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A Prospective, International Cohort Study of Invasive Mold Infections in Children

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Background. Invasive mold infections (IMIs) are a leading cause of mortality in immunocompromised children, yet there has never been an international epidemiologic investigation of pediatric IMIs.
Methods. This international, prospective cohort study was performed to characterize the epidemiology, antifungal therapy, and outcomes of pediatric IMIs. Children (≤18 years) with proven or probable IMIs were enrolled between August 2007 and May 2011 at 22 sites. Risk factors, underlying diagnoses, and treatments were recorded. Outcomes were assessed at 12 weeks after diagnosis using European Organization for Research and Treatment of Cancer/Mycoses Study Group response criteria.
Results. One hundred thirty-one pediatric patients with IMIs were enrolled; the most common IMI was invasive aspergillosis ([IA] 75%). Children with IA and those with other types of IMIs had similar underlying risk factors, except that children with IMIs caused by non-*Aspergillus* species were more likely to have received mold-active antifungal agents preceding diagnosis. The most commonly used antifungal classes after diagnosis were triazoles (82%) and polyenes (63%). Combination therapy was used in 53% of patients. Use of combination therapy was associated with an increased risk of adverse events (risk ratio, 1.98; 95% confidence interval, 1.06–3.68; $P = .031$), although there was no detectable difference in outcome.
Conclusions. Although risk factors for IMIs are similar across specific subtypes, preceding antifungal therapy may be an important modifier. Combination antifungal therapy requires further study to determine its true risks and benefits.

Key words. antifungal; aspergillosis; mucormycosis; outcome; treatment.

Invasive mold infections (IMIs) are a leading cause of morbidity and mortality in immunocompromised children [1–5]. Most knowledge regarding the epidemiology and treatment of IMIs is derived from studies in adults. Previous studies of pediatric IMIs have largely focused on invasive aspergillosis (IA) or a specific patient population, typically children with cancer [3–7]. Because infections in other patient populations and with other mold species are relatively uncommon at any single center, a multicenter approach is needed to capture the spectrum of IMIs seen in children.

Whereas amphotericin B formulations (polyenes) were once the mainstay of therapy for IMIs, analysis of

prescribing trends has demonstrated increasing use of triazoles (voriconazole and posaconazole) and echinocandins (caspofungin, micafungin, and anidulafungin) and diminished use of polyenes in children [8]. Increased options for antifungal therapy and preclinical data have also generated interest in combination therapy for refractory fungal disease [9, 10]. However, detailed characterization of newer agents and their use in combination for IMIs is lacking in children.

The International Pediatric Fungal Network ([PFN] www.ipfn.org) was formed to advance understanding of invasive fungal disease in children through multicenter collaborative studies. This study leveraged this large international network to characterize the current epidemiology and spectrum of IMIs occurring in children with varied underlying conditions and to understand patterns of antifungal therapy and contemporary outcomes.

METHODS

Study Design and Population

We conducted a prospective study of children (≤18 years and >120 days) diagnosed with proven or probable IMIs between August 1, 2007 and May 31, 2011. Eligible individuals were enrolled at any of the 23 US and 19 international PFN sites. Patients were eligible for inclusion if they had proven or probable IMIs. Patients with endemic fungal disease or those with unidentified mold infections were excluded. Each patient contributed 1 episode. Invasive mold infections were diagnosed according to European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [11]. Proven disease required mycological and/or histological documentation from a normally sterile site, and probable disease required a host factor, clinical features, and mycological evidence, which for IA could include a positive galactomannan assay [11]. The study was approved by the institutional review boards at the coordinating center (Duke University) and at each of the PFN sites. Informed consent was obtained in accordance with the requirements of each site.

Data Collection

Data collection methods for the PFN registry have been previously described [12]. Investigators entered primary data using REDCap [13]. The date of diagnosis was considered to be the date IMI was established by the clinical team at the study site. Antifungal agents administered preceding diagnosis were reported, but not with specific doses, durations, or clinical indication (ie, prophylaxis vs empiric therapy). Detailed information was collected regarding the first 5 systemic antifungal therapies administered after diagnosis, including agent, dose, frequency, formulation, dates received, and adverse events (AEs) that occurred with that agent. Adverse events were categorized by severity and organ system according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 [14]. Data were not collected regarding surgery after diagnosis. Outcomes at 12 weeks after IMI diagnosis were reported by site investigators using EORTC/MSG response criteria and characterized as complete response, partial response, stable response, progression of disease, or death [15]. Enrolling sites were contacted to complete missing data fields. Information on the mold pathogen for each event was captured, but fungal isolates were not collected.

Statistical Analysis

Continuous variables were summarized using medians and interquartile ranges (IQRs) and compared using the Wilcoxon rank-sum test. Categorical variables were summarized with frequencies and compared using Fisher's exact test or χ^2 test as appropriate.

