficult in children. In patients who do not benefit from initial antifungal therapy, options include switching to another agent with a different mechanism of action or combination therapy. Further research is required to better establish optimal approaches to the management of pediatric patients with invasive aspergillosis recalcitrant to initial primary therapy.

### Introduction

Invasive aspergillosis is a major cause of morbidity and mortality in severely immunocompromised individuals. Moreover, the incidence and burden of invasive aspergillosis have increased in recent times<sup>1–5</sup>, paralleling increases in the number of immunocompromised patients. A retrospective review of the records of 5589 patients who underwent hematopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center in Seattle from 1990 through 1998 revealed a greater than 200% increase in invasive aspergillosis incidence for both allograft and autograft recipients<sup>4</sup>. Additionally, a 2001 US study examining trends in mortality due to invasive mycoses reported a 357% increase in deaths due to aspergillosis from 1980 to 1996<sup>6</sup>. Another recent US study reported that invasive aspergillosis in immunocompromised children was associated with increased length of hospital stay and total hospital charges compared with immunocompromised children without invasive aspergillosis<sup>7</sup>.

Although approximately 185 different species of *Aspergillus* have been identified, only a small subset causes human disease<sup>8</sup>. *Aspergillus fumigatus* is the most common cause of invasive aspergillosis in the US, followed by *Aspergillus flavus*. Less-frequent causes include *Aspergillus niger* and *Aspergillus terreus*. *Aspergillus* spp. are primarily airborne pathogens and, hence, most cases of invasive aspergillosis involve the sino-pulmonary tract. More rarely, *Aspergillus* spp. may enter the body via the gut or skin and cause gastrointestinal or cutaneous infection. The most common site of disseminated aspergillosis is the central nervous system

(CNS)<sup>9</sup>, although the cardiovascular system and other tissues may be rarer sites of dissemination.

A small but growing body of work has focused on invasive aspergillosis in pediatric patients. Further research is required to study and establish optimal approaches to the initial management of children with invasive aspergillosis as well as those recalcitrant to primary therapy. This case-based review examines the current literature to explore critical issues related to the epidemiology, diagnosis, and treatment of pediatric invasive aspergillosis. A case study will be used to illustrate these issues. However, it is not intended to be a comprehensive review of the issues highlighted.

### Case study: pediatric patient with AML

*A 7-year-old boy presented with a history of acute myeloid leukemia (AML) and development of relapse after failed response to induction therapy. He was subsequently treated with umbilical cord blood transplantation (UCBT), and engrafted well on day +28 post-transplant. However, on day +79, he developed grade II skin and gut graft-versus-host disease (GVHD), which was treated with corticosteroid pulses plus maintenance tacrolimus therapy. The patient now exhibits minor respiratory symptoms.*

This case study represents a child with multiple risk factors for invasive aspergillosis, and symptoms possibly consistent with pulmonary aspergillosis. A 2006 study of invasive aspergillosis among hospitalized immunocompromised children yielded a total annual incidence of 0.4%, but three quarters of those cases occurred in children with a malignancy. Moreover, the highest incidence was observed in children who had undergone allogeneic bone marrow transplantation (4.5%) or those with AML (4.0%)<sup>7</sup>. Similarly, a 2008 French study reported that the highest incidence of invasive aspergillosis in a pediatric hematology department was observed in children with AML (5.4%) or leukemia relapse (4%)<sup>10</sup>. Likewise, the I<sup>3</sup> Aspergillus Study Group examined completed case forms on 595 patients with proven or probable aspergillosis infection, and identified bone marrow transplantation (32%) and hematologic malignancy (29%) as the major risk factors for invasive aspergillosis<sup>11</sup>. The boy in the case study has relapsed AML that was treated with UCBT, a form of allogeneic bone marrow (or hematopoietic stem cell) transplantation, placing him at elevated risk for invasive aspergillosis. He also developed GVHD, which was treated with corticosteroid therapy, further elevating his risk for invasive aspergillosis.

