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Major Article

Invasive fungal infection in patients with hematologic disorders in a Brazilian tertiary care hospital

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Abstract

Introduction: Invasive fungal infections (IFIs) are an important complication in immunocompromised individuals, particularly neutropenic patients with hematological malignancies. In this study, we aimed to verify the epidemiology and diagnosis of IFIs in patients with hematologic problems at a tertiary hospital in *Goiânia*-GO, Brazil. **Methods:** Data from 117 patients, involving 19 cases of IFIs, were collected. The collected data included diagnosis methods, demographics, clinical characteristics, and *in vitro* susceptibility to different antifungal agents. Among the 19 cases, 12 were classified as proven IFI and 7 as probable invasive aspergillosis with detection of galactomannan in blood and presence of lung infiltrates in radiographic images. Logistic regression analysis showed that the proven and probable IFIs were associated with increased risk of death. Statistical analysis demonstrated that age, sex, and underlying disease were not independently associated with risk of death in IFI patients. **Results:** Most bloodstream isolates of *Candida* spp. exhibited low minimum inhibitory concentrations (MICs) to all antifungal agents tested. Voriconazole and amphotericin had the lowest MICs for *Aspergillus* spp. and *Fusarium* spp., but *Fusarium* spp. showed the least susceptibility to all antifungals tested. Amphotericin B, fluconazole, and itraconazole were found to be inactive *in vitro* against *Acremonium kiliense*; but this fungus was sensitive to voriconazole. **Conclusions:** Considering the high number of IFI cases, with crude mortality rate of 6%, we could conclude that IFIs remain a common infection in patients with hematological malignancies and underdiagnosed ante mortem. Thus, IFIs should be monitored closely.

Keywords: Invasive fungal infections. Hematologic disease. *In vitro* susceptibility.

INTRODUCTION

Invasive fungal infections (IFIs) are an important complication in immunocompromised individuals, particularly in neutropenic patients with hematological malignancies[1]. Over the last decades, this group of patients has presented severe infections, which has been the cause of high mortality rate[2,3].

Invasive aspergillosis (IA) and invasive candidiasis are the main invasive fungal diseases associated with bloodstream infections. Although invasive yeasts, such as *Candida* spp., and molds, such as *Aspergillus* spp., are the predominant pathogens of IFIs, other uncommon and difficult-to-treat molds, such as Mucorales, *Fusarium* spp., and phaeohyphomycetes, have emerged in patients with hematological malignancies[4,5]. The clinical presentation of IFIs is nonspecific, and the diagnostic criteria are poorly defined in critically ill populations, rendering the diagnosis of the disease challenging. In this study, we aimed to verify the epidemiology and diagnosis of IFIs in patients with hematologic problems at a tertiary hospital in *Goiânia*-GO, Brazil.

METHODS

Patients and study setting

This study assessed the prevalence (16.2%) of IFIs among 117 patients with hematologic malignancies in Hospital Araújo Jorge/Associação de Combate ao Câncer em Goiás (ACCG) of *Goiânia-GO* from December 2009 to July 2011. The setting was a 170-bed tertiary care hospital with medical and surgical wards, intensive care units, and an emergency department. The following variables were recorded and analyzed: sex, age, and underlying disease. This study was approved by the Ethics Committee of ACCG. Hospital prophylactic protocols included antifungal drugs such as fluconazole or itraconazole for every patient.

The only cases included in the data analysis were those classified as proven or probable according to the European Organization for Research and Treatment of Cancer/Mycosis Study Group[6] (EORTC). IFIs were defined as proven when

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cultures from blood were positive for fungi (at least twice at different times). IFIs were defined as probable IA when new lung infiltrates were evidenced on high-resolution chest computed tomography scans and galactomannan (GM) was detected in serum with the Platelia *Aspergillus* enzyme immunoassay.

Blood cultures were performed to detect fungal species by using a biphasic brain-heart infusion (BHI) medium. Five milliliters of blood were inoculated into the culture bottles and incubated in an upright position for 30 days at 25°C. Cultures were examined daily and subcultured onto Sabouraud dextrose agar slant medium for identification. The isolated filamentous fungi were identified with standard morphological characteristics of their conidiophores and conidia. For species identification, a slide culture method was used (microculture) in potato agar and the culture was incubated at 25°C for 5 days. Confirmation of fungi as etiologic agent was accomplished by reproducible growth from the blood samples at different times by repeated culture attempts. *Candida* isolates were identified by microscopic morphology on cornmeal tween 80 agar and by biochemical methods using the API 32C AUX system (Biomerieux, Marcy l'Etoile, France).

Antifungal susceptibility tests to four antifungal agents, namely amphotericin B, itraconazole, voriconazole, and fluconazole, were performed using the broth microdilution assay. These tests were carried out according to the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) M27-A3, M38-A2, and M27-S4 documents[7-9].

