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Prevention and Monitoring of Invasive Fungal Infections in Pediatric Patients with Cancer and Hematologic Disorders

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Background. The occurrence of invasive fungal infection (IFIs) in a pediatric hematology/oncology unit after renovation of the ventilation system, and initiating routine azole antifungal prophylaxis was monitored. In addition, the value of serial screening for *Aspergillus* galactomannan (GM) for diagnosing invasive aspergillosis was assessed. ***Procedure.*** A total of 98 consecutive high-risk pediatric patients were prospectively surveyed for signs of IFI and weekly monitored for serum GM. The data was not made available to treating physicians. ***Results.*** Only 2 patients had proven and 27 possible IFI based on the European Organization for Research and Treatment of Cancer/Mycoses Study Group definitions. The incidence of proven IFI was 1/31 (3.2%) in the allogeneic stem cell transplant (SCT) (*Aspergillus* spp), 0/26 in the autologous SCT, and 1/60 (1.6%) in the induction therapy group (*C. krusei*). GM was detected at least in one tested sample in 12/98 patients (12.2%), in five patients in two or more sequential samples. In the latter group, IFI was proven in one patient and could not be excluded in the others. Four of the five patients belonged to the 31 allogeneic and one to the 26 autologous SCT patients. In patients with only one positive GM test none developed signs of IFI and only one received empirical amphotericin B. ***Conclusions.*** With the currently used preventative and prophylactic measures, IFI is uncommon in children with high-risk for infection. Regular screening for GM could be useful among allogeneic SCT patients and two positive samples should prompt further investigative procedures and preemptive antifungal therapy. Pediatr Blood Cancer 2007;48:28–34.

Key words: *Aspergillus* infection; enzyme-linked immunosorbent assay; galactomannan; hemato-oncology children; invasive fungal infection

INTRODUCTION

Invasive fungal infections (IFIs) have become more frequent as a consequence of increasing intensity of modern cancer chemotherapy [1–3]. This may be especially due to the introduction of modalities which manipulate the immune system profoundly, such as the use of T-cell depleted stem cell grafts, antilymphocyte globulin, and high-dose chemo-radio therapies. Establishing a reliable diagnosis remains difficult and morbidity, and mortality of IFI is still high even though new antifungal drugs may bring some improvement [4,5]. Early diagnosis is expected to improve results of treatment and promising results of earlier detection of invasive aspergillosis using DNA detection by PCR or fungal antigen detection have been reported [6–9]. Although there have been dramatic improvements in antifungal therapy, the prevention of IFI is the ultimate goal.

We have previously reported the incidence of IFI to be 16% in allogeneic stem cell transplant (SCT) patients and 8% in children with autologous transplant in our center [10,11]. In order to diminish these infections, air ventilation renovation was performed and each two-door isolation room of our pediatric hematology/oncology and transplant unit was provided with positive pressure ventilation and high-efficiency particulate air (HEPA) filtration [12,13]. In addition, prophylactic systemic antifungal medication was started in all high-risk patients. After commencing these prophylactic measures, we started a prospective study aiming to determine the current frequency of IFI and to evaluate the new monitoring methods, especially fungal antigen tests, in the diagnostic work-up.

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PATIENTS AND METHODS

Study Population

The study comprised all consecutive pediatric patients who were treated at the hematology/oncology unit of the Hospital for Children and Adolescents, University of Helsinki, from January 2000 to June 2002 and at increased risk for developing IFI. Eligible patients were those receiving therapy for remission induction of acute leukemia or myeloablative high-dose chemo-radiotherapy followed by SCT. The patients with leukemia induction were treated according to the Nordic protocols [14,15]. The patients ($n = 98$) could be included in the study on more than one occasion, the number of episodes being 117, that is, 60 patients undergoing induction therapy of acute lymphoblastic leukemia (ALL) ($n = 46$), acute myeloid leukemia (AML) ($n = 2$), or ALL relapse ($n = 12$), 31 in allogeneic SCT and 26 in the autologous SCT groups. All patients were under 17 years of age. The duration of neutropenia $<500 \times 10E6/L$ varied between 4 and 61 (mean 20) days, in