### Risk factors for invasive aspergillosis

Acute myelogenous leukemia or other hematologic malignancies are frequently treated with bone marrow/stem

cell transplantation. This procedure involves the transfer of stem cells harvested from bone marrow or blood of another individual (allogeneic) or previously collected from the same individual (autologous) into the patient with a hematologic malignancy, after a conditioning regimen of high-dose chemotherapy and with immunosuppression. As its name implies, UCBT is a form of allogeneic stem cell transplantation in which the transplanted stem cells are harvested from umbilical cord blood<sup>12</sup>. Most commonly, UCBT is used in pediatric rather than adult patients. Transplanted stem cells are expected to then repopulate the bone marrow of the patient and produce new immune cells important for cancer surveillance. Hematologic malignancy, relapsed hematologic malignancy (leukemia), and allogeneic (vs autologous) bone marrow transplantation (particularly as the number of human leukocyte antigen [HLA] mismatches increases) are all risk factors for invasive aspergillosis<sup>3,10,13–17</sup>.

Graft-versus-host disease is a potential complication of allogeneic hematopoietic bone marrow transplantation in which immune cells that originate from or develop from the donor's bone marrow attack the recipient's tissues<sup>16,18,19</sup>. It is well known that GVHD is a risk factor for invasive aspergillosis<sup>3,13–17,19</sup>. The immunosuppressive agents tacrolimus or cyclosporine are typically used to prevent GVHD development, occasionally in combination with other agents<sup>18</sup>. When GVHD does develop, it is usually treated with high-dose corticosteroids (e.g., prednisone) and continuation of tacrolimus or cyclosporine<sup>18</sup> – as was observed in the case study here. The typical prednisone dose for severe acute GVHD is  $\geq 2$  mg/kg/day, often administered for 30 days or longer. High-dose corticosteroid or other immunosuppressive treatment is also a well-recognized risk factor for invasive aspergillosis<sup>3,13–15,17,19</sup>. The risk of invasive aspergillosis progressively increases with increases in corticosteroid dose to 2.0 mg/kg/day and above<sup>14,15</sup>. Interestingly, the association of corticosteroid therapy with increased invasive aspergillosis risk may go beyond the immunosuppressive effects of these compounds. At least one in vitro study has suggested that corticosteroids may also stimulate or otherwise facilitate the growth of *A. fumigatus* and *A. flavus*<sup>20</sup>, underscoring the risk of invasive aspergillosis with steroids.

Other factors associated with increased risk of invasive aspergillosis include persistent neutropenia, impaired lymphocyte engraftment following bone marrow transplantation, cytomegalovirus (CMV) disease, respiratory virus infection, increasing age, hyperglycemia, cytotoxic chemotherapy, prior antibiotic therapy, and *Aspergillus* colonization<sup>3,13,14,16,17,19,21</sup>. In addition to individuals with hematologic malignancies or recipients of stem cell transplants, other patient populations at increased risk for invasive aspergillosis include recipients of solid-organ

transplants, chronic granulomatous disease, myelodysplastic syndrome, aplastic anemia, pre-existing lung disease (e.g., asthma, chronic obstructive pulmonary disease [COPD], or emphysema), AIDS, or other immunodeficiency conditions or syndromes<sup>3,7,14,17</sup>. Critically ill patients in the intensive care unit (ICU) may also be at increased risk for invasive aspergillosis, even in the absence of hematologic malignancy<sup>22,23</sup>. This is particularly so for ICU patients receiving corticosteroids or those with COPD or cirrhosis of the liver.

### Mortality and risk factors for mortality in pediatric patients

Invasive aspergillosis is associated with a high overall and attributable mortality (case-fatality) rate in patients of all ages<sup>24</sup>. A 2001 systematic review reported an overall invasive aspergillosis case-fatality rate of 58% when including patients of all ages, and an even higher rate of 68% when examining only patients aged 20 years or younger<sup>25</sup>. The overall case-fatality rate was highest for patients with disseminated disease or CNS involvement (88.1%), recipients of a bone marrow transplant (86.7%), and patients with HIV infection or AIDS (85.7%). The largest pediatric analysis by Burgos and colleagues of 139 cases of pediatric invasive aspergillosis diagnosed at six major medical centers in the US between January 1, 2000, and July 1, 2005, reported an overall mortality rate of 52.5% (i.e., percentage of patients who died during treatment)<sup>26</sup>. A 2007 systematic review of reported cases of CNS aspergillosis in children reported an overall mortality rate of 65.4%, but a significantly lower rate for cases after 1990 vs before 1990 (39.5% vs. 82.8%; odds ratio [OR] = 0.14; 95% confidence interval [CI] = 0.042–0.43;  $p = 0.0004$ )<sup>9</sup>. The reason for the difference is unclear, but likely related to improved therapeutic agents and diagnostic capabilities.