Analysis of antifungal therapy was restricted to the first 12 weeks after diagnosis. Combination therapy was defined as concurrent use of 2 or more agents for ≥3 days. Primary therapy was defined as the first agent received after diagnosis. To compare underlying conditions and risk factors, patients were categorized as having IA if any *Aspergillus* species was reported (even in the presence of other mold species) and as having non-*Aspergillus* IMIs when no *Aspergillus* species was reported.

For analysis of predictors of treatment outcome at 12 weeks, survey logistic regression with enrolling site as the clustering variable was used to adjust for clustering by enrolling site. To identify independent predictors of treatment failure, we performed multivariable logistic regression including all predictor variables that were associated with treatment failure in bivariable analysis at $P \leq .1$. We also analyzed the relationship between any combination therapy within 12 weeks after diagnosis and treatment failure at 12 weeks using multivariable logistic regression to adjust for confounding. Potential confounders were identified based on a bivariable association with both the outcome and exposure at $P \leq .1$. For all comparisons, P values were considered significant at $\alpha = 0.05$. All statistical analysis was performed using Stata version 12 (StataCorp, College Station, TX).

RESULTS

Characteristics of the Study Population

We enrolled 131 children with IMIs from 22 study sites (12 United States, 10 international), with a median of 3 patients per site (IQR, 1–10) (Supplementary Table 1).

Table 1. Characteristics of Children With Invasive Mold Infections

Characteristic	All IMI (n = 131)	IA (n = 98)	Non-IA (n = 33)	P[a]
Age (median, IQR)	10.6 (5.6, 15.0)	10.6 (5.7, 14.7)	11.0 (5.6, 5.0)	.947
Male (no., %)	60 (46)	42 (43)	18 (55)	.313
Race (no., %)				.094
White/Caucasian	72 (55)	53 (54)	19 (58)	
Asian	19 (15)	11 (11)	8 (24)	
Other/unknown/mixed	40 (31)	34 (35)	6 (18)	
Ethnicity (no., %)				.028
Hispanic/Latino	34 (26)	31 (32)	3 (9)	
Underlying Condition (no., %)				.592
Hematologic malignancy[b]	73 (56)	54 (55)	19 (58)	.087
Acute lymphoblastic leukemia	36 (27)	22 (22)	14 (42)	
Myeloid malignancies[c]	31 (24)	26 (27)	5 (15)	
Lymphoma	6 (5)	6 (6)	0	
Other hematologic condition	21 (16)	18 (18)	3 (9)	.603
Aplastic anemia	10 (8)	8 (8)	2 (6)	
Hemophagocytic lymphohistiocytosis	5 (4)	4 (4)	1 (3)	
Other[d]	6 (5)	6 (6)	0	
Solid tumor malignancy[e]	14 (11)	9 (9)	5 (15)	
Inherited immunodeficiency[f]	12 (9)	10 (10)	2 (6)	
Solid-organ transplant[g]	4 (3)	3 (3)	1 (3)	
Surgery/trauma[h]	2 (2)	1 (1)	1 (3)	
Other[i]	3 (2)	2 (2)	1 (3)	
No known underlying diagnosis	2 (2)	1 (1)	1 (3)	
HSCT recipient (no., %)[j]	43 (33)	33 (35)	10 (30)	.674
Autologous	4 (3)	2 (2)	2 (6)	.878
Allogeneic-matched related	8 (6)	6 (6)	2 (6)	
Allogeneic-mismatched related	6 (5)	5 (5)	1 (3)	
Allogeneic-unrelated	18 (14)	14 (14)	4 (12)	
Allogeneic-unknown	7 (5)	6 (6)	1 (3)	

Abbreviations: EBV, Epstein-Barr virus; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis, including patients with other mold species; IMI, invasive mold infection; IQR, interquartile range; non-IA, patients with other mold species but without aspergillosis.

[a]*P* values are for comparisons between IA and non-IA IMIs and were calculated using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables.

[b]Of patients with hematologic malignancy, 19 (26%) were newly diagnosed, 24 (33%) were relapsed, 22 (30%) were in remission, and 8 (11%) had unknown malignancy status. Distribution of malignancy status did not differ between patients with IA versus other types of IMI (*P* = .614).

[c]The majority had acute myelogenous leukemia; category also includes patients with chronic myelogenous leukemia and myelodysplastic syndrome.

[d]Includes 3 patients with Fanconi anemia, 2 with beta thalassemia, and 1 with congenital amegakaryocytic thrombocytopenia.

[e]Includes 5 patients with neuroblastoma, 4 with brain tumors, 2 with Wilm's tumor, 1 with hepatocellular carcinoma, 1 with retinoblastoma, and 1 with hepatoblastoma. Four (29%) were newly diagnosed, 5 (36%) were relapsed, 4 (29%) were in remission, and 1 had unknown status at the time of IMI diagnosis. Distribution of malignancy status did not differ significantly between patients with IA versus other types of IMI (*P* = .999).

