Distribution of filamentous fungi causing invasive fungal disease at the Haematological Unit, Hospital de Clínicas de Porto Alegre, Brazil

ABSTRACT

Very limited data are available in the literature to elucidate the aetiology of invasive mould infections in Latin America. Here we report that *Aspergillus* species caused only half of such cases in a cohort study conducted over 21 months in a university hospital in Porto Alegre, Southern Brazil. *Fusarium* spp. were the second most prevalent moulds (20.7%), followed by Zygomycetes (13.8%). The importance of obtaining local epidemiological data for adequately guiding empirical antifungal therapy is reinforced.

Keywords: *Aspergillus*, *Fusarium*, mold infections, epidemiology, zygomycosis.

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INTRODUCTION

Several authors have reported an increasing incidence of invasive fungal diseases (IFDs), especially in patients with haematological malignancies and in those undergoing haematological stem cell transplantation (HSCT).[1-6] Since these are difficult to diagnose conditions, with an associated high mortality rate, empirical antifungal therapy is widely used for patients with a suspected IFD. However, the effectiveness of any empirical treatment depends on knowledge of causative agents. *Candida* species are by far the most common fungi causing IFDs in humans.[7] However, many centres over the past decade have reported a marked reduction in the incidence of *Candida* bloodstream infections as a result of fluconazole prophylaxis.[8] In contrast, there has been an increment in the incidence of invasive mould infections,[9,10] particularly due to species of *Aspergillus*, *Fusarium*, and Zygomycetes.[2,3,9,10]

The purpose of this study was to describe the fungal agents isolated from patients admmitted to a haematological unit in Brazil. All cases included in this cohort occurred before the installation of High-Efficiency Particulate Air (HEPA) filtration in the haematological unit.

MATERIAL AND METHODS

This was a retrospective cohort study conducted to assess the distribution of filamentous fungi causing IFD in patients with haematological malignancies and those who had undergone HSCT. The study was performed at the Hospital de Clínicas de Porto Alegre (HCPA), a 750-bed tertiary-care university hospital located in Porto Alegre, Southern Brazil. The files from the Mycology Laboratory were hand searched from February 2004 to December 2006, in an attempt to select for cases of IFD caused by moulds. Medical records from these patients were reviewed. Patients with endemic mycoses or yeast infections were not included in the study, as well as those in whom the agent was not identified in culture. All IFDs were classified according to the revised EORTC/MSG consensus criteria,[11] and only cases of proven/probable infections were studied.

Standard mycological procedures were performed for the analysis of clinical samples, including microscopy and culture. Descriptive statistics were used to summarise the data. Statistical analysis was performed with SPSS 16.0. Normally distributed quantitative data were analysed with Student's

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t-test, while the non-parametric Mann-Whitney test was used for variables with asymmetric distribution. Categorical data were evaluated with chi-square or Fisher's exact test. Survival analyses were performed 6 weeks after IFD was diagnosed using Kaplan-Meyer analysis and the log rank test. P-values of < 0.05 were considered statistically significant. This article is part of a more comprehensive project submitted to and approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (GPPG 07068).

During the study period, HSCT patients received antifungal prophylaxis with fluconazole at 200 mg daily. Patients on high doses of chemotherapy for acute myeloid leukaemia, acute lymphoblastic leukaemia, or high risk myelodisplasic syndrome received itraconazole capsules (200 mg once a day with an acid drink).

RESULTS

A total of 29 patients with IFD caused by moulds were identified. Patients were mostly female (n = 15) and mean age was 31.7 year-old. Sixteen patients (55.2%) had been submitted to HSCT, including allogeneic (n = 12; 41.4%) and autologous (n = 4; 13.8%) recipients. Only 24.1% (n = 7) of patients were not neutropenic at the time of IFD diagnosis.

As shown in Table 1, only 58.6% of cases were caused by *Aspergillus* species (n = 17). *Fusarium* spp. were the second most prevalent filamentous fungi (n = 6; 20.7%), followed by Zygomycetes (n = 4; 13.8%). Other fungi included species of *Curvularia* and *Trichoderma* (one case each; 3.4%). Most fungal infections were classified as proven IFD (n = 20, 69.0%) and 9 cases were probable (31.0%). Most IFDs occurred in the context of febrile neutropenia (n = 22; 75.9%). Clinical samples were paranasal sinus biopsy (n = 15; 51.7%), respiratory tract specimen (bronchoalveolar lavage/sputum) (n = 9; 31%), blood culture (n = 2; 6.9%), skin biopsy (n = 2; 6.9%), and lung biopsy (n = 1; 3.4%). *Aspergillus* species were recovered in 60% of cases of invasive fungal sinusitis (9/15), followed in frequency by Zygomycetes (n = 3; 20%), *Fusarium* species (n = 2; 13.3%), and *Curvularia* spp. (n = 1; 6.7%). Surgical debridement of paranasal sinuses was performed for most patients with invasive fungal rhinosinusitis (93.3%).

