

New findings and unique aspects in pediatric aspergillosis

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There is a paucity of specific data on pediatric invasive aspergillosis. While the underlying predisposing patient diseases and treatments differ in children and adults, it also appears that there is a heterogeneity of invasive aspergillosis disease that extends to children. These aspects extend in some reports to the *Aspergillus* spp. distribution as well as the fundamental pathophysiology of the disease in different age groups. For instance, the newer diagnostic tools hold great promise for adult patients but it appears that they have limited usefulness in children. Only through dedicated pediatric study will clinicians fully discover the nuances and unique findings in children with this deadly disease.

Keywords aspergillosis, pediatric

Lessons from clinical trials

Unfortunately there has been little investigation into invasive aspergillosis (IA) in pediatric patients. In fact, many clinical trials or antifungal compassionate release programs actually specifically exclude children. The aspergillosis field has witnessed three large-scale randomized clinical trials for the antifungal treatment of IA [1–3], but has acquired limited pediatric data. In the pivotal study comparing voriconazole to amphotericin B deoxycholate (AmB) [1], there were 277 total patients in the modified intent-to-treat analysis and patients were eligible for enrollment if they were ≥ 12 years old. However, despite allowing children to enroll in the clinical trial, the mean age in the voriconazole arm was 48.5 years (range 13–79 years) while the mean age in the AmB arm was 50.5 years (range 12–75 years). In the second clinical trial comparing amphotericin B colloidal dispersion to conventional AmB [2], patients were eligible if they were >2 years old. However, despite this lowered eligibility age to include children, for the 174 evaluable patients in this study the mean age in the amphotericin B colloidal dispersion arm was 48 years (range 7–81 years), and the mean age in the AmB arm was 44 years (range 0–81 years). Finally, a

European study comparing two doses (1 mg/kg/d vs. 4 mg/kg/d) of liposomal amphotericin B (L-AmB) evaluated 87 patients enrolled who were >1 year old [3]. The mean age in the 1 mg/kg/d L-AmB arm was 51 years (range 14–74 years), and the mean age in the 4 mg/kg/d L-AmB arm was 46 years (range 15–81 years). Unfortunately, in all three of these landmark trials that have helped shape the new landscape of IA treatment, no pediatric-specific epidemiologic or outcome data were analyzed, therefore missing a tremendous prospective, randomized opportunity.

Aside from those crucial randomized clinical trials, other important open trials for the treatment of IA have been similarly heavily weighted toward inclusion of largely only adult patients. In the Mycoses Study Group multicenter itraconazole trial there was no age eligibility restriction for enrollment of the total 76 evaluable patients. However, the mean age in the group with pulmonary disease was 47.5 years, while the mean age in the group with extrapulmonary disease was 48.9 years [4]. Another open, non-comparative multicenter study examining the safety and efficacy of voriconazole against IA evaluated 116 patients who were ≥ 14 years old with a mean age of 52 years (range 18–79 years). In both of these studies, while some children were enrolled, the children clearly constituted a small percentage of the overall number of patients and, most importantly, even in these smaller studies there was no stratification of pediatric patient outcome.

Looking beyond controlled clinical trials, there has been very little investigation into even the treatment

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practice patterns or outcomes of pediatric IA patients. The largest study of treatment practices and outcomes in IA reviewed 595 patients treated by 89 physicians. While the patients analyzed were from 0–86 years old, the mean age was 42.3 years [5]. In a European review of diagnosis and therapeutic outcome, there were 123 patients analyzed with a mean patient age of 46 years (range 9–83 years) [6]. Finally, while neither an older report of 2,121 published cases [7] nor a report on therapeutic outcome in 1,223 cases of IA [8] did not specifically exclude children, like all other previous studies there were no specific data supplied on the pediatric patients with IA.