[f]Includes 6 patients with chronic granulomatous disease, 2 with severe combined immunodeficiency, 2 with X-linked lymphoproliferative syndrome, 1 with Job syndrome, and 1 with Griscelli syndrome.

[g]Includes 2 liver transplant recipients, 1 heart transplant recipient, and 1 lung transplant recipient.

[h]Both patients had orthopedic and soft tissue surgery and/or trauma.

[i]Other underlying diagnoses were mitochondrial disease, Crohn's disease with EBV-associated lymphoproliferative disorder, and systemic lupus erythematosus.

[j]HSCT status was missing for 4 patients.

The majority (n = 96, 73%) of patients were enrolled from 6 sites, with 1 site enrolling 33 patients. The median age was 10.6 years (IQR, 5.6–15.0) and the most common underlying condition was hematologic malignancy, reported in 73 (56%) patients (Table 1). Sixty-four (49%) patients had proven IMIs and 67 (51%) had probable IMIs. Ninety-eight (75%) patients had IA (34 proven, 64 probable), 17 (13%) had mucormycosis (14 proven, 3 probable), and 22 (17%) had other IMIs (18 proven, 4 probable). Eight patients had multiple mold species identified, and 5 had IMIs in overlapping categories.

Among 43 patients who underwent hematopoietic stem cell transplantation (HSCT), 27 (63%) underwent myeloablative conditioning. Of the 36 HSCT recipients who had complete information regarding timing of IMI diagnosis from transplantation, 5 (14%) were diagnosed before or on the day of HSCT and 31 (86%) were diagnosed at a median of 168 days after HSCT (IQR, 25–247). The distribution of interval from HSCT to IMI diagnosis was approximately bimodal, with 11 IMIs diagnosed within the first 30 days, 2 diagnosed between 30 and 90 days, 15 diagnosed between 90 and 365 days, and 3 diagnosed more than 1 year post-HSCT (Supplementary Figure 1). Among solid-organ transplant recipients, median time from transplantation to IMI diagnosis was 24 days (IQR, 12–60 days).

Table 2 shows immunologic risk factors, concurrent infections, and antifungal therapy preceding IMI diagnosis.

Overall, underlying conditions and risk factors were similar in patients with IA versus other IMIs, except that those with other IMIs were more likely to have received antifungal agents with mold activity before diagnosis (67% vs 40%; $P = .009$), and specifically were more likely to have received voriconazole, posaconazole, or an echinocandin in the 14 days before diagnosis. Although the association between mold type and preceding antimold therapy did not reach statistical significance when patients with mucormycosis were considered separately from those with other non-*Aspergillus* IMIs, both groups had a trend toward greater exposure to preceding mold therapy when compared with patients with IA (68% vs 40%, $P = .054$ for mucormycosis; 62% vs 40%, $P = .088$ for other IMIs).

Table 2. Immunologic Risk Factors, Concurrent Infection, and Antifungal Exposure Before Invasive Mold Infection Diagnosis

	No. (%)			
Exposure	All IMI (n = 131)	IA (n = 98)	Non-IA (n = 33)	*P*[a]
Neutropenia[b]	90 (69)	65 (66)	25 (76)	.388
ANC <100	76 (58)	52 (53)	24 (73)	
ANC 101–500	14 (11)	13 (13)	1 (3)	
Corticosteroid therapy[b]	51 (39)	42 (43)	9 (27)	.149
<2 mg/kg per day (prednisone equivalents)	22 (17)	17 (17)	5 (15)	
≥2 mg/kg per day (prednisone equivalents)	29 (22)	25 (26)	4 (12)	
Other immunosuppression[c,d]	36 (27)	30 (31)	6 (18)	.185
Calcineurin inhibitor-based regimen	28 (21)	24 (24)	4 (12)	
Other regimen	8 (6)	6 (6)	2 (6)	
GVHD[d,e]	10 (8)	9 (9)	1 (3)	.602
Acute	4 (3)	3 (3)	1 (3)	
Chronic	6 (5)	6 (6)	0	
Concurrent infection[d,e]	70 (53)	55 (56)	15 (45)	.318
Bacterial	41 (31)	28 (29)	13 (40)	
Viral	31 (24)	28 (29)	3 (9)	
Fungal	9 (7)	7 (7)	2 (6)	
Antifungal use for ≥3 days[d,e]	95 (73)	72 (73)	23 (70)	.659
Triazole				
Fluconazole	42 (32)	38 (39)	4 (12)	.005
Voriconazole	26 (20)	14 (14)	12 (36)	.010
Posaconazole	4 (3)	1 (1)	3 (9)	.049
Itraconazole	4 (3)	4 (4)	0	.572
Polyene	24 (18)	16 (16)	8 (24)	.309
Echinocandin	21 (16)	10 (10)	11 (33)	.004
Flucytosine	1 (1)	1 (1)	0	.999

Abbreviations: ANC, absolute neutrophil count; GVHD, graft-versus-host disease; IA, invasive aspergillosis, including patients with other mold species; IMI, invasive mold infection; non-IA, patients with other mold species but without aspergillosis.

