Invasive Aspergillosis in Hematopoietic Stem Cell Transplant Recipients: A Retrospective Analysis

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Invasive aspergillosis (IA) currently is an important cause of mortality in subjects undergoing hematopoietic stem cell transplants (HSCT) and is also an important cause of opportunistic respiratory and disseminated infections in other types of immunocompromised patients. We examined the medical records of 24 cases of proven and probable invasive aspergillosis (IA) at the Hospital de Clinicas of the Federal University of Parana, Brazil, from January 1996 to October 2006. During this period occurred a mean of 2.2 cases per year or 3.0 cases per 100 HSTC transplants. There was a significant relationship between structural changes in the bone marrow transplant (BMT) Unit and the occurrence of IA cases (p=0.034, relative risk (RR) = 2.47). Approximately 83% of the patients died due to invasive fungal infection within 60 days of follow up. Some factors tended to be associated with mortality, but these associations were not significant. These included corticosteroid use, neutropenia (<100 cells/mm^3) at diagnosis, patients that needed to change antifungal therapy because of toxicity of the initial first-line regimen and disseminated disease. These factors should be monitored in BMT units to help prevent IA. Physicians should be aware of the risk factors for developing invasive fungal infections and try to reduce or eliminate them. However, once this invasive disease begins, appropriate diagnostic and treatment measures must be implemented as soon as possible in order to prevent the high mortality rates associated with this condition.

Key-Words: Invasive aspergillosis, transplant recipients, immunocompromised.

Invasive Aspergillosis (IA) currently is an important cause of mortality in subjects undergoing Hematopoietic Stem Cell Transplants (HSCT) and is an important cause of opportunistic respiratory and disseminated infection in other types of immunocompromised patients [1]. The risk factors for invasive fungal disease occurrence have been described, including those related to environmental factors, which may play an important role in the development of this condition [2]. An aggressive diagnostic approach in patients at risk and prompt institution of antifungal therapy are essential for patient survival [3]. Most IA studies have involved mainly adult patients; few analyses have included pediatric patients. We examined the epidemiological, clinical, microbiological and radiological characteristics of HSCT patients, including both pediatric and adult patients, who had proven or probable IA at the Hospital de Clinicas of the Federal University of Paraná, Brazil. We also looked for local environmental factors that could be related to the occurrence of this condition.

Meterial and Methods

This retrospective study was conducted in a 15 beds BMT Unit. The patient selection process was divided into two steps. First, an infectologist and a hematologist reviewed the charts of all patients who had filamentous fungus diagnosed in microbiological tests (culture and/or direct microscopic), based on data from the Mycology Laboratory Database, from January 1996 to October 2006. Then, using the medical chart information, only patients that had documented at least one site with proven or probable disease caused by *Aspergillus* were considered for analysis. Patients that were classified as having only possible IA were excluded, but patients with at least one proven or probable IA and other possible site involvement were accepted. Data were collected on epidemiological, clinical, microbiological and radiological aspects.

Standard definitions were used for proven and probable IA [4]. The *Aspergillus* galactomannan test was not available at the time. The sites of infection were classified as pulmonary, sinusal, cutaneous and cerebral. Patients with more than one affected site without evidence of contiguous spread were considered to have disseminated infection. The day of diagnosis of IA was the day when the first diagnostic test was performed. The first day of a symptom that could indicate IA was considered as the beginning of disease. Death due to IA was considered when there was clinical evidence that it was the main cause. Host demographics included sex, age and underlying disease. The pediatric group included all patients less than 18 years old. Primary antifungal therapy was defined as therapy with a systemic antifungal agent with anti-*Aspergillus* activity given for at least three consecutive days, prescribed either empirically or therapeutically. Neutropenia was defined as at least two days with neutrophil counts less than 500 cells/mm^3; neutrophil counts less than 100 cells/mm^3 were observed separately. Corticosteroid use was considered as a risk factor when a patient had used it for three or more weeks before the IA diagnosis and continued using during fungal infection treatment. The environmental factors considered were the documented histological data about structural changes and air conditioner system cleaning.

A logistic regression model was used to analyze the relationship between cases of IA and environmental factors. Fisher's exact test was applied to look for differences in clinical manifestations between pediatric and adult patients. A chi-

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square test with Yates correction was used to examine differences in radiological presentations. Overall survival was estimated with Kaplan-Meier curves. Multiple analyses (based on the Cox model) were performed to determine factors associated with survival time. A significant p value was considered at level <.05 with 95% confidence interval.