The retrospective study by Burgos and coworkers also examined risk factors for overall mortality in the 139 cases of pediatric invasive aspergillosis<sup>26</sup>. Table 1 highlights factors significantly associated with overall mortality in the

study. Antifungal therapy before diagnosis approached, but did not reach, statistical significance ( $p = 0.059$ ). A multivariate regression analysis identified allogeneic bone marrow transplant (adjusted OR = 6.58; 95% CI = 2.67–16.21) and surgery (adjusted OR = 0.22; 95% CI = 0.06–0.85) as independent predictors of overall mortality. Infection with a particular *Aspergillus* spp. (*A. fumigatus* or *A. flavus*) was not a significant risk factor for overall mortality. The *Aspergillus* spp. most frequently recovered by culture was *A. fumigatus*, followed by *A. flavus* (15.7%), *A. terreus* (4.7%), and *A. niger* (4.7%). This is consistent with other pediatric studies in which most patients have infection with *A. fumigatus* (59% in the Burgos study). *A. flavus* may be the predominant species in studies in which skin is the primary clinical site of invasive aspergillosis<sup>27</sup>.

Table 2 presents the results from a retrospective cohort study that used information from the 2000 Kids Inpatient database to evaluate the rate of in-hospital mortality by underlying condition for immunocompromised pediatric patients with or without invasive aspergillosis<sup>7</sup>. The mortality rate varied by underlying condition and was substantially higher in immunocompromised patients with invasive aspergillosis compared with those without invasive aspergillosis.

Considering what is known about risk factors for invasive aspergillosis, the information from the case study indicates that the child presented in the case study is at high risk, and his respiratory symptoms are consistent with the lungs as the primary site of infection. Importantly, subtle clinical signs, such as mild respiratory symptoms, are paramount in a severely immunocompromised patient. Further diagnostic tests are warranted to determine the exact etiology of the symptoms. If the child does have invasive pulmonary aspergillosis, his risk of death is high and prompt initiation of antifungal treatment is required.

### Case (continued): diagnosis of invasive aspergillosis in children

A computed tomography (CT) scan of the chest was obtained and revealed three peripheral nodules. The child was not currently undergoing antifungal therapy, but had a history of febrile neutropenia during the engraftment period that was treated with liposomal amphotericin B for 10 days. A galactomannan (GM) test was performed, yielding a value of 0.72. Results from bronchoalveolar lavage (BAL) revealed the presence of *A. fumigatus*.

The overall high mortality rate of invasive aspergillosis is probably due, in large part, to diagnostic delay. Diagnosis of invasive aspergillosis in immunocompromised patients is difficult, but early diagnosis and initiation of appropriate treatment is critical for successful resolution.

**Table 1.** Risk factors for overall mortality in 139 cases of pediatric invasive aspergillosis.

Characteristic	Survival (n = 66)	Death (n = 73)	p-value
Bone marrow transplant, n (%)			0.001
Autologous	1 (2)	1 (1)	
Allogeneic	11 (17)	40 (55)	
GVHD, n (%)	3 (5)	20 (27)	0.010
Steroid therapy, n (%)	42 (64)	62 (85)	0.033
Immunosuppression, n (%)	22 (33)	47 (64)	0.001
Surgery post diagnosis, n (%)	38 (58)	23 (32)	0.045

GVHD = graft-versus-host disease.  
Adapted from Burgos *et al.*, 2008<sup>26</sup>.

Table 2. In-hospital mortality rate by underlying condition.

Underlying condition*	Mortality rate, %		RR (95% CI)	p-value
	Patients with IA (N = 666)	Patients without IA (N = 151,537)		
Malignancy	21	1	13.5 (10.9–16.8)	<0.001
Solid tumor	18	1	14.0 (6.8–28.6)	<0.001
Leukemia	21	2	11.0 (8.5–14.2)	<0.001
ALL	21	1	14.9 (10.2–21.7)	<0.001
AML	20	3	5.0 (3.3–7.4)	<0.001
Lymphoma	29	2	13.5 (6.7–27.1)	<0.001
Other malignancy (NOS)	19	2	9.5 (5.3–17.0)	<0.001
Hematologic disorder (aplastic anemia)	22	3.4	5.3 (3.7–7.5)	<0.001
Immunodeficiency†	6	2.3	2.4 (1.1–5.2)	0.2
Solid-organ transplant	33	6	4.7 (0.9–23.6)	NA
Bone marrow transplant	44	8	3.8 (2.6–5.6)	<0.001
Allogeneic	45	11	3.3 (2.2–4.8)	<0.001
GVHD	44	10	3.4 (2.2–5.3)	<0.001
Non-GVHD	52	13	3.0 (1.4–6.1)	<0.001
Autologous	66	3	–	NA

\*Patients may have presented with >1 condition.