[a] *P* values are for comparisons between IA and non-*Aspergillus* IMIs and are calculated using Fisher's exact test.

[b] Refers to exposure within 14 days preceding diagnosis of IMI. Concurrent fungal infection refers only to concurrent nonmold infections and includes 7 patients with concurrent infection with yeast species (3 with *Aspergillus* spp and *Candida* spp; 1 with *Curvularia* spp and *Candida* spp; 1 with *Exserohilum* spp and *Candida* spp; 1 with *Aspergillus* spp, *Rhizopus* spp, *Paecilomyces* spp, and *Saccharomyces* spp; and 1 with *Aspergillus* spp, *Penicillium* spp, and *Trichosporon* spp), and 2 who had concurrent infection with *Aspergillus* spp and *Pneumocystis jirovecii*.

[c] Immunosuppressive agents (besides steroids and calcineurin inhibitors): sirolimus (n = 5, 4%), mycophenolate mofetil (n = 6, 5%), azathioprine (n = 2, 2%), and biologic agents antithymocyte globulin (n = 5, 4%), basiliximab (n = 3, 2%), etanercept (n = 3, 2%), infliximab (n = 2, 2%), alemtuzumab (n = 1, 1%).

[d] Refers to the 30-day period preceding diagnosis of IMI.

[e] Categories are not mutually exclusive.

Diagnosis

Diagnostic methods used in evaluation of IMIs were recorded in 104 patients and included computed tomography scan (n = 77, 74%), galactomannan assay (n = 65, 63%), culture (n = 46, 44%), biopsy (n = 30, 29%), and β-D-glucan assay (n = 24, 23%). Of the 64 patients with probable IA, the diagnosis was established via galactomannan assay in 42 (66%) without positive culture or pathology. In patients with IA, *Aspergillus fumigatus* was the most common identified *Aspergillus* species, and the most common non-*Aspergillus* mold was *Rhizopus* spp (Table 3). The most commonly reported site of infection was the lungs in 91 (72%) patients, followed by the sinuses in 17 (13%). In contrast to patients with IA, in whom 82 (84%) infections were pulmonary, pulmonary infections were identified in only 6 (35%) patients with

Table 3. Causative Agents of Pediatric Invasive Mold Infections

	No. (%)[a]
Aspergillus spp	98 (75)
Aspergillus fumigatus	26 (20)
Aspergillus flavus	7 (5)
Aspergillus niger	6 (5)
Aspergillus terreus	5 (4)
Aspergillus nidulans	2 (2)
Other	3 (2)
Unknown	56 (43)[b]
Mucormycoses	17 (13)
Rhizopus spp	9 (7)
Mucor spp	3 (2)
Absidia spp	2 (2)
Other	2 (2)
Unknown	2 (2)[c]
Other mold	22 (17)
Curvularia spp	4 (3)
Exserohilum spp	4 (3)
Fusarium spp	4 (3)
Bipolaris spp	3 (2)
Alternaria spp	2 (2)
Paecilomyces spp	2 (2)
Other	6 (5)[d]

[a] Categories are not mutually exclusive due to multiple mold species identified in 8 patients: *Aspergillus* spp and unidentified mold; *Aspergillus fumigatus* and *Absidia* spp; *Aspergillus* spp and unknown Mucorales spp; *Aspergillus* spp, *Bipolaris* spp and *Curvularia* spp; *Mucor* spp and unknown Mucorales spp; and *Alternaria* spp and *Verticillium* spp; *Aspergillus* spp, *Rhizopus* spp, and *Paecilomyces* spp; and *Aspergillus* spp, and *Penicillium* spp.

[b] Includes 42 infections diagnosed by indirect test (galactomannan assay) with negative culture and 9 infections diagnosed by pathology with negative culture.

[c] Unknown mucormycoses were diagnosed by pathology with negative culture.

[d] Additional mold types isolated in single cases in the cohort were *Penicillium* spp, *Pseudallescheria* spp, *Scedosporium* spp, *Scytalidium dimidiatum*, *Verticillium* spp, and *Wangiella dermatitidis*.

mucormycosis and 9 (32%) patients with other IMIs. The majority of patients had single organ involvement, whereas 19 (15%) had multiple sites involved. All of the patients with mixed infections had at least 1 nonsterile site involved.