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79% of patients neutropenia lasted for 10 days or more. All transplanted patients, except those with myeloid malignancies, received myeloid growth factor G-CSF, starting on day +1 post transplant and continuing until neutrophil recovery (>500 × 10E6/L). During induction therapy, G-CSF was used in life threatening infections only. Corticosteroid therapy was given to all patients undergoing ALL induction (prednisolone 2 mg/kg/day for 42 days) and for treatment of graft-versus-host disease (GVHD) when needed. In total, 72 patients (63%) received corticosteroids >0.5 mg/kg/day for 3–139 (mean 40) days (Table I).

Patients were monitored for the development of the signs and symptoms of infections. Diagnostic procedures included daily physical examination, total blood count, C-reactive protein, conventional chest, and sinus X-rays on admission and, thereafter, as clinically indicated. Blood specimens for bacterial, fungal, and viral isolation were drawn when an infection was suspected and daily during fever. Weekly surveillance specimens for fungal culture from stool and throat were obtained. High-resolution pulmonary CT and MRI scans were performed on clinical indications. Bronchoalveolar lavage (BAL) or pulmonary biopsies were not performed. During neutropenia patients received empirical broad-spectrum antibacterial therapy prompted by fever >38.5°C. If the fever persisted after modification of antibacterial therapy, antifungal therapy, usually amphotericin B, was added to the antibiotic regimen. Autopsy was performed on fatalities unless refused by the parents. Fungal disease was classified as proven, probable, or possible according to European organization for Research Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definitions [16].

TABLE I. Characterization of Patients During Episodes with a High-Risk of Invasive Fungal Infections (IFIs)

No of patients		98
No of episodes		117
Mean age, years (range)		6.5 (1.0–16.5)
Leukemia induction		60
ALL, at diagnosis	46	
ALL, at relapse	12	
AML	2	
Allogeneic SCT		31
Donor:		
URD	18	
MFD	13	
Ac GVHD gr II–IV	13	
CMV infection	8	
Autologous SCT		26
Mean duration of neutropenia		
<200 × 10E6/L, days (range)		16.4 (1–46)
<500 × 10E6/L, days (range)		19.8 (4–61)
Corticosteroid therapy >0.5 mg/kg/d		
No (%)		72 (63%)
Mean duration, days (range)		40 (3–139)

Data per episode unless otherwise noted.
URD, unrelated donor; MFD, matched family donor.

Prophylactic Methods for IFI

All patients were hospitalized in single reverse-isolation rooms in a unit equipped with HEPA filters. Antifungal prophylaxis was given throughout the period of induction therapy of ALL and during neutropenia in other patients. It was continued in patients receiving prolonged immunosuppressive therapy. The aim was to use itraconazole in oral solution 5 mg/kg/day in two divided doses but due to its gastrointestinal side effects and severe interactions with other medications oral or iv fluconazole 5–8 mg/kg/day was often used instead of itraconazole. Thus, either itraconazole (n = 24), fluconazole (n = 44), or both (n = 44) sequentially, were used as prophylaxis for 4–196 (median 32) days. Monitoring of itraconazole levels was not performed systematically. Only five patients received no prophylaxis.

Environmental Surveillance

Monthly air samples were collected at ten locations in the patient area using a SAS Super100 air sampler. One cubic meter air was impacted onto malt agar and then incubated at 30°C for 5 days. All fungal isolates were identified by morphological criteria. Water samples from taps, showers, and toilets were collected at intervals and cultured in a similar manner. Settled dust, collected in an open plastic container, was analyzed once a month by resuspending the dust in phosphate buffered saline and culturing on malt agar.