Epidemiology of pediatric invasive aspergillosis

While it is clear that there are no specific data on outcomes or even treatment practices in pediatric aspergillosis, there is unfortunately also very little specific information on the fundamental epidemiology of pediatric IA. While one large study examined 621 patients with IA in the greater Paris area, the mean age was 40.3 years (range 6 days to 89.7 years) and there were no specific pediatric data [9]. Another study examined risk factors for mould infection in 230 bone marrow transplant patients and enrolled patients from age 3 months to 54 years, but the mean age was approximately 29 years and there was no specific pediatric analysis [10]. One study examined 409 patients for early infections after hematopoietic stem cell transplantation (HSCT) and enrolled patients from ages 6 months to 65 years (mean age 32 years), but again with no pediatric analysis [11]. Another study of 173 allogeneic HSCT patients after non-myeloablative conditioning analyzed patients with a mean age of 53 years (range 0–72 years) but also did not comment on pediatric disease [12]. Only one study of 327 patients with IA from 1985–1999 stratified patients into three separate age groups [13]. A total of 13% of patients were less than 19 years old, with 34% between 19–40 years old and 53% of patients more than 40 years of age. While this leans toward establishing a pediatric figure, the number of transplants performed in the youngest age group was not reported, so a true incidence of pediatric disease cannot be calculated. Therefore, the true incidence of IA in children remains unknown despite numerous epidemiologic studies of the disease.

Unfortunately, even large-scale studies of infections in pediatric HSCT patients do not answer the fundamental questions. In a study of 148 pediatric HSCT patients from 1986–1996, eight patients had proven IA

but, the age range of the infected children (six from allogeneic transplants, two from autologous transplants) were not detailed. Additionally, there were 48 patients with suspected invasive fungal infection, but the results were not stratified between *Candida* and *Aspergillus* infection and there were no specific IA analyses on any of the patients [14]. Another larger report reviewed 510 pediatric HSCT recipients in 485 patients from 1990–1998 [15]. There were 26 cases with IA (4.79% of infections) in 584 culture-proven infections in the first year post-transplant. This pediatric report was actually the first to employ tools beyond descriptive statistics to analyze pediatric IA, as a multivariable analysis showed that IA was more likely to be associated with severe graft-versus-host disease (RR 7.5%; 95% CI 3.0–18.4). Additional analyses revealed that there were ten cases of IA in the first 30 days, 13 cases from 31–100 days post-transplant, and only three cases from days 101–365 post-transplant. While limited, here is the beginning of a proposed pediatric epidemiologic picture.

Autopsy data from Japan for IA patients from years 1989, 1993, and 1997 analyzed the patients based on age into single decade blocks [16]. From a total of 412 IA patient autopsies over those three years, there was a total of 14 IA cases in children aged 0–9 years, and 24 in those aged 10–19 years. Comparatively, there were 92 cases from age 50–59 years and 102 cases from ages 60–69 years. The study did not report the total number of children in each range who underwent autopsy, so it is again impossible to accurately calculate an incidence of pediatric IA. In an excellent review of IA case fatality rates pooled from 1,941 patients from clinical trials, cohort or case-control studies, and case series of ≥ 10 patients with definite or probable IA from 1995–1999, there was also some stratification of case fatality rate by decade of life [17]. The mean age of all patients was 44.2 years [range 3–91 years], and the youngest cohort (≤ 20 years) had a case fatality rate of 68.2% [15/22], while the next highest case fatality rate was 59.3% in the age group of 21–30 years old. This study concluded there was little variation in mortality by age, but the one ‘pediatric’ case fatality rate was considerably higher than the other age cohorts. This suggests the epidemiologic possibility that pediatric and adult IA differ in outcome.

While the basic epidemiology of IA in adults has been established through several studies, there are only two published reviews of childhood cases. Each suffers from the limitations of time as the diagnostic and therapeutic tools available to pediatric clinician have dramatically improved. In 1993 the Hospital for Sick Children in Toronto reviewed 39 cases of pediatric IA

from 1979–1988 [18]. Of those 39 cases, 24 were proven IA and 15 were probable IA. The median age of the cases was 10 years old (range 22 days to 18 years), and 74% had a hematologic malignancy or were a bone marrow transplant (BMT) recipient. A total of 31 of 36 patients had an absolute neutrophil count (ANC) of <500 cells/ μl with the mean duration of an ANC <1000 cells/ μl of 20 days. Of the 24 proven IA cases, disease presented as cutaneous ($n=10$), lung ($n=3$), esophagus ($n=1$), and bowel ($n=1$) and autopsy lung ($n=2$) or autopsy disseminated ($n=7$). All except one patient was immunocompromised owing to an underlying condition, and 31 of 39 patients had an ANC <500 cells/ μl at the time of diagnosis.