Therapy

The most frequently used antifungals for initial monotherapy were voriconazole (34%) followed by polyenes (31%) (Table 4). Primary combination therapy was used in 33 (25%) patients, and it was given more often in those who had received antimold therapy before IMI diagnosis (41% vs 12% for those without preceding antimold therapy; $P < .001$). During the 12 weeks after diagnosis, the most frequently used antifungal for patients with IA was voriconazole (82%). The most frequently used agents in patients with mucormycosis were polyenes (82%). In patients with other IMIs, the most frequently used agents were voriconazole (64%) and polyenes (64%).

Seventy (53%) patients received combination therapy with 2 or more concurrent agents sometime within the 12 weeks after diagnosis. In those who did not receive primary combination therapy, the median time to start combination therapy was 5 days (IQR, 3–12 days) from the start of initial therapy and was similar between different IMI categories. Combination therapy was given for a median of 25 days (IQR, 13–58 days). Combinations of triazole and polyene agents were used most frequently (28%); in patients with mucormycosis they were used in 24% of the episodes, while in patients with other IMIs they were used in 36% of the episodes. In patients with IA, the most frequently used regimens were combinations of triazoles and echinocandins (32%) and of triazoles and polyenes (28%).

There were 47 AEs reported during therapy for IMIs, including 5 severe events (3 hepatic, 1 skin, 1 central nervous system [CNS]), 22 moderate events (2 skin, 2 CNS,

Table 4. Systemic Antifungal Therapy After Invasive Mold Infection Diagnosis

Antifungal Treatment	All IMI (n = 131)	Aspergillosis[a] (n = 98)	Mucormycosis[a] (n = 17)	Other Mold[a] (n = 22)
Initial therapy		No (%)		
Monotherapy	97 (74)[b]	76 (78)	12 (71)	14 (64)[b]
Triazole	49 (37)	42 (43)	4 (24)	6 (27)
Voriconazole	45 (34)	39 (40)	2 (12)	5 (23)
Posaconazole	3 (2)	2 (2)	2 (12)	1 (5)
Itraconazole	1 (1)	1 (1)	0	0
Polyene	40 (31)	30 (31)	7 (41)	4 (18)
Echinocandin	8 (6)	4 (4)	1 (6)	4 (18)
Combination therapy[c]	33 (25)[b]	22 (22)	5 (29)	7 (32)[b]
Triazole/polyene	19 (15)	13 (13)	3 (18)	4 (18)
Triazole/echinocandin	9 (7)	7 (7)	0	2 (9)
Polyene/echinocandin	2 (2)	1 (1)	1 (6)	0
Triazole/polyene/echinocandin	3 (2)	1 (1)	1 (6)	1 (5)
Therapy 12 wks after diagnosis		No (%)		
Triazole	107 (82)	84 (86)	10 (59)	18 (82)
Voriconazole[d]	95 (73)	80 (82)	4 (24)	14 (64)
Posaconazole[e]	22 (17)	8 (8)	7 (41)	10 (45)
Itraconazole	1 (1)	1 (1)	0	0
Polyene[f]	83 (63)	58 (59)	14 (82)	14 (64)
Echinocandin[g]	51 (39)	39 (40)	4 (24)	9 (41)
Terbinafine	1 (1)	0	0	1 (5)
Combination therapy[c]	70 (53)	53 (54)	7 (41)	12 (55)
Triazole/polyene	37 (28)	27 (28)	4 (24)	8 (36)
Triazole/echinocandin	36 (27)	31 (32)	1 (6)	5 (23)
Polyene/echinocandin	11 (8)	8 (8)	2 (12)	1 (5)
Triazole/polyene/echinocandin	10 (8)	6 (6)	1 (6)	3 (14)
Other[h]	6 (5)	3 (3)	1 (6)	3 (14)

Abbreviations: IMI, invasive mold infection; IQR, interquartile range.

[a]Categories are not mutually exclusive: 5 patients with mixed infections were included in multiple categories.

[b]Initial monotherapy and initial combination therapy groups do not add up to total because 1 participant with *Fusarium* did not receive treatment due to death near the time of diagnosis.

[c]Combination therapy is defined as overlap of 2 or more agents for at least 3 days. Some patients received multiple types of combination therapy during the 12 weeks after diagnosis.

[d]Median voriconazole starting dose 12.2 mg/kg per day (IQR, 9.8–14.3 mg/kg per day) for patients <12 years old, 400 mg/day (IQR, 400–560 mg/day) for patients ≥12 years old. Sixty-four (67%) started therapy with oral formulation.

[e]Median posaconazole starting dose 20.8 mg/kg per day (IQR, 17.9–26.0 mg/kg per day) for patients <12 years old, 800 mg/day for all but 1 participant ≥12 years.

[f]Of 83 patients who received polyenes, 75 (90%) received liposomal amphotericin B, with median starting dose 5.0 mg/kg per day (IQR, 3.1–5.3 mg/kg per day).