Overall mortality 6 weeks after the diagnosis of IFD was 41.9%. Similar mortality rates were observed for patients with invasive aspergillosis (35.3%), fusariosis (50.0%), and zygomycosis (50.0%) (p = 0.283). Mortality was 50.6% for patients with proven IFD and 21.4% for those with probable IFD (p = 0.127).

DISCUSSION

The medical importance of IFDs has markedly increased in the last decades. The causes for that are multifactorial. In special, there has been an increment in the proportion of individuals at risk as a result of immunosuppression, particularly amongst haematological patients. In addition, aggressive medical interventions have allowed critically ill patients to survive longer, increasing their overall risk to acquire fungal infections.[5,12]

In the present study, we showed the distribution of filamentous fungi causing invasive fungal diseases at the haematology unit of HCPA, a reference medical centre in Southern Brazil. Roughly, half of infections were caused by *Aspergillus* species, especially *A. fumigatus* and *A. flavus* (the latter usually in association with invasive fungal rhinosinusitis). A large proportion (41.4%) of IFDs was associated with fungi other than Aspergilli. *Fusarium* species caused 20.7% of invasive mould infections, followed in frequency by Zygomycetes (13.8%). *Fusarium* species are known to cause a broad spectrum of infections in humans, including superficial, locally invasive and disseminated infections, particularly in immunocompromised patients with prolonged neutropenia.[13] In addition, *Fusarium* spp. are highly resistant to antifungal drugs.[14] Zygomycosis, which seems to be an emerging problem for many medical centres,[4,6] is also a very difficult to treat infection, usually requiring a combination of surgical debridement and high dose treatment with amphotericin B. Because voriconazole is inactive against the Zygomycetes, the emergence of these fungi might complicate voriconazole use in the empirical setting.

The emergence of mould infections have been documented in several studies. Similarly to our findings, Neofytos *et al.*[5] documented that most (59.2%) IFDs in HSCT recipients were caused by *Aspergillus* species, followed in frequency by *Candida* spp. (24.8%), Zygomycetes (7.2%), and other moulds (6.8%). In the study by Horn *et al.*[20] *Aspergillus* spp. caused 14.8% of all IFDs in a cohort of 1710 patients enrolled in 22 sites in the United States (PATH Alliance study). A small number of IFDs caused by other fungi was also observed, including species of *Paecilomyces*, *Scedosporium*, *Alternaria*, *Acremonium*, *Fusarium*, and Zygomycetes. Garcia-Vidal *et al.*[21] observed that *Aspergillus* species caused 88% of invasive mould infections following allogenic HSCT. Several authors have stressed that IFDs caused by different moulds may require specific strategies in terms of diagnosis, treatment, and prophylaxis. Obtaining local epidemiological data is therefore a crucial step in organising health care units and planning therapeutic interventions.

Although numerous studies in the last 10 years have documented the epidemiology of Candidaemia in Brazil,[15-17] little information is available on invasive aspergillosis (IA) or other invasive mould diseases. A previous study performed in Porto Alegre demonstrated that 82% of cases of IA were not diagnosed before autopsy.[18] This reality seems similar in several other countries: it is estimated that 20-80% of IA cases are not diagnosed before death, frequently because of lack of clinical suspicion.[9,19] Since the frequency of autopsy is at a decrease in the western world, physicians risk ending

Table 1. Patient demographic data and main agents of invasive fungal disease caused by moulds