†All immunodeficiency conditions combined.

IA = invasive aspergillosis; RR = relative risk; CI = confidence interval; ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; NOS = not otherwise specified; NA = not available.

Adapted from Zaoutis *et al.*, 2006<sup>7</sup>.

Development of improved diagnostic techniques for early diagnosis would certainly enhance outcomes by enabling earlier institution of effective antifungal therapy.

A chest CT is commonly used in the early diagnosis of invasive pulmonary aspergillosis, but the results are often inconclusive and further diagnostic testing is required. In addition, there may be differences between CT scans from pediatric and adult patients with invasive pulmonary aspergillosis that require consideration. In this case, the identification of multiple peripheral nodules on the CT scan are consistent with several conditions that must be differentiated, including lung malignancy, viral pneumonia (e.g., adenovirus or CMV), bacterial pneumonia (e.g., *Nocardia* spp.), or fungal pneumonia (e.g., *Aspergillus* spp. or *Zygomycetes*, among others). Differentiation of zygomycosis from aspergillosis is important because the recommended pharmacotherapy for invasive aspergillosis (voriconazole) has little or no activity against *Zygomycetes*<sup>28</sup>.

In general, diagnostic testing should begin with noninvasive methods and only move to more invasive approaches as needed. Diagnostic options include conventional or high-resolution CT (as was initially performed in this case), magnetic resonance imaging (MRI), positron emission tomography (PET), serum GM assay, BAL, and tissue biopsy. At this time, MRI and PET are more research-oriented than commonly used clinical approaches. The utility of standard blood cultures for *Aspergillus* spp. is limited because of a high percentage of false-negative results, even in patients with disseminated aspergillosis<sup>29</sup>. Of the listed options, the GM serum assay is the least invasive, and lung biopsy the most invasive.

## Radiology: use of CT scans

The chest CT scan is still the radiologic cornerstone for the detection of invasive pulmonary aspergillosis, and is more sensitive and specific than traditional chest radiographs in the early disease course<sup>16,17,30</sup>. Computed tomography is capable of detecting the 'halo sign' and 'air-crescent sign' that are characteristic but not diagnostic of invasive pulmonary aspergillosis. The halo sign appears as an area of haziness or ground-glass opacity surrounding a nodule and, in the correct clinical context, is more specific than the air-crescent sign for invasive aspergillosis<sup>16,17,31</sup>. The air-crescent sign appears as a crescent-shaped area of radiolucency in a region of nodular opacity. Other radiographic findings frequently observed in patients with invasive pulmonary aspergillosis include a nodule (the earliest radiographic sign), consolidation, wedge-shaped infarcts, and cavitation<sup>17</sup>. In conjunction with other clinical or host factors, the presence of each of these signs is suggestive of invasive pulmonary aspergillosis. The air-crescent sign is frequently not seen in neutropenic patients, but may appear during the recovery phase<sup>31</sup>. Of note, some studies suggest that cavitation and the air-crescent sign are more likely to be observed in older children, and may frequently be absent from CT scans obtained from younger children with pulmonary invasive aspergillosis<sup>27,30</sup>. This warrants consideration when interpreting chest CT scans in children suspected of having invasive aspergillosis.

When obtaining serial CT scans, it is also important to realize that radiologic signs of invasive pulmonary aspergillosis follow a characteristic time course that does not necessarily correspond with treatment effectiveness or

eventual outcome. For example, Brodoefel and coworkers reported that time until complete radiologic remission and outcome are independent of initial or maximum lesion size and number in patients with invasive pulmonary aspergillosis<sup>32</sup>. Irrespective of antifungal therapy, serial CT scans in patients with invasive pulmonary aspergillosis show a distinctive pattern characterized by an initial rise in number and size of lesions, followed by a plateau in lesion size, and gradual reduction. Unlike lesion size and number, cavitation formation was strongly predictive of time until radiologic remission and beneficial outcome. In particular, time to complete radiologic remission was 2.5 times (CI = 1.3–5.7) longer in patients with vs without cavitary lesions, but their chance to survive was also much greater (OR = 8.4; CI = 1.07–176).