Patient Monitoring

The study period covered the time of induction therapy for ALL, the total time of chemotherapy of AML, the inpatient period with neutropenia in transplanted patients and after that as outpatients until resolution of GVHD and/or cessation of immunosuppressive therapy. During the study period, a blood sample for serum *Aspergillus* galactomannan (GM), a polysaccharide cell component, was taken once a week. The tests were performed on sera that had been stored frozen and the results were not available to the clinician during patient care. The ELISA for detection of GM was performed according to the manufacturer's (Platelia *Aspergillus*, Bio-Rad, Hercules, CA) instructions. Antigen levels were recorded as negative, positive borderline. The absorbances obtained for individual tested sera were interpreted using two reference sera provided in the kit, a threshold/calibrator serum, and a positive control serum included in each assay. Values below that of the threshold/calibrator serum were designated as negative, those above that of the positive control were considered as positive and those between the values of the two references as borderline. After the study period of collecting the samples for fungal tests the clinical follow-up continued on an outpatient basis for 1 year. Written informed consent was obtained from all patients or their parents under the guidelines of the institutional review board.

RESULTS

Patients with Proven IFI

IFI was proven in 2 of these 98 children with 117 high-risk episodes. A newly diagnosed 6-year-old boy with ALL was on fluconazole prophylaxis when he became colonized with *Candida krusei* and 2 weeks later, that is, after 5 weeks induction therapy, the yeast was cultured from blood and clinical signs of infection were also apparent. C-reactive protein was 210 mg/L. Abdominal ultrasound was normal, and no CT or MRI scans were performed. *C. krusei*was sensitive to neither fluconazole nor itraconazole. He made a quick recovery during amphotericin B treatment. He subsequently had allogeneic SCT 6 months later with no signs of IFI and all colonization tests were negative. Another 8-year-old boy was transplanted for relapsed ALL using an unrelated donor (Patient number 10 in Fig. 1). He suffered from severe acute and chronic GVHD with prolonged steroid therapy. He also had bacterial septicemias and reactivation of CMV and was treated with long courses of antimicrobials. Two months post SCT A*spergillus niger* was isolated from stool on two occasions and at the same time the serum GM test was positive in three consecutive blood samples. The GM result became negative during itraconazole prophylaxis and 4 weeks later the patient received amphotericin B for 18 days for a suspected fungal infection. No findings suggestive of invasive aspergillosis were seen in chest X-ray and IFI was not documented at this phase. After multiple problems with chronic GVHD and CMV, he died from gastrointestinal bleeding 11 months after SCT having received itraconazole for several months before that. C-reactive protein varied from 45 to 62 mg/L during the last week. No CT or MRI scans were performed at that time. At autopsy, no pulmonary abscesses were seen but histological examination of the lung showed fungal hyphe morphologically suggestive of *Aspergillus* spp. Immunohistochemical staining was positive for *A. fumigatus*. GM was not investigated during this phase. This patient was the only one with documented invasive *Aspergillus* infection.

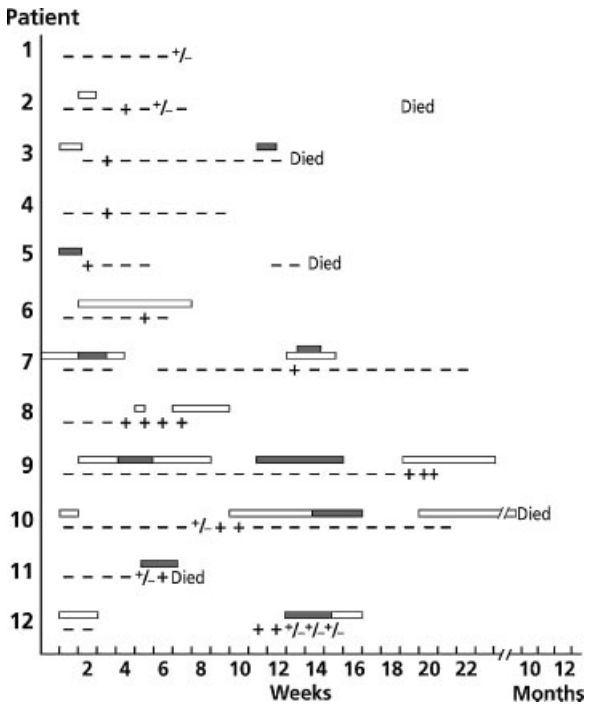

Fig. 1. Patients with at least one positive galactomannan test among 98 high-risk patients. Timing of positive (+), borderline (+/−), and negative (−) galactomannan tests, and of therapy with amphotericin B (hatched bar) or itraconazole (open bar) indicated.