Patient	Sex	Age	Underlying disease	HSCT	Neutropenia at diagnosis	Sites of infection	Agent	EORTC/ MSG criteria	Survival status (at 6 weeks)
1	M	32	ALL	Allogeneic	Yes	Paranasal sinuses	*A. fumigatus*	Proven	Died
2	F	17	HD	Autologous	No	Lungs	*A. fumigatus*	Probable	Survived
3	M	33	ALL	Allogeneic	Yes	Paranasal sinuses	*A. fumigatus*	Proven	Died
4	F	52	MM	-	Yes	Paranasal sinuses	*A. flavus*	Proven	Died
5	F	15	AML	Allogeneic	No	Lungs	*A. fumigatus*	Probable	Survived
6	F	37	AML	Allogeneic	No	Paranasal sinuses	*A. fumigatus*	Probable	Survived
7	M	18	ALL	Allogeneic	Yes	Skin	*Fusarium* sp.	Proven	Died
8	M	21	ALL	Allogeneic	Yes	Paranasal sinuses	*Fusarium* sp.	Proven	Died
9	F	81	MM	-	Yes	Bloodstream	*Fusarium* sp.	Proven	Survived
10	M	40	AML	-	Yes	Lungs	*A. fumigatus*	Proven	Died
11	M	24	HD	Autologous	No	Paranasal sinuses	*Absidia* sp.	Proven	Died
12	M	25	AML	-	Yes	Paranasal sinuses	*Aspergillus* sp.	Proven	Died
13	M	45	AML	Allogeneic	Yes	Paranasal sinuses	*Aspergillus* sp.	Proven	Died
14	F	11	ALL	-	Yes	Paranasal sinuses	*A. flavus*	Proven	Survived
15	M	39	AML	-	Yes	Paranasal sinuses	*A. fumigatus*	Proven	Died
16	M	35	ALL	-	Yes	Lungs	*A. fumigatus*	Probable	Died
17	F	15	ALL	-	Yes	Skin	*Fusarium* sp.	Proven	Died
18	F	38	Aplasia	-	No	Paranasal sinuses	*Fusarium* sp.	Proven	Died
19	F	44	CML	Allogeneic	Yes	Paranasal sinuses	*Rhizopus* sp.	Proven	Died
20	M	5	Aplasia	-	Yes	Paranasal sinuses	*Mucor* sp.	Proven	Survived
21	M	63	ALL	-	Yes	Lungs	*A. fumigatus*	Probable	Survived
22	F	31	AML	-	Yes	Paranasal sinuses	*A. niger*	Proven	Died
23	M	46	CLL	Allogeneic	Yes	Lungs	*Trichoderma* sp.	Probable	Died
24	F	57	NHL	Autologous	Yes	Bloodstream	*Fusarium* sp.	Proven	Died
25	F	26	Aplasia	Allogeneic	No	Lungs	*Rhizopus* sp.	Probable	Died
26	M	40	AML	Autologous	No	Lungs	*A. fumigatus*	Probable	Survived
27	F	29	ALL	-	Yes	Paranasal sinuses	*Curvularia* sp.	Proven	Died
28	F	40	AML	Allogeneic	Yes	Lungs	*A. fumigatus*	Proven	Died
29	F	15	AML	Allogeneic	Yes	Lungs	*A. fumigatus*	Probable	Survived

ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; BAL, bronchoalveolar lavage; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; EORTC/MSG: European Organization for Treatment of Cancer/Mycoses Study Group; F, female; HD, Hodgkin disease; HSCT, haematological stem cell transplantation; M, male; MM, multiple myeloma; NHL, non-Hodgkin lymphoma.

up with an incorrect perception that invasive mould infections rarely occur in the clinical practice.

To the best of our knowledge, only two cohort studies have so far documented the epidemiology of IFD in Brazil. The distribution of *Aspergillus* species causing IFD at Hospital de Clínicas da Universidade Federal do Paraná – another referral centre for allogeneic HSCT in Southern Brazil – was recently published.[12] A total of 24 cases of proven/probable IA were detected over a 10-year period in that investigation, including 11 proven IA cases. The reported incidence of 3% was probably underestimated, since galactomannan testing was not available at the time of the study. Overall mortality was 70% after 30 days of follow-up. Non-*Aspergillus* moulds were recovered from 163 additional

patients but no details were provided. Partial results from a multicenter survey study performed in Brazil were recently presented in abstract form.[22] Considering patients with proven/probable infections only, the incidence of IFD in HSCT recipients and patients with acute myeloid leukaemia and myelodysplastic syndrome was 6.7% in that investigation. Twenty-five patients (out of 460) had proven IFD. Also, this may also be underestimated, since serum galactomannan testing was not routinely performed. Similarly to our findings, fusariosis was the second leading cause of invasive mould disease, being more common than zygomycosis.

One remarkable finding of our study was the elevated proportion of proven IFD cases (69.0%), in comparison to probable cases. This was achieved due to an aggressive policy of computed tomography imaging of the paranasal sinususes in patients with febrile neutropenia, which frequently triggers the performance of paranasal sinuses biopsies. Accordingly, approximately 50% of patients in the current series had invasive fungal rhinosinusitis. The diagnosis of these conditions is actually dependent on demonstration of tissue invasion by fungi, and the paranasal sinuses are more easily reached for invasive medical procedures than the lungs.

CONCLUSION

In conclusion, here we demonstrated that 41.4% of invasive mould infections were associated with fungi other than those belonging to the *Aspergillus* genus. *Fusarium* spp. was the second most frequently recovered fungi. Of concern is the fact that Zygomycetes were the second aetiology of invasive rhinosinusitis in this study. Epidemiological surveillance is necessary in order to monitor the dynamics of these infections.

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