In 41% (16/39) of the total patients, the *Aspergillus* infection was cutaneous disease which was first suspected as a skin lesion. These lesions were described as tender, erythematous macules or vesicles which frequently progressed to necrotic eschars. The lesions typically presented at sites of trauma related to arm-boards or intravenous sites (69%). Skin lesions resolved in 56% (9/16) of patients, but in all cases resolution was coincident with recovery from neutropenia. In another 41% of patients, IA was first suspected based on pulmonary findings of fever and an abnormal chest X-ray (15 cases) or pleuritic pain (1 case) despite broad spectrum antibiotics. Patients were hospitalized for a mean of 47 days (range 0–180 days) in the six months preceding diagnosis. The overall survival rate was only 23.1% (9/39), which is similar to the large case fatality rate of 68.2% in the age group of patients ≤ 20 years from the case review and generally less than many adult studies.

The second pediatric IA study was a review of 66 cases of proven IA from approximately 9,500 children treated from 1962–1996 at St. Jude's Children's Hospital in Memphis [19] with a median age of 11.2 years (range 1.3–21.6 years). The ANC for these pediatric patients was <500 cells/ μl for a median of 14 days (1–402 days), and the onset of underlying disease and IA was a median of 16 months (0–180 months). Sixty-six per cent of patients were hospitalized for a median of 36 days (1–52 days) before the onset of clinical disease, and clinical symptoms were a median of 11 days (0–69 days) before the diagnosis of IA. This report did calculate a true incidence of IA in specific pediatric subpopulations, and found that 8% (2/25) of the patients with myelodysplastic syndrome had IA, followed by an IA incidence of 7% (1/14) in chronic granulomatous disease patients, 6% (1/16) in choriocarcinoma, 4.6% (2/43) in aplastic anemia, 4% (26/647) in acute myelogenous leukemia, 4% (1/24) in chronic myelogenous leukemia, and 1% (29/2659) in acute

lymphoblastic leukemia. The survival was 58% at the end of one month, 25% after two months, and only a 15% survival rate after 10 months. This again is a lower survival rate than most adult studies have reported, and the authors found that pulmonary IA fared worse than non-pulmonary disease, with an overall median time of 29 days (3–312 days) between diagnosis and death.

Interestingly, the species distribution of pediatric vs. adult isolates appears different in those two studies compared to previous work with adult patients. A large Bacteriology and Mycoses Study Group study reviewed 256 isolates of *Aspergillus* species from patients with IA from 24 medical centers [20]. *Aspergillus fumigatus* comprised 67% (171/256) of isolates while *Aspergillus flavus* was the second most common isolate at 16% (41/256). This parallels the species distribution in the large voriconazole clinical trial [1] where 77% (85/110) of isolates were *A. fumigatus* and 6% (7/110) were *A. flavus*. However, in both the Toronto [18] and St. Jude [19] studies, *A. flavus* was the predominant pathogen. In the Toronto study, 65% (17/26) of isolates were *A. flavus*, followed by 15% (4/26) *A. fumigatus*. In the St. Jude study, 72% (28/39) of isolates were *A. flavus*, followed by 38% (15/39) *A. fumigatus* isolates.

Diagnosis of pediatric aspergillosis

Despite recent advances in non-invasive surrogate markers of disease, radiologic evaluation remains the cornerstone of diagnosing IA. In adult series of pulmonary IA, approximately 50% of cases show cavitation and air crescent formation is found in 40% of cases [21]. In one 10-year review of 27 consecutive pediatric patients (mean age 5 years), there was central cavitation of small nodules in only 25% of children and no evidence of air crescent formation within any area of consolidation [22]. In another pediatric series there was a 22% (6/27) rate of cavitation on chest X-ray [23] and in a further study there was a 43% (6/14) rate of cavitation on CT [24]. However, in these other two pediatric series the mean ages were higher, suggesting that there is a spectrum of radiologic disease presentation that is directly related to age, and that perhaps cavitation and air crescent formation is more likely in the older child and adult than in the younger child.

Diagnosis of pediatric IA with newer antigen tests remains difficult owing to repeated differences in use of the galactomannan [GM] assay that is approved for use in adult patients. For instance, in one prospective study from 1995–1998 of 450 adult allogeneic HSCT patients (3883 samples) and 347 children with hematologic malignancies (2,376 samples), the false positive rate in

adult patients was 2.5% (10/406) while in children it was up to 10.1% (34/338). Additionally, while the sensitivity and specificity of the test using an OD of >1.5 in at least two sequential samples was 88.6% and 97.5%, respectively, in adult patients, the sensitivity increased to 100% and the specificity dropped to 89.9% in children [25]. In another study of 797 episodes, including 48 pediatric patients, the false-positive rate in the fever of unknown origin group was 0.9% in adults and 44.0% ($P < 0.0001$) in children. Additionally, the overall specificity of the test was again lower in children at 47.6% compared to 98.2% ($P < 0.0001$) in adult patients [26]. While there are numerous theories as to the increased false-positivity in children, ranging from *Bifidobacterium bifidum* spp. in the gut microflora which mimics the epitope recognized by the EB-A2 in the ELISA kit [27] to GM-positive infant formula used in pediatric patients [28], the complete answer remains elusive.