Serum GM antigen levels were measured weekly using enzyme linked immunosorbent assay (ELISA) with the Platelia *Aspergillus* test (Bio-Rad, Paris, France). GM testing was conducted in accordance with the manufacturer's recommendation for testing serum samples. A cutoff optical density index of 0.5ng/mL or higher was considered positive for GM in serum in two sequential samples[1,10].

Statistical analysis

All statistical analyses were performed with SPSS® software (version 16 for Windows). Univariate analysis, which was determined using chi-square (χ^2) analysis, identified variables predicting mortality. The following variables were recorded and analyzed: sex, age, and underlying disease. The significance level was set at 5%. Sensitivity and specificity were reported for GM testing.

RESULTS

The mean age of the 117 patients was 35.7 years, with a range of 21 to 60 years. Of these, 52.1% were male and 24.7% had acute myeloid leukemia (**Table 1**). Ninety percent of the patients were severely neutropenic at the time of symptom

TABLE 1

Analysis of characteristics and risk factors of 117 patients with hematopoietic stem cell transplant for invasive fungal infections (proven/probable).

		Proven*/probable**		**Proven***				
		***Aspergillus* spp.**		***Candida* spp.**		**other molds*****		
Total		**survival**	**death**	**survival**	**death**	**survival**	**death**	**p-value**
Age (years)								0.48
0-20	26	2	-	-	2	-	-	
21-40	39	5	2	-	-	2	1	
41-60	38	-	1	3	-	-	-	
>60	14	-	-	-	1	-	-	
Sex								1.00
male	61	3	3	2	2	-	-	
female	56	2	2	1	1	-	-	
Underlying diseases***								0.89
AML	29	2	1	1	2	2	-	
CML	12	1	-	-	-	-	-	
ALL	24	2	1	-	1	-	-	
LH	15	1	-	-	-	-	-	
NHL	19	-	1	1	-	-	-	
MM	14	1	-	1	-	-	1	
AA	2	-	-	-	-	-		
MDS	1	-	-	-	-	-	-	
HPN	1	-	-	-	-	-	-	

AML: acute myeloid leukemia; **CML:** chronic myeloid leukemia; **ALL:** acute lymphoid leukemia; **LH:** Hodgkin's lymphoma; **NHL:** non-Hodgkin lymphoma; **MM:** multiple myeloma; **AA:** aplastic anemia; **MDS:** myelodysplastic syndrome; **HPN:** paroxysmal nocturnal hemoglobinuria. ***Proven:** positive culture for fungi. ****Probable:** clinical illness consistent with IFI with supporting radiographic image and detection of galactomannan in serum. *****Other molds:** *Fusarium* spp. (2 cases), *Acremonium kiliense* (1 case).

onset. IFIs were identified as proven in 12 (10.2%) patients, defined by growth of *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and *Acremonium kiliense* on BHI medium. Among the culture-documented infections, Aspergillus *fumigatus* accounted for two samples, *A. flavus* for one sample, and *C. albicans* was responsible for the majority (3 cases) of yeast invasive infections, followed by *C. parapsilosis* and *C. tropicalis* (**Table 2**). Probable IA was found in seven patients (6%), with detection of GM in blood and presence of lung infiltrates in radiographic images (**Figure 1**). All patients diagnosed with IFIs had used fluconazole as the prophylactic agent. The median time of IFI diagnosis was 45 days (range 7-84 days) since hospitalization. Out of the 10 patients with proven and probable IA, nine (90%) exhibited at least one symptom that was suggestive of pulmonary disease and one had disseminated infection. Disseminated infection with *Fusarium* spp. occurred from skin as a portal of entry, whereas *A. kiliense* fungemia had involvement of the lungs.

The overall mortality rate for hematologic disorders with proven/probable IFI was 6% (7/117). In the analysis of underlying diseases alone or together, the p values were >0.05. Demographic characteristics, underlying disease, and outcomes of patients associated with IFIs found in this study are outlined in **Table 1**.

As shown in **Table 2**, most *Candida* bloodstream isolates exhibited low minimum inhibitory concentrations (MICs) to all antifungal agents tested. Amphotericin B had the lowest MICs ranging from 0.125 to 0.5µg/mL for all species, while resistance to all azoles tested was observed in one strain of *C. albicans*. The MICs for filamentous fungi ranged markedly among antifungal agents and organisms. Voriconazole and amphotericin B had the lowest MICs against *Aspergillus* spp. However, *Fusarium* spp. were the least susceptible against all antifungals tested, inhibited only at high concentrations of itraconazole and fluconazole. Amphotericin B, fluconazole, and itraconazole were found to be inactive *in vitro* against *A. kiliense*, but *A. kiliense* was sensitive to voriconazole.