[g]Median starting doses: caspofungin, 50 mg/m^2 per day (IQR, 49.1–73.4 mg/m^2 per day) in patients <12 years old, 50 mg/day in all patients ≥12 years old; micafungin, 3.1 mg/kg per day (IQR, 2.9–4.4 mg/kg per day) in patients <12 years old, 150 mg/day (IQR, 110–175 mg/day) in patients ≥12 years old.

[h]Other combination regimens used were as follows: voriconazole and posaconazole in 1 participant with aspergillosis; voriconazole, posaconazole and micafungin in 1 participant with aspergillosis; liposomal amphotericin B, voriconazole, and posaconazole in 1 participant with aspergillosis and mucormycosis (unknown species) and 1 participant with *Exserohilum* spp; liposomal amphotericin B and terbinafine 1 participant with *Fusarium*; and liposomal amphotericin B, voriconazole, posaconazole, and micafungin in 1 participant with *Exserohilum* spp.

9 hepatic, 5 renal, 5 other), and 20 mild events (2 skin, 5 hepatic, 9 renal, 3 other). Adverse events were most frequently reported with voriconazole (n = 22, 47%) and liposomal amphotericin B (n = 17, 36%). Compared with patients who did not receive combination therapy, those who received any type of combination therapy within 12 weeks after diagnosis were more likely to have AEs (18% vs 36%; risk ratio, 1.98; 95% confidence interval [CI], 1.06–3.68; $P = .031$).

Forty-eight (37%) patients received additional therapies that may have augmented the immune response to invasive fungal infection. The most frequently used immunomodulatory agent was granulocyte colony-stimulating factor (n = 41, 31%), followed by granulocyte transfusions (n = 8, 6%), granulocyte macrophage colony-stimulating factor (n = 7, 5%), and interferon gamma (n = 1, 1%).

Outcomes

Sixty percent of patients had a complete or partial response to therapy, and the overall mortality rate for all types of IMIs was 30% at 12 weeks (Table 5). Of the 39 patients who died, 33 (85%) had active fungal disease at the time of death, and active fungal disease was the main attributed cause of death in 11 (28%) of these. When only patients with a single type of mold infection were analyzed, there was no difference in 12-week treatment success ($P = .170$), progression-free survival ($P = .494$), or mortality ($P = .623$) between patients with IA, mucormycosis, or other types of IMIs. Of the 8 patients who had multiple mold species reported, all but 1 survived to 12 weeks.

In bivariable analysis, increased odds of treatment failure were noted with receipt of corticosteroids (odds ratio [OR], 3.27; 95% CI, 1.77–6.03; $P = .001$) or other immunosuppression (OR, 2.10; 95% CI, 1.10–4.02; $P = .026$) before diagnosis (Table 6). Preceding neutropenia was associated with decreased odds of treatment failure (OR, .50; 95% CI, .27–.93; $P = .030$). After multivariable adjustment for other predictors associated with treatment outcome (with $P \leq .1$), both preceding corticosteroid therapy (adjusted OR, 2.73; 95% CI, 1.17–6.37; $P = .023$) and preceding neutropenia (adjusted OR, .48; 95% CI, .25–.93; $P = .031$) remained significantly associated with treatment outcome. In bivariable analysis, combination therapy was not associated with treatment outcome (OR, 1.17; 95% CI, .55–2.44; $P = .664$). After multivariable adjustment for confounding by preceding neutropenia, preceding immunosuppression, preceding mold therapy, and disseminated infection, we found no significant association between combination therapy and treatment outcome (adjusted OR, .93; 95% CI, .44–1.97; $P = .839$).

DISCUSSION

This prospective cohort study of pediatric IMIs is unique in its international scope and inclusion of children with any type of IMI. Although underlying conditions and risk factors were similar between patients with IA and other IMIs, children with non-*Aspergillus* IMIs were more likely to have received antifungal agents with mold activity before diagnosis. Although mucormycosis and other non-*Aspergillus* IMIs are classically thought to develop in more immunocompromised patients, previous analysis of clinical predictors of mucormycosis in adult cancer patients with either pulmonary aspergillosis or pulmonary mucormycosis identified only concurrent sinus involvement and voriconazole prophylaxis as independent predictors of mucormycosis, whereas other factors such as active malignancy, HSCT status, and corticosteroid use were not independently associated with mucormycosis [16]. Our findings are similar in that children with mucormycosis and other non-*Aspergillus* IMIs differed from those with IA primarily in exposure to prior mold-active agents, but not in immunologic risk factors. Other studies have shown prior exposure to voriconazole to be a risk factor for mucormycosis and other non-*Aspergillus* IMIs [5, 17–20]. Breakthrough infection with non-*Aspergillus* molds has also been reported in patients receiving other agents such as caspofungin and posaconazole [21, 22].