Results

Two hundred and twenty-four patients with filamentous fungus found in a microbiological test were selected for chart review. Sixty-one of these patients had a positive exam (culture or direct examination) for *Aspergillus*. Thirty-seven patients were excluded for lack of evidence of IA. Twenty-four patients that had proven or probable disease after chart review, were selected for analysis.

From January 1996 to October 2006 there was a mean of 2.2 cases per year or 3.0 cases per 100 HSTC transplants (Figure 1). An analysis using a logistic regression model demonstrated a significant relationship between structural changes in the Bone Marrow Transplant (BMT) Unit and the occurrence of cases of IA (p=0.034, RR = 2.47).

Fifty percent (n = 12) of the patients were less than 18 years old (median seven years) and 50% were 18 or more years old (median 34). The most common baseline diseases observed before transplant were aplastic anemia 45.8% (n = 11), chronic myeloid leukemia 16.6% (4) and acute myeloid leukemia 8.3% (2) (Table 1). Concerning clinical aspects, 12 cases (50%) were classified as proved and 12 cases (50%) as probable, based on EORTC/MSG criteria [4]. Seventy-nine percent of the patients (n = 19) had at least some evidence of pulmonary disease (one proven case, 12 probable and six possible). The other infection sites included 37.5% (n = 9) with signs of sinus involvement (three proven, three probable and three possible), 33.3% (n = 8) cerebral (two proven and six possible), 29.7% (n = 7) cutaneous (five proven and two possible) and 4.17% (n = 1) involved a proven renal case. In this case of renal infection, the patient had only hematuria as a symptom, and an abdominal ultrasound yielded renal lesions compatible with an infarct; the biopsy culture was positive for *Aspergillus fumigattus*.

Half of the patients had documented evidence of two or more non-contiguous sites being involved and were classified as disseminated disease.

When patients were analyzed separately according to whether they were adult or pediatric cases (Table 2), there were no differences in clinical manifestations, except for skin involvement, which was more frequent in the adult group (p=0.01, Chi-square test).

All patients received allogenic hematopoietic stem cell transplants; the source was bone marrow in 70.8% of the cases and umbilical cord in 29.2%. Most patients (87.5%) were neutropenic at the time of diagnosis or had been neutropenic (<100 neutrophils per mm^3 within the last 60 days for more than 10 days). Among patients who were neutropenic at diagnosis 88% had neutrophil counts less than $100/mm^3$. Three patients did not have neutropenia as a risk factor; but in these cases, the patients had GVHD (Graft *versus* host disease) or had been subjected to prolonged corticosteroid treatment. The mean time between first clinical manifestations and confirmed diagnosis was 14 days (SD = 17.4 days).

Fifty-nine percent of the patients did not have species identification, 29% were *A. fumigattus* (7), 8% were *A. niger* (2) and 4% *A. flavus* (1) (Figure 2).

In the analysis of radiological findings (Table 3), nodules with or without a halo sign were the most common presentation. Specifically, the halo sign was identified in four of the 16 patients (25%) with pulmonary disease based on computed tomography of the thorax. There were no differences between the pediatric and adult patients (p = 0.63, chi-square test with Yates correction).

At the time of first manifestation of invasive disease, 95.8% (23) of the patients were using some antifungal therapy; most of them (65.2%) used fluconazol for prophylactic treatment. Treatment for invasive fungal infection was started a mean of 4.6 days after the first clinical manifestation (SD = 5.9). When we examined treatment option, 91.6% of the patients began with Amphotericin B deoxicholate (n = 22); however, 37.5% of them needed to change this first choice, principally because of nephrotoxicity (55.6%) and failure (33%). Five patients (55.6%) of those with sinus disease (n = 9) needed complementary surgical treatment.

Approximately 70% of the patients died due to invasive fungal infection within 30 days of follow up, and 83.3% died within 60 days (Kaplan-Meier survival curve, Figure 3).

Some factors appeared to contribute to the mortality rate based on a multiple analysis (Cox model, Table 4). Patients that had used corticosteroids during the three weeks before diagnosis and continued using it without changing the dose during invasive aspergillosis treatment appeared to be more likely to succumb to invasive aspergillosis; however, due to the small number of patients in this sample, this tendency was not significant (p=0.49). Other factors that appeared to be correlated, but also non significant for the same reason, were neutropenia (<100 cells/mm^3) at diagnosis, patients that needed to change antifungal therapy because of toxicity of first-line regimen and disseminated disease. Being an adult appeared to be a protector factor.