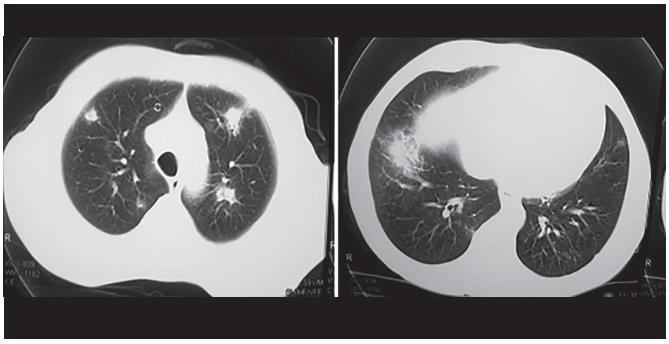

FIGURE 1 - Chest computed tomography: pulmonary infiltrates scattered throughout the parenchyma of both lungs evidenced in probable cases of invasive aspergillosis.

Statistical analysis and diagnostic performance of serum galactomannan assay

Statistical analysis revealed that age, sex, and underlying disease were not independently associated with risk for IFI. Hematologic diseases were associated with increased risk for IFI by molds ($p<0.01$). Seven (6%) died among the IFI patients, and analysis identified the association of IFIs with increased risk of death ($p<0.01$) in these patients.

The performance of the GM test was evaluated with proven and probable IA. At the index cutoff value of ≥0.5 ng/mL, the ELISA tests had a sensitivity of 90% (9 of 10) and a specificity of 85.8% (94/107).

TABLE 2

Antifungal susceptibility of bloodstream isolates from patients with hematologic malignances.

	MIC (µg/mL)			
Species (n)	**Fluconazole**	**Voriconazole**	**Amphotericin B**	**Itraconazole**
Candida parapsilosis (2)	0.125	<0.003	0.125	0.5
	2.0	0.25	0.5	1.0
Candida albicans (3)	0.5	<0.003	0.25	<0.003
	0.5	0.19	0.5	0.125
	>64	16	0.5	>16
Candida tropicalis (1)	8	0.006	0.5	>0.003
Aspegillus fumigatus (2)	16	0.25	2	0.12
	32	0.5	4	>16
A spegillus flavus (1)	>64	0.5	2	0.25
Fusarium spp. (2)	16	4	2	8
	32	4	2	8
Acremonium kiliense (1)	>64	0.125	>16	>16

MIC: minimum inhibitory concentration.

DISCUSSION

Invasive mycoses are life-threatening opportunistic infections and have emerged as a major cause of morbidity and mortality in critically ill patients[11]. This study is the first to verify the prevalence of IFIs in hematologic malignancy patients in Goias State (Midwest of Brazil). In our survey, aspergillosis and candidemia were the major IFIs detected in patients with hematologic malignances, but other infections, such as fusariosis and *A. kiliense* fungemia with involvement of the lungs, were observed.This study focused on the epidemiology of fungal diseases, their considerable morbidity in patients with hematologic malignances, and low sensitivity of the culture methods for the detection of *Aspergillus* infections. We observed IFIs in 19 (16.2%) patients with hematologic disorders, with molds present in 13 and yeasts in 6 patients. An increased incidence of IFIs that present hematologic problems was reported by some researchers[12,13]. Singh and Paterson[14] verified that IA is the most frequent IFI; it can occur in 8-15% of patients with hematologic disease. In the present study, *Aspergillus* infections were considered as proven/probable in 10 (8.5%) of 117 hematologic problem patients. The diagnosis of proven IA infection represented a problem in this study. IA diagnosis is a challenge because clinical and radiological signs are nonspecific, and tissue biopsies are invasive and not always a possible means of diagnosis[10]. Among the 10 patients with suspected aspergillosis, infection was proven in only 30% of cases by blood culture and probable according to EORTC criteria in 70%. In these patients, we detected GM antigenemia and lung infiltrates using standard chest radiography and high-resolution computerized tomography.

The impact of early diagnosis leading to appropriate therapy may result in improved prognosis of patients. In this way, the detection of GM, a component of cell wall of *Aspergillus* sp. in body fluids hematogenously released during hyphal growth, becomes a useful aid in IA diagnosis[10,15]. The assay has gained widespread acceptance as a sensitive method to undertake prospective surveillance in risk population[16]. The sensitivity and specificity of GM testing found in our study (with at least two consecutive positive samples) were 90% (9 out of 10) and 85.8% (94/107), respectively, when IA was defined using clinical characteristics and radiological findings suggestive of aspergillosis and even taking into account the positive culture. The Platelia *Aspergillus* GM EIA was a disappointment in one case where the culture was positive. Marr et al.[17] suggested that antifungal therapy decreases the sensitivity of the *Aspergillus* GM enzyme immunoassay on serum