The appearance of cavities on serial CT scans (frequently accompanied by the appearance of the air-crescent sign as neutropenia resolves) may be indicative of patient recovery. Similarly, if antifungal therapy is initiated and subsequent scans show an increase in the number or size of lesions, this is more likely a reflection of the typical progression of disease rather than failed therapy. Antifungal therapy should not be terminated or otherwise changed on the basis of these radiologic signs alone.

### Serum assays: galactomannan antigen testing

Infection by *Aspergillus* can be detected by using an enzyme immunoassay (Platelia *Aspergillus* enzyme immunoassay [EIA]; BioRad Laboratories, Redmond, WA) to discern the presence of GM in circulating blood<sup>29,30,33</sup>. A component of the *Aspergillus* cell wall, GM is released into the surrounding environment by growing *Aspergillus* spp. Serum levels of GM correlate with fungal burden in lung tissue from animals with experimental pulmonary aspergillosis<sup>34</sup>, and – according to the 2009 invasive aspergillosis clinical practice guidelines from the Infectious Diseases Society of America (IDSA) – may be considered as a surrogate marker for detection of invasive aspergillosis<sup>29</sup>. Some data suggest that serial measures of serum GM can also be used for therapeutic monitoring in adult and pediatric patients with invasive aspergillosis<sup>35,36</sup>. The IDSA guidelines state that the duration of antifungal therapy should not be determined solely by the disappearance of GM from serum, but also by resolution of clinical and radiologic findings<sup>29</sup>.

The GM EIA has been best studied in the hematologic malignancy and bone marrow transplantation populations. Both the specificity and sensitivity of the GM EIA for invasive aspergillosis are high for infected, neutropenic adult patients from these populations. There are some indications that the assay may perform less well in non-neutropenic patients<sup>16,29,33</sup>. In one recent review, the sensitivity, specificity, positive-predictive value, and

negative-predictive value of the GM EIA for aspergillosis in adults were listed as 71% to 100%, 85% to 100%, 54% to 100%, and 97% to 99%, respectively<sup>30</sup>. In addition to a non-neutropenic state, other factors that may reduce the sensitivity of the GM EIA for aspergillosis include too high an assay cutoff value (1.0 or 1.5), infection with a slow-growing species (e.g., *A. terreus*), use of mould-active antifungal therapy, and presence of chronic granulomatous disease<sup>29,33,37</sup>. Studies have shown that using an index cutoff for positivity of 0.5 vs greater indices substantially increases sensitivity, with only minimal loss in specificity<sup>38</sup>.

Factors that can decrease the specificity of the assay and lead to false-positive results include a low cut-off value ( $\leq 0.5$ ), invasive infection with other fungi (e.g., *Penicillium* spp., histoplasmosis, and blastomycosis), colonization with *Bifidobacterium bifidum*, or use of piperacillin/tazobactam or amoxicillin/clavulanate antibiotic therapy<sup>29,33,37</sup>. The false-positivity is due to cross-reactivity of the assay with GM associated with these other fungi, bacteria, or antibacterial compounds. One study showed that piperacillin/tazobactam, amoxicillin, or amoxicillin/clavulanate use was associated with false-positive results that lasted up to 5 days after termination of antibiotic therapy in hematology patients with *Aspergillus*<sup>39</sup>.

The GM EIA was approved by the US Food and Drug Administration (FDA) in 2003 for the diagnosis of invasive aspergillosis in patients. There are some older data suggesting there may be differences in sensitivity of the GM EIA when used in pediatric vs adult patients. In particular, a number of studies have reported a high percentage of false-positive results with use of GM EIA in children compared with adults<sup>27,30</sup>. In a recent review of the literature, the false-positive rates in adult and pediatric patients with aspergillosis were listed as 3% to 10% and 10% to 44%, respectively<sup>30</sup>. Additionally, there are some indications that the specificity of the test may be lower in pediatric than adult patients (48% vs 98%,  $p < 0.0001$ , in one study<sup>40</sup>). However, another study reported high rates of both sensitivity (100% vs 88.6% in adults) and specificity (89.9% vs 97.5% in adults) in pediatric patients with hematologic malignancies<sup>41</sup>.