Compared with previous cohort studies of IA in children, we found more frequent use of extended spectrum triazoles as well as echinocandins, with voriconazole replacing liposomal amphotericin B as the predominant agent for treatment of IA [3–5]. This result likely reflects incorporation of evidence primarily in adults showing

Table 5. Treatment Outcomes of Invasive Mold Infections

Outcome (per EORTC criteria)	No. (%)			
	All IMI (n = 131)	Aspergillosis[a] (n = 98)	Mucormycosis[a] (n = 17)	Other Mold[a] (n = 22)
Success – complete response	35 (27)	24 (25)	3 (18)	9 (41)
Success – partial response	44 (34)	38 (39)	4 (24)	6 (27)
Failure – stable response	10 (8)	3 (3)	4 (24)	3 (14)
Failure – progression of disease	3 (2)	3 (3)	0	0
Failure – death	39 (30)	30 (31)	6 (35)	4 (18)

Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; IMI, invasive mold infection.
[a]Categories are not mutually exclusive due to inclusion of 5 patients with mixed infections.

Table 6. Risk Factors for Treatment Failure in Children With Invasive Mold Infections

Predictor	Bivariable Association		Multivariable Adjusted Association	
	Odds Ratio (95% CI)[a]	P[b]	Odds Ratio (95% CI)[c]	P[b]
Malignancy	1.00 (.62–1.63)	.990	–	–
HSCT recipient	1.88 (.57–6.19)	.277	–	–
Neutropenia[d]	.50 (.27–.93)	.030	.48 (.25–.93)	.031
Corticosteroid therapy[d]	3.27 (1.77–6.03)	.001	2.73 (1.17–6.37)	.023
Other immunosuppression[d]	2.10 (1.10–4.02)	.026	1.30 (.49–3.50)	.583
Concurrent infection[d]	1.33 (.65–2.74)	.421	–	–
Preceding mold therapy	1.85 (.98–3.48)	.054	1.78 (.75–4.21)	.179
Invasive aspergillosis	.62 (.28–1.35)	.210	–	–
Invasive mucormycosis	2.45 (.82–7.28)	.095	1.95 (.49–7.77)	.323
Other IMI	.66 (.27–1.63)	.350	–	–
Proven IMI	1.23 (.56–2.68)	.594	–	–
Disseminated infection[e]	2.21 (.89–5.51)	.080	1.67 (.75–3.72)	.199
Combination therapy[f]	1.17 (.56–2.44)	.664	.93 (.44–1.97)	.839
Immunomodulatory therapy[g]	1.71 (.88–3.32)	.104	–	–

Abbreviations: CI, confidence interval; EORTC, European Organization for Research and Treatment of Cancer; HSCT, hematopoietic stem cell transplantation; IMI, invasive mold infection.

[a]Bivariable odds ratios were calculated comparing odds of treatment failure at 12 weeks (EORTC response categories 3 [stable response], 4 [progression of disease], or 5 [death]) in patients with vs those without the predictor (all predictor variables were dichotomized) and were adjusted for clustering by enrolling site.

[b]χ^2 test, P values were adjusted for clustering by enrolling site.

[c]Adjusted odds ratios and P values for neutropenia, steroid therapy, other immunosuppression, preceding mold therapy, invasive mucormycosis, and disseminated infection were derived from a multivariable logistic regression model containing all of these variables as predictors and treatment failure as the outcome. The adjusted odds ratio and P value for combination therapy were derived from a multivariable logistic regression model with the association between combination therapy and treatment failure adjusted for confounding by neutropenia, other immunosuppression, preceding mold therapy, and disseminated infection.

[d]Refers to exposure within 14 days before diagnosis of IMI.

[e]More than 1 clinical site identified.

[f]Combination therapy was defined as overlap with 2 or more antifungal agents by ≥3 days at any time within 12 weeks after diagnosis.

[g]Immunomodulatory therapy given at any time after diagnosis.

superiority of voriconazole to amphotericin B deoxycholate in terms of outcome and tolerability [23]. Similar trends in antifungal utilization in US children's hospitals have been demonstrated using administrative data [8].

Although data for combination antifungal therapy in children with IMIs are sparse, it is frequently used in practice, as evidenced by our study and by other recent pediatric cohort studies that have reported rates of combination therapy ranging from 32% to 91% [3–5]. We found that primary combination therapy was more likely to be used in patients who had received antimold agents before diagnosis. Higher utilization of combination therapy in this setting may reflect (1) higher concern for drug resistance or (2) poor response to empiric therapy leading to use of salvage regimens. The extensive use of combination therapy and the variety of combinations used in this cohort highlight the degree of uncertainty and risk that clinicians face in treating children with IMIs.