Discussion

A substantial increase in the number of patients at risk of developing IA in the last 20 years has been observed, especially in HSCT patients. This condition appears to be a major direct or contributory cause of death. The incidence of IA varies widely from center to center; while within centers, the disease tends to occur sporadically [5,6]. Previous studies related to IA mainly focused on clinical and therapeutic aspects [7,8]; there are few publications on local prevalence of this disease, or those that investigate contributing environmental factors for its development, except during outbreaks [9]. We documented 2.2 cases per year or 3.0 cases of proven and probable cases per 100 HSTC transplants in a 15-bed bone marrow transplant unit that makes a mean of 70 transplants per year.

Table 1. Demographic and clinical characteristics of 24 allogeneic transplant recipients with invasive aspergillosis.

PN	Age	Gender	BD	Font	Species	Site Evolvement
1	1	M	AML	UC	*Aspergillus niger*	Sinuses [2]
2	2	M	WA	UC	*Aspergillus* sp.	Sinuses [2]
3	6	F	AA	BM	*A. fumigattus*	Sinuses [1] Cerebral [3]
4	6	M	FA	UC	*Aspergillus* sp.	Pulmonary [2]
5	7	M	ALL	UC	*Aspergillus* sp.	Pulmonary [2]
6	7	M	AA	BM	*A. flavus*	Pulmonary [1] Cerebral [3]
7	8	M	AA	BM	*Aspergillus* sp.	Pulmonary [2]
8	9	M	AA	BM	*Aspergillus* sp.	Cerebral [3] Pulmonary [2] Sinuses[3]
9	11	F	AA	UC	*Aspergillus* sp.	Pulmonary [2]
10	14	M	FA	UC	*A. fumigattus*	Pulmonary [2]
11	15	F	AA	BM	*A. fumigattus*	Pulmonary [2] Sinuses [3] Cerebral [3]
12	15	M	CML	BM	*Aspergillus* sp.	Sinuses [1] Pulmonary [3]
13	19	M	AA	BM	*Aspergillus* sp.	Sinuses [1] Skin[3]
14	20	F	AA	BM	*A. fumigattus*	Skin [1] Pulmonary [3]
15	24	F	AA	BM	*Aspergillus* sp.	Pulmonary [2]
16	32	F	AML	BM	*A. niger*	Pulmonary [2]
17	33	M	AA	BM	*Aspergillus* sp.	Cerebral [1] Pulmonary [2] Sinuses [2] Skin [3]
18	33	M	BL	BM	*Aspergillus* sp.	Pulmonary [2]
19	35	M	CML	BM	*Aspergillus* sp.	Skin [1] Cerebral [3] Pulmonary [3]
20	37	M	MD	BM	*A. fumigattus*	Skin [1] Pulmonary [3]
21	38	F	NHL	UC	*A. fumigattus*	Skin [1] Cerebral [3] Pulmonary [3] Sinuses [3]
22	38	M	CML	BM	*Aspergillus* sp.	Pulmonary [2] Sinuses [3]
23	40	M	AA	BM	*Aspergillus* sp.	Skin [1] Pulmonary[3]
24	42	M	CML	BM	*A. fumigattus*	Renal [1]

PN: Patient Number; BD: Baseline Disease; M: Male; F: Female; AML: Acute Myeloid Leukemia; CML: Chronic Myeloid Leukemia ALL: Acute Lymphocytic Leukemia; AA: Aplastic Anemia; FA: Fanconi Anemia; MD: Myelodisplasia; BL: Biphenotipic Leukemia; WA: Wiscott Aldrich; NHL: Non-Hodgkin's Lymphoma; 1: proven disease; 2: probable disease; 3: possible disease.

Table 2. Frequency of specific site involvement in adult and pediatric allogeneic transplant recipients.

Site	Pediatric (N = 12)	Adult (N= 12)
Sinuses	5 (41.7%)	4 (33.3%)
Pulmonary	9 (79%)	10 (83.3%)
Cutaneous	0	7 (58%)
Cerebral	3 (25%)	7 (33.3%)

Table 3. Radiological findings in patients with pulmonary invasive aspergillosis.

Finding	Pediatric (N= 9)	Adult (N= 7)
Nodule without halo sign	3 (33.3%)	4 (57.1%)
Nodule with halo sign	2 (22.2%)	2 (28.6%)
Pneumothorax	1 (11.1%)	0.0
Unspecific infiltrate	3 (33.3%)	1 (14.3%)

Table 4. Multiple analyses using the Cox model for risk factors for death of hematopoietic stem cell transplant patients infected with invasive aspergillosis.