The reasons for the reported age-related differences in GM EIA results have not been fully elucidated, but results from two recent prospective studies suggest that the GM EIA is very useful in children<sup>42,43</sup>. In the 2007 Steinbach study, 826 serum samples were collected from 64 pediatric bone marrow transplantation recipients and analyzed using the GM EIA<sup>42</sup>. Twenty of 811 samples were positive for GM on repeat testing (specificity = 97.5%; 95% CI = 96.2–98.4), including samples from 8 of 63 patients without clinical evidence of invasive aspergillosis (specificity = 87.3%; 95% CI = 76.9–93.4; false-positive rate of 12.7% [by patient]). Eleven of the patients in the study had received piperacillin/tazobactam therapy, and 4 of 11 had a

positive GM EIA value coinciding with the dates of piperacillin/tazobactam administration. After excluding these patients from the analyses, the specificity of the GM EIA increased to 98.4% (95% CI = 97.2–99.1), and the false-positive rate dropped to 8.5% by patient (and from 2.5% to 1.6% by sample). The second study, by Hayden and coworkers, demonstrated the GM EIA test detects GM antigen with a high degree of sensitivity in pediatric patients with proven or probable invasive aspergillosis (65.7%; 95% CI = 38.2–85.8)<sup>43</sup>. The false-positive rate in this study was 12.8% (by patient).

The IDSA guidelines currently recommend using the GM EIA in conjunction with CT scans for early, noninvasive diagnosis of invasive aspergillosis in high-risk patients<sup>29</sup>. The test should be performed serially, probably twice per week through the periods of highest risk, whether the periods involve neutropenia or active GVHD. The results from the 2007 study suggest the GM EIA might be able to play a similar role in pediatric as in adult patients at high risk for invasive aspergillosis, provided safeguards are undertaken to improve the specificity and lower the risk of false-positive results. Further validation of the GM EIA for the early diagnosis of invasive aspergillosis in high-risk pediatric patients is warranted, and would best be obtained in a prospective, larger-scale, pediatric-dedicated trial examining GM EIA separately in allogeneic bone marrow transplant recipients and high-risk leukemia patients.

### Other fluid and tissue specimens: bronchoalveolar lavage and lung biopsy

Bronchoalveolar lavage is a more invasive procedure than the GM assay or using radiologic techniques to image the lungs of patients suspected of having pulmonary aspergillosis. *Aspergillus* spp. can often be detected in BAL fluid<sup>17,29,37</sup>, either by the GM EIA or following culturing. Such a finding, in conjunction with a positive GM EIA serum test and radiologic and clinical results, may be used to conclude a diagnosis of invasive pulmonary aspergillosis. The GM EIA has been used to detect GM in BAL fluid from patients suspected of having pulmonary aspergillosis or in cerebrospinal fluid from patients at risk for CNS aspergillosis, and appears to possess higher sensitivity in BAL fluid than serum. However, these are not approved uses and should only be considered investigational at this time<sup>29</sup>.

Tissue biopsy is the most invasive diagnostic procedure for invasive aspergillosis. While lung biopsy is the gold standard for the diagnosis of invasive pulmonary aspergillosis, this procedure is difficult to perform in critically ill patients with significant respiratory compromise, particularly if they are also thrombocytopenic<sup>17,30</sup>. However, the biopsy results from a complicated case can be important in

firmly establishing a diagnosis that enables prompt determination of potentially life-saving therapy, including both the form and duration of such therapy.

With respect to the case study, the chest CT revealed three peripheral nodules, consistent with invasive pulmonary aspergillosis, but also consistent with other diagnoses. The results from the GM EIA yielded a value of 0.72, which was suggestive of invasive aspergillosis, particularly in the context of the CT findings. A diagnosis of invasive pulmonary aspergillosis was cemented by the results from culturing of BAL fluid, which revealed the presence of *A. fumigatus*. A lung biopsy was therefore deemed unnecessary.