Treatment of pediatric aspergillosis

There has never been a dedicated, prospective, large-scale investigation into treatment or diagnosis of pediatric IA. Only one large dataset has individually analyzed both adult and pediatric IA outcome. The adult data were an analysis from the open-label, multicenter clinical study of emergency use of amphotericin B lipid complex (ABLC) at 5 mg/kg/d from 1990–1995 in the treatment of patients with proven or probable invasive fungal infections, who either failed to respond to previous systemic antifungal therapy or developed toxicity [29]. This study examined a total of 551 patients with 556 courses of ABLC therapy and the mean age of all enrolled patients was 37.2 years (range 21 days to 93 years). There were a total of 130 cases of IA, with a complete or partial response rate of 42%, stable response in 12%, and failure in 45% of patients. Specifically, the complete or partial response rates of pulmonary IA ($n = 74$) was 38%, disseminated IA ($n = 27$) was 30%, sinusitis ($n = 14$) was 64%, and single-organ extrapulmonary IA ($n = 15$) was 67%.

While that larger analysis of 556 treatment episodes predominantly in adults did not stratify children, a subsequent analysis was performed on 54 of 111 pediatric (<18 years old) patients from that same study [30]. The mean patient age was 9.3 years (range 21 days to 16 years). There were only 25 cases of IA, with a complete or partial response rate of 56%, stable response in 8%, and failure in 36% of patients. Specifically, the complete or partial response rates of pulmonary IA ($n = 10$) was 50%, disseminated IA ($n =$

7) was 29%, sinusitis ($n = 5$) was 100%, and single-organ extrapulmonary IA ($n = 3$) was 67%.

An analysis of the compassionate open label use of voriconazole in children <16 years old for refractory invasive fungal infection with clinical or radiologic progression of disease after ≥ 7 days of systemic antifungal therapy for children revealed 42 patients with proven or probable IA [31]. The mean age of all 58 children with invasive fungal infections was 8.2 years (range 9 months to 15 years), and the analysis of the 42 patients with IA revealed a complete or partial response rate of 43%, stable response in 7% of patients, 10% of patients intolerant to therapy, and 40% failing therapy. Specifically, the complete or partial response rates of pulmonary IA ($n = 12$) was 33%, central nervous system ($n = 6$) was 50%, disseminated IA ($n = 7$) was 86%, sinusitis ($n = 7$) was 29%, and single-organ (bone, liver, or skin) IA ($n = 10$) was 30%. The epidemiology of species distribution in this study again more closely paralleled previous adult studies, with *A. fumigatus* (26/42 patients), *A. flavus* (6/42 patients), and *A. nidulans* (3/42 patients) the most common species, rather than the previous Toronto or St. Jude pediatric studies.

Conclusion

There has never been a large-scale dedicated pediatric IA study for diagnosis or treatment. Additionally, while children have been enrolled in randomized and open clinical trials for the treatment of IA, they constitute a small percentage of the total patients and pediatric-specific data have not been analyzed. The basic pathophysiology of IA may differ in children compared with adult patients, as the distribution of *Aspergillus* sp. from two dedicated pediatric reviews appears to differ from adult studies and the outcomes appear worse in some series but slightly better in other series. This basic difference may extend to disease presentation, where in one review of 89 cases of cutaneous IA, 63% (56/89) of the cases were in children [18]. There are also clear differences in diagnosis of IA in children, with radiologic presentation differing from adults, and diagnosis by antigenemia definitely less helpful than it is in adult patients. Finally, there are the obvious nuances of antifungal management in pediatrics owing to differing pharmacokinetic and pharmacodynamic principles, including possible differences in efficacy. It is clear that contemporary, prospective, pediatric-focused studies will have to be performed to accurately understand the true pathophysiology and optimal diagnostic and treatment strategies for pediatric IA.

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