We did not find an association between receipt of combination therapy and improved treatment outcome. However, there were several limitations in analyzing this association. The use of combination therapy was heterogeneous in terms of the combinations used, timing, and duration. The association between combination therapy and treatment response is also subject to confounding by indication. Confounding by indication can obscure a treatment benefit when severely ill individuals are more likely to receive aggressive therapy, but are also more likely to have poor outcomes. We adjusted for identified confounders including neutropenia, immunosuppression, preceding mold therapy, and disseminated infection, but we were unable to control for unmeasured confounders such as severity of illness and end organ dysfunction.

We found that children who received combination therapy were more likely to experience AEs related to antifungal therapy. The specific timing of the AEs with each agent was not documented, so we could not determine whether the events actually occurred during combination therapy. It is possible that combination therapy was used more commonly after an AE to administer agents at lower doses and reduce toxicity. However, we found that final doses of the most commonly used agents were equivalent between children who received combination therapy and those who received solely monotherapy. It is also possible that this association was seen due to confounding by severity of illness, which may increase AE risk and be associated with increased combination therapy utilization, but we could not control for this because severity of illness was unmeasured.

The overall 12-week success rate was 60%, and 68% of patients were alive without disease progression at 12 weeks after diagnosis. There were no statistically significant

differences in global treatment success, progression-free survival, or mortality when comparing patients with different IMI types, but small numbers of patients with mucormycosis or other IMIs limit conclusions regarding outcome by subtype. The 12-week mortality rate of 30% in our cohort is similar to the 31% 12-week mortality rates from recent single-center cohort studies of IMIs in children with cancer or receiving HSCT [5, 24]. The long-term mortality rate reported for one of these studies was much higher at 73%, and prior pediatric cohort studies, primarily focusing on IA, have reported similarly poor long-term mortality rates, ranging from 50% to 88% [3–5, 7, 25, 26]. A systematic review of pediatric mucormycosis cases found a 36% mortality rate during the disease course for those who received antifungal therapy and an 88% mortality rate for those who did not [27]. In that systemic review, disseminated disease was associated with higher mortality compared with localized disease, and surgery was protective [27]. Our cohort included a relatively small number of children with mucormycosis; all received antifungal therapy and the majority had localized disease. We did not collect data on surgical treatment, but it is possible that this also contributed to the observed mortality rate.

Receipt of corticosteroids before diagnosis was independently associated with increased odds of treatment failure. It has been previously shown that receiving corticosteroids, particularly doses $\geq$2 mg/kg per day, is associated with increased mortality in pediatric and adult HSCT recipients with IA [28, 29]. We were surprised to find that neutropenia preceding diagnosis was independently associated with lower odds of treatment failure. Neutropenia has been previously associated with increased mortality in adult and pediatric HSCT recipients, and shorter duration of neutropenia and recovery from neutropenia have been associated with improved survival in children and adults with IA in the setting of acute myelogenous leukemia [29, 30]. We did not collect information on duration of neutropenia or occurrence of neutrophil recovery in this cohort. However, when compared with patients who were neutropenic at diagnosis, those who were not neutropenic at diagnosis were more likely to have inherited immunodeficiencies, surgery, or solid-organ transplantation as the primary underlying condition, and HSCT recipients who were not neutropenic at diagnosis were more likely to have graft-versus-host disease. Therefore, the finding that neutropenia at diagnosis was associated with lower odds of treatment failure is likely due to the greater prevalence of less modifiable immunologic risk factors in the patients who were not neutropenic at diagnosis, or perhaps a higher degree of suspicion in neutropenic patients that led to early initiation of antifungal therapy.

There are several limitations to this study, as with most multicenter registries. There is potential for confounding by enrolling site because the majority of patients with IMIs were enrolled from 6 sites. We attempted to control for potential confounding by site by adjusting for clustering by site in the analysis of predictors for treatment failure. It is also possible that some of the patterns of risk factors, causative agents, and therapy that we found are more reflective of the patterns at the centers with highest enrollment than they are of children with IMIs overall. In terms of combination therapy utilization, 18 of 22 sites reported use of combination therapy for at least 1 of the enrolled patients, so this likely reflects a true finding. There is also potential for measurement bias due to (1) differences in the reporting of AEs between different sites and (2) different interpretations of definitions such as for day of diagnosis and for certain underlying risk factors. Finally, differences among study sites may be a product of different approaches to diagnostic evaluation. This could result in the study not capturing all patients with true IMI. Therefore, the study findings may be less generalizable to children who have IMIs but do not meet the criteria for proven or probable disease.

Despite these limitations, inclusion of children from multiple international sites has enabled a more comprehensive understanding of IMIs in immunocompromised children. Utilization of new antifungal agents has increased, and these agents are frequently used together in combination regimens. Although the impact of combination therapy on outcomes is uncertain, our findings highlight a critical need to better understand the benefits and risks of combination antifungal therapy. Robust comparative studies with defined criteria for use of combination therapy, standardized regimens, and rigorous assessment of toxicity are needed to define the role of combination therapy in management of IMIs in children.

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