### Case (continued): management of pediatric aspergillosis

*The child is started with intravenous (IV) voriconazole, 7 mg/kg twice daily.*

As discussed, the collection of results from various diagnostic tests indicates the boy in the case study has invasive pulmonary aspergillosis. Hence, a decision was made to treat with voriconazole, which is consistent with recommendations from the 2008 clinical practice guidelines from IDSA<sup>29</sup>. The IDSA guidelines recommend early initiation of antifungal therapy in patients even strongly suspected of invasive aspergillosis while diagnostic evaluation is still being conducted. (Note that a GM EIA may yield a false-negative result when antifungal prophylaxis or empiric therapy is involved.) Once a diagnosis of invasive pulmonary aspergillosis is made, the IDSA guidelines recommend primary treatment with IV or oral voriconazole for most patients, with a preference for the IV formulation in seriously ill patients. A 2001 study by Herbrecht and coworkers demonstrated a higher response rate, improved survival, and fewer severe adverse effects with voriconazole vs. amphotericin B in patients with invasive aspergillosis<sup>44</sup>. Surgery is only indicated if the lesion is impinging on a great vessel. Similar recommendations are made for patients with other (nonpulmonary) forms of invasive aspergillosis.

For pediatric patients, the current recommended dose of voriconazole is 7 mg/kg IV every 12 hours. This is a higher dose than used for adults because a pharmacokinetic study showed that elimination of voriconazole is linear in pediatric patients unlike adults<sup>45</sup>. This suggests that even higher dosages than currently recommended may be required in some pediatric patients to achieve similar exposures as adults receiving their recommended dosage (6 mg/kg IV q12 hours for 1 day, followed by 4 mg/kg IV q12 hours). A subsequent study by the same group indicated that exposure was lower in children receiving 8 mg/kg twice daily compared with adults

receiving 4 mg/kg twice daily (area under the curve [AUC] = 34,681 vs. 42,000 ng · h/mL)<sup>46</sup>. Interpatient pharmacokinetic variability was high in the study, but there were no significant differences in the 2- to 6- and 6- to 12-year-old groups, except at the 8 mg/kg dose. After oral therapy, children aged 2 to 6 years had a lower AUC and maximum drug concentration ( $C_{max}$ ) than older children, and the oral bioavailability was less in pediatric than adult patients (65% vs. 96%). For most pediatric patients receiving voriconazole for invasive aspergillosis, it will be best to start with 7 mg/kg IV q12 hours and continue this same dose for maintenance daily therapy for the full course of therapy. In some cases, an even higher dosage may be required and further study of voriconazole dosing in pediatric patients is warranted.

The IDSA guidelines state measurement of serum voriconazole levels may be useful in some patients receiving voriconazole therapy, to evaluate for potential toxicity or to document adequate drug exposure, particularly in patients receiving oral therapy<sup>29</sup>. Therapeutic drug monitoring may be especially important in pediatric patients receiving voriconazole therapy, given the relatively limited database upon which dosing recommendations are based and the recognized differences in pharmacokinetics between adult and pediatric patients. However, caution is needed in interpreting voriconazole serum levels as it is unclear what level correlates with clinical efficacy.

Alternative therapies recommended by the IDSA include lipid formulations of amphotericin B (liposomal amphotericin B [L-AmB] and amphotericin B lipid complex [ABLC]), echinocandins (caspofungin and micafungin), posaconazole, and itraconazole<sup>29</sup>. The recommended dosages of L-AmB and ABLC in both adults and pediatric patients with invasive aspergillosis are 3 to 5 mg/kg/day IV and 5 mg/kg/day IV, respectively. Caspofungin is indicated for the treatment of invasive aspergillosis in pediatric ( $\geq 3$  months) or adult patients who are refractory to or intolerant of other pharmacotherapies<sup>29</sup>. The recommended maintenance dosage (after the loading dose of 70 mg/m<sup>2</sup>/d) of caspofungin in pediatric patients is 50 mg/m<sup>2</sup>/day. Although micafungin has activity against *Aspergillus* spp. and is a recommended therapy by the IDSA, it does not currently have an indication for treatment of invasive aspergillosis in pediatric or adult patients. Dosage recommendations have not been established for micafungin in adult or pediatric patients with invasive aspergillosis. A recommended dosage of posaconazole in pediatric patients is currently being studied<sup>29</sup>. Dosing of itraconazole to achieve therapeutic drug levels can be difficult and should generally not be used. Fluconazole is not active against *Aspergillus* spp.<sup>47</sup> and is not indicated for the treatment of invasive aspergillosis<sup>48</sup>.