Patients with Possible IFI

In 27/117 (23%) episodes there was a clinical suspicion of IFI, that is, prolonged fever not responding to broad spectrum antibacterial therapy resulting in initiation of empirical antifungal therapy. This therapy was either amphotericin B, conventional (n = 10, dose 0.7 mg/kg/day), or liposomal amphotericin B (AmBisome^{R}) (n = 18, dose 1–3 mg/kg/day), or caspofungin (n = 1, dose 50 mg/d), and the treatment lasted for 1–67 (mean 15) days. Possible IFI was treated in 12/31 (39%) of allografted, 3/26 (11%) of autografted, and 12/60 (20%) of induction therapy patients (Table II). IFIs was not documented in any of them.

Patients Without IFI

In 88/117 (75%) episodes no signs of IFI was noticed and no therapeutic antifungal medication was given. Most of these patients were in induction therapy and autologous SCT groups where 70/86 (81%) of the patients belonged to non-IFI group compared with 18/31 (58%) in allogeneic SCT group (Table II).

Overall Mortality

A total of 23/98 (23%) patients died during the 1-year observation period, 6 during the study period, and 17 subsequently. The cause of death was relapse in 15 patients and in 8 patients treatment related toxicity. Of these eight autopsy was performed on five patients, in one of them IFI was documented (Patient 10 in Fig. 1), in the other four no signs of IFI were seen The three patients not autopsied had multiple clinical problems after SCT such as

TABLE II. IFIs in Children During High-Risk Episodes

IFI	Allogeneic SCT	Autologous SCT	Induction	Total
Proven/probable	1	0	1	2
Possible	12	3	12	27
Non-IFI	18	23	47	88
	31	26	60	117

non-engraftment (n = 2), veno-occlusive disease (n = 1), hemolytic-uremic syndrome (n = 2), thrombotic microangiopathy (n = 2), chronic GVHD (n = 1), and septic infection (n = 3), which were the main causes of death (Patients 3 and 11 in Fig. 1). Without autopsy, the possibility of IFI could not be excluded.

The incidence of proven IFI in the different treatment groups was 1/31 (3.2%) in allogeneic SCT group, 0/26 in autologous SCT group, and 1/60 (1.6%) in induction therapy group (Table II). The three patients who died of treatment-related reasons and were not autopsied belonged to the allogeneic SCT with possible IFI group. Had an IFI been documented in them, the incidence would have been 4/31 (12.9%) in the allogeneic SCT group.

Colonization with *Aspergillus*

Aspergillus spp were cultured from the stool in three patients during the 117 episodes. In addition to patient number 10 in Figure 1 positive for *A. niger* (see text above), two other patients had one weakly stool sample positive for *A. ustus* and *A. glaucus*, respectively. Subsequent stool samples and serum GM tests were negative. All throat samples were negative for *Aspergillus* spp.

Galactomannan Tests

The samples for GM test were taken weekly during the 2–28 (mean 7.5)-week study period. During each of the 117 episodes, 2–32 (mean 8.0) samples were investigated in total 932 samples. The results of the GM tests were not available at the time when a decision regarding treatment was made. In total, during 12 of 117 (10.3%) episodes at least one sample tested positive for GM, that is, in 7/31 in the allogeneic, 2/26 in the autologous, and 3/60 in the induction therapy group. In seven of them (Patients 1–7) only one test and in five (Patients 8–12) two to five consecutive positive tests were recorded (Fig. 1).

Of the seven patients with only one positive test, five became GM negative and responded well without any antifungal medication (Patients 1–5). Two were receiving prophylactic itraconazole at the time of the test. One of them received also empirical amphotericin B. In the next sample both were GM negative and continued without any symptoms of IFI (pats 6 and 7). Colonization samples from throat and stools were negative for *Aspergillus* spp in all of these patients.

Of the five patients with at least two consecutive positive GM tests, three were receiving and continued on prophylactic itraconazole at the time of seroconversion, and did not develop any clinical signs of IFI during the following weeks (pats 8–10, see Patient no. 10 in the section Patients with proven IFI). Two patients received empirical amphotericin B due to suspected IFI: Patient no 11 died at day +25 after unrelated donor SCT with septicemia and non-engraftment having received amphotericin B for 10 days. Autopsy was denied by the parents. Patient no 12 cleared the symptoms of infectious disease and recovered from the autologous SCT. The incidence of patients with at least two consecutive positive GM test varied according to treatment groups: 4/31 (12.9%) in allogeneic SCT, 1/26 (3.8%) in autologous SCT, and 0/60 in induction therapy group (Pearson Chi-square $P = 0.016$).

The association of positive GM tests with the clinical fungal infection groups is shown in Table III. The only patient with confirmed IA was GM positive in three samples, several months earlier (Patient no 10 in Fig. 1). In the non-IFI group, 5/88 (5.7%) had one and 1/88 (1.1%) had at least two consecutive positive GM test.

Environmental Surveillance

During the study period filamentous fungi were isolated very infrequently from the air and dust samples collected from the patient areas. The species recovered were *Pencillium chrysogenum* and *Aspergillus niger*. No fungi were isolated from the water samples.

DISCUSSION

In this prospective study of high-risk pediatric patients, the incidence of proven/probable IFI was lower than expected. Only two patients suffered from verified IFI: 1 of 31 patients (3.2%) with allogeneic SCT (*Aspergillus* spp), no IFI in 26 patients who received an autologous SCT, and 1 of 60 (1.6%) who was undergoing induction chemotherapy for acute leukemia (*Candida krusei*). In previous studies on young transplanted patients incidences of 5% for *Aspergillus* and 6%–8% for *Candida* infection have been reported [3,17,18]. A wide range from 0% to over 20% in the incidence of IFI during chemotherapy for acute leukemia has been reported [19,20]. The previous retrospective analysis in our own hospital revealed documented IFI in 18 of 148 (12%) transplanted children, that is, 12/73 (16%) in allogeneic and 6/75 (8%) in autologous stem cell recipients, 8 infections with *Aspergillus* spp and 10 with *Candida* spp [11]. Accordingly, approximately 10 patients with IFI were expected to be found in the current study. Due to difficulties in diagnosing fungal infections our current result may be underestimating the real incidence. There may be other cases among those patients who received amphotericin B who did not have documented IFI or in those who died and were not

TABLE III. Results of ELISA Screening for Galactomannan of High-Risk Pediatric Patients

	Proven IA n = 1	Proven IFI n = 1	Possible IFI n = 27	Non-IFI n = 88
No. with >2 positive GM	1	0	3	1
No. with one positive GM	0	0	2	5
No. with negative GM	0	1	22	82

autopsied. The same problem, however, is reflected in previous studies. In the transplant group of the current study, the proportion of patients with an allogeneic transplant (54% vs. 49%) and, particularly with unrelated donor grafts (58% vs. 31%), both high-risk factors for IFI, was higher than in our previous retrospective study. However, not only the proportion of patients with proven IFI but also the proportion of those patients with possible IFI decreased from 32% to 26%, thus increasing the proportion of non-IFI patients in the transplanted group from 55% to 72% compared with our previous study. Accordingly, the reported increase in incidence of IFI in cancer patients in some centers cannot be documented in our unit. In contrast, the observed rate of IFI was lower than in our previous study in the same unit. The preventive procedures undertaken between our two studies were the initiation of systematic antifungal prophylaxis with fluconazole or itraconazole, and air ventilation renovation, including thorough cleaning of the ventilation system and providing each SCT patient with a high-pressure ventilated, HEPA filtered room. According to environmental measurements performed in the department during the present study period there was very little airborne contamination as reflected by the absence of filamentous fungi in air and dust samples, and no contamination of the patients' water supplies. The effect of the measures taken cannot be analyzed separately in this investigation but they all together might have helped to decrease the IFI incidence.

Establishing the diagnosis of IFI is extremely difficult, especially in children for whom most procedures, such as BAL, biopsies, CT, and MRI, may necessitate general anesthesia. Furthermore, when X-ray changes are prominent, the diagnosis of IFI and starting of antifungal therapy may be relatively late. Thus, in addition to prevention of these infections, their early, reliable, and non-invasive diagnosis is necessary to decrease the related morbidity and mortality. Several reports have been published on screening for GM, a polysaccharide cell component released to serum by all pathogenic *Aspergillus* species during its growth in tissues. The sensitivity and specificity of this test have been shown to be high in most series but considerable discrepancies between the studies have been reported [21–25]. The practice followed in most previous studies and that recommended by the EORTC/MSG classification is that two consecutive positive results in blood samples are required to consider the test as a microbiological criterion for IA [16]. Our findings are in accordance with this: only one among the seven patients with single positive test received a short course of amphotericin B for suspected fungal infection and none subsequently had signs of IA. On the other hand, when analyzing the clinical data of our five patients with two or more consecutive positive GM results, one cannot exclude the possibility of *Aspergillus* infection, possibly in very early stage, in any of them. It is possible that itraconazole alone, administered to three of them, was enough to inhibit the fungal disease at that point (Fig. 1).

False positive results in the tests for *Aspergillus* GM antigen have been reported especially in children [21,22,24]. False positive results were previously observed in 11/25 (44%) of children with fever of unknown origin while in only 2/235 (0.9%) of the corresponding adult group. Furthermore, 3 of the 11 children, but none of the adults, had repeatedly positive tests. In the same study, surveillance of SCT recipients revealed 9 false positive results (75%) in the group of 12 children and, in 7 of them, results were repeatedly positive. No false negative results were recorded in these pediatric groups [21]. The negative predictive value was also 100% in another study on pediatric hematology patients where false positive tests were recorded in 10% of children [22]. The presence of high amounts of GM in cereals and their derivatives, and in milk [26] or the presence of mucositis and passage of intestinal GM to blood have been suggested as causes of these false positives [22]. Other possible explanation for false positives is ELISA cross-reactivity with other fungi or bacteremia [27]. In our study, food might explain some single positive samples during induction therapy, when children often eat well, but during a SCT period oral intake is often scanty and may not cause any GM load in the intestine. No patient was found to be colonized with *Aspergillus* species or possible cross-reactive fungi. Patients treated with piperacillin-tazobactam have been reported to have positive GM tests [28], but no patient in our material was receiving this antibiotic at the time when tests were positive.

Once-a-week GM screening is probably optimal since GM usually remains positive from 1 week to 2 months [22]. Screening of induction therapy patients did not reveal any positive patients in our samples and may not be indicated. Among recipients of allogeneic SCT, 12.9% had a positive GM test and this group could be a target for screening as has been suggested in several studies in order to make the diagnosis more timely [6,7,22,29]. On the other hand, the benefit of prospective screening of SCT patients has not been shown to be clinically useful for an early diagnosis in all previous studies [23] and our findings were in concert with this observation. Another alternative for clinical purposes could be to take several (2–3) daily samples at the very early phase of symptoms and continue the close follow-up in positive cases in order to monitor the fungal disease and its response to therapy [6,30]. Effective antifungal therapy should be started without delay and two consecutive positive GM tests should be taken into account when making this decision. The discontinuation of anti-fungal therapy, often a difficult clinical decision, might also be guided in part by serial GM testing [22]. Due to false negative and false positive results the GM tests do not make other diagnostic methods, such as pulmonary CT scan, unnecessary but may be a good additional tool and possibly diminish the need for more invasive diagnostic procedures, which are undesirable especially in children. New non-invasive methods for early diagnosis of IFI, for instance, the Glucatell-test for

beta-glucan test, have been studied and promising results in adults been reported [31]. No studies have been reported in children.

According to our results, documented IFI is relatively infrequent among intensively treated children in a hematology/oncology ward with currently adopted protocols for prevention and prophylaxis incorporating environmental isolation and empirical anti-fungal policy. Regular screening of GM may be useful in allo SCT patients. Frequent serial monitoring at an early stage of symptoms is highly recommended. Two positive samples should indicate thorough clinical investigations with CT scan or MRI, and strongly support addition of specific antifungal chemotherapy.

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