Variable	Risk rate	p value
Adult	0.45	0.40
Disseminated disease	2.72	0.16
Neutrophils <100 cells/mm^3	4.96	0.11
Changing initial AF due to toxicity	4.27	0.16
CTC > 3 weeks and continue using	1.93	0.49

CTC: corticosteroid; AF: antifungal.

A logistic regression model established a significant relationship between structural changes in the BMT Unit and the occurrence of IA cases ($p = 0.034$, RR = 2.47). It is well known that appropriate environmental control measures are important in preventing or arresting an outbreak of nosocomial aspergillosis, especially because the most frequent settings of nosocomial invasive aspergillosis involve granulocytopenic patients following respiratory infection from an airborne source, associated with hospital construction activity or contaminated ventilation systems [9].

Figure 1. Occurrence of invasive aspergillosis cases according to the number of hematopoietic stem cell transplants realized per year in the bone marrow transplant unit.

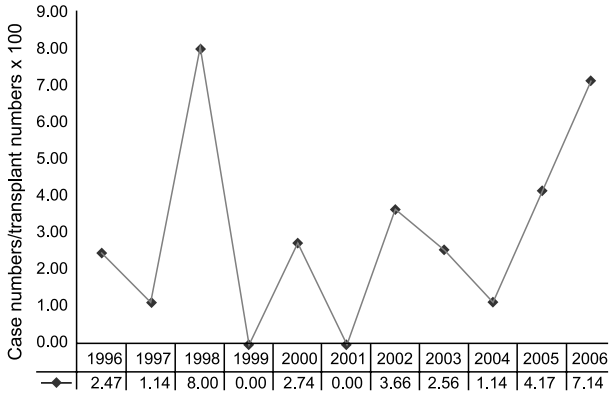


Figure 2. Species distribution in 24 cases of invasive *Aspergillus* infection.

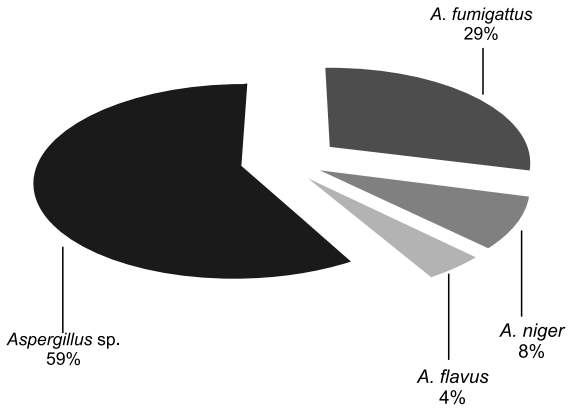
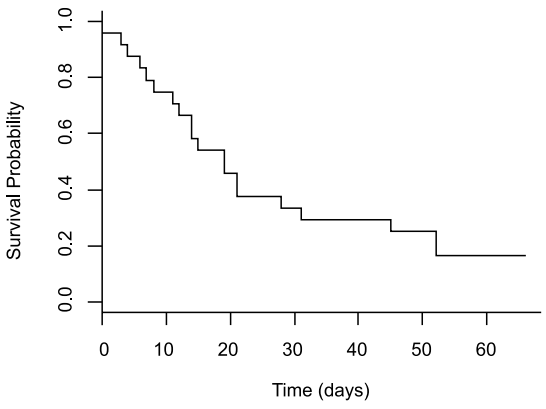


Figure 3. Kaplan-Meier Survival Probability curve in 24 invasive aspergillosis cases.



High-Efficiency Particulate Air (HEPA) filtration has been recommended to avoid fungal infection in highly immunosuppressed patients. A systematic review of this technique was published in 2006 by Eckmanns et al. [10]; they found no significant advantages of HEPA filtration for reducing mortality of hematological malignancy patients who have severe neutropenia. The use of this filter to protect patients with bone marrow transplants appeared to be beneficial, but no definite conclusion could be drawn from the data. The BMT unit in our study had an HEPA filter; however, it did not always operate and because these periods were not well documented, we were not able to analyze this factor.

Because *Aspergillus* conidia are typically airborne, the most common site of invasive aspergillosis in immunocompromised patients is the lungs. According to a review published by Denning in 1998 [6], the lungs are usually the most affected site in allogeneic bone marrow or peripheral stem cell transplants (80%-90%); the other involved sites are the sinuses (5%-10%) and the central nervous system (5%-20%). Therefore, as we expected, most of our patients had pulmonary (80%) or sinus involvement (37%).