The IDSA does not recommend routine primary combination therapy due to lack of clinical support for such an approach at this time<sup>29</sup>. The best approach for

management of a patient with invasive aspergillosis who does not benefit from initial antifungal monotherapy is unclear. A switch to an alternative therapy with a different mechanism of action is an option, and as discussed, caspofungin is indicated for the treatment of antifungal-refractory invasive aspergillosis. The IDSA guidelines state that either a switch to another drug class or addition of another agent may be considered for salvage therapy in individual patients refractory to initial monotherapy<sup>29</sup>. There is little available data to guide the use of initial or adjuvant combination therapy at this time.

The optimal duration of antifungal treatment of invasive aspergillosis has not been established in pediatric or adult patients<sup>29</sup>. *Aspergillus* is a slow-growing mould that is more difficult to eradicate than *Candida* and, hence, treatment duration will need to be longer for invasive aspergillosis than for invasive candidiasis. The IDSA guidelines generally recommend a minimum duration of 6 to 12 weeks, and continuation throughout the period of immunosuppression in immunosuppressed patients, until resolution of lesions<sup>29</sup>. Sometimes a given patient may require treatment for a year or more.

Treatment should be monitored by periodic radiologic imaging (typically CT), together with serial clinical evaluation of signs and symptoms<sup>29</sup>. In some cases, serial GM EIA has been used with other measures for therapeutic monitoring, although this is not an established approach at this time. Disappearance of GM from serum should not be used as the sole determinant for termination of treatment.

A recent retrospective analysis of 139 cases of pediatric invasive aspergillosis demonstrated that L-AmB was the most commonly used antifungal therapy (57.3%), followed by voriconazole (52.7%), caspofungin (42%), ABLC (25.2%), and itraconazole (21.4%)<sup>26</sup>. Interestingly, most patients received two or more agents concurrently for 3 days or more after diagnosis of invasive aspergillosis: 45.8% received at least three concurrent agents, 33.6% received two agents concurrently, and only 20.6% received monotherapy.

It is important to recognize that there are relatively few randomized trials of treatment for invasive aspergillosis, and none for treatment of the disease in pediatric patients. Hence the current recommendations for voriconazole and other treatment options are based on limited clinical trials, supplemented by opinions and experience of respected experts in the field. Additional clinical trials are warranted to compare voriconazole with other treatment options in adult and pediatric patient populations with invasive aspergillosis.

## Conclusions

Optimal treatment of invasive aspergillosis involves early diagnosis and treatment utilizing the most current

diagnostic technologies and antifungal strategies. Where possible, empiric therapy of high-risk patients with suspected invasive aspergillosis is warranted. Invasive aspergillosis is most readily defeated when it is attacked early in the disease process. The same general approaches to management apply in pediatric as in adult patients, although there may be some differences in diagnostic testing based on age that need to be considered, particularly as they apply to chest CT scans and use of the GM EIA. In addition, higher dosages of particular antifungal agents are required in pediatric patients to achieve similar exposures as in adults receiving a lower dosage. When initial therapy fails, one of the first questions should be whether the child is receiving an optimal dose of antifungal therapy. Voriconazole appears to be the best primary therapy in pediatric as in adult patients.

Optimal therapy in patients refractory to initial monotherapy is unclear, and much more research is needed in this area. Options include switching to another antifungal with a different mechanism of action or adding one or more antifungal agents to initial therapy. Caspofungin was recently approved for the treatment of invasive aspergillosis in pediatric patients refractory or intolerant to other therapies. Other options for primary or salvage therapy include L-AmB, ABLC, micafungin, posaconazole, and itraconazole, although pediatric dosages have not been established for micafungin and posaconazole, and itraconazole dosing can be difficult in pediatric patients. Micafungin is not currently approved for the treatment of invasive aspergillosis in pediatric or adult patients but would likely have similar efficacy as caspofungin.

There are more recent publications of voriconazole and other treatment options, and more extensive published literature on L-AmB and ABLC in aspergillosis that was not reviewed here, as it is beyond the scope of a case-based presentation.

## Transparency

### Declaration of funding

This activity is supported by an educational grant from Merck & Co., Inc.

### Declaration of financial/other relationships

W.J.S. has disclosed that he has received consulting fees from, and has contracted research for, Astellas and Merck. In addition, he has disclosed that he has received fees for non-CME services from Merck and Pfizer.

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