Among other sites, the brain is involved in 33.3%, according to the literature. Skin involvement was observed in 29% of our patients (7/24), all in the adult group. This finding was the only difference in clinical manifestation between the adult and pediatric groups (p=0.01). In a study conducted by Abbasi et al. in 1999 [11], 20% (13/66) of the children who had cancer (mostly hematological malignancies), with documented invasive aspergillosis, had skin involvement of the latter. They reasoned that since armboards would be used more frequently for children than for adults, to stabilize intravenous infusion sites, armboard use could have contributed to this problem. The lesion can develop from direct inoculation of the fungus from an environmental source as a result of trauma or may result from movement of fungus to areas of stasis during fungemia. We did not document trauma as an entry mechanism in cutaneous cases, and we did not observe dissemination via the circulatory system to the skin in the pediatric group, possibly because of the small number of patients.

When we examined the radiological pulmonary presentation, nodules with or without a halo sign on computed tomography (CT) scan were the most common finding. According to Greene [12], at baseline, the macronodule is by far the most common CT finding, and one or more are present in 90% of the patients. On the other hand, macronodules exhibiting halo signs have been identified in almost two-thirds of patients with confirmed diagnosis of invasive pulmonary aspergillosis (IPA). Also, consolidations occur in about one quarter of the patients, and air crescent signs are found in about one tenth. The halo sign consists of an opaque nodule with a surrounding ground-glass area and is widely adopted as a specific early indicator of probable IPA in patients who are at high risk of such infections [13]. In our study, the halo sign was identified in 25% of the patients that had a CT scan (4/16). Gasparetto et al. [14], in a review of 12 cases, identified

the halo sign in 5 of 12 patients (41%); bilateral and asymmetric distribution of lesions was the dominant pattern (66%). When we compared the pediatric and adult groups, we did not find significant differences (p=0.63, chi-square test with Yates correction).

Aspergillus fumigatus was the most common species identified; this fact is closely related to the most common site affected, the respiratory tract. This species has some characteristics that contribute to this pathogenicity, such as the very small conidia size (3-5μ), which allows the conidia to penetrate deeply into the lungs. Also, among all pathogenic species, *A. fumigatus*, in particular, binds laminin and fibrinogen more efficiently than other species, presumably allowing greater adhesion in the airways before invasion [11, 15].

For many years, Amphotericin B was the only choice for treatment of IA, but since 2002 Voriconazole has become the first line therapy for this condition [16]. However, for economic reasons, Amphotericin B was the most frequently prescribed antifungal drug in our sample, though it had to be discontinued in almost 40% of the patients because of toxicity or treatment failure. A recently published 400-case review study found that Voriconazole was associated with reduced risk of IA-related death among patients who did not have severe underlying organ impairment [8]. In our sample, because of the small number of patients who used Voriconazole in the first treatment regimen, it was not possible to analyze this aspect; however, a new study is being conducted to evaluate this data.

Clearly, the observed mortality rate of 83% in our study is extremely high. Several factors could be implicated in this poor outcome. This kind of patient could have significant immune compromise from cumulative insults, including underlying disease, previous cytotoxic chemotherapies and conditioning regimens, and prophylaxis and therapy for graft-*versus*-host disease. The diagnosis is often made late in the course of infection, when the fungal burden is high and antifungal treatment is less likely to be efficacious. For this reason, we examined many variables in this group of patients, in an attempt to establish some prognostic factors. However, because of the small number of patients in this sample, we did not find any significant trends. We did observe that some factors, such as corticosteroid use in the last three weeks before diagnosis and its maintenance during invasive aspergillosis treatment, neutropenia (<100 cells/mm^3) at diagnosis, the need to change antifungal therapy because of toxicity of the first line regimen that was used, and disseminated disease, had a tendency to be correlated with poor prognosis. Some authors have reported that HLA mismatch, elevated creatinine and bilirrubin levels, treatment with corticosteroids at 2 mg/kg per day and disseminated and late IA are independently associated with higher risks of mortality [8]. It is known that creatinine and bilirrubin levels are closely related to complications that this kind of patient can present, such as GVHD or renal failure due to drug toxicity, for example. We did not check these parameters in our study but we included associated conditions that clearly could contribute to death.

In conclusion, it is important to be aware of local factors in a BMT unit that could be controlled in order to prevent IA. Also, physicians should pay attention to the risk factors for developing invasive fungal infections and try to reduce or eliminate them. However, once this invasive disease begins, appropriate diagnostic and treatment measures must be implemented as soon as possible in order to avoid the high mortality associated with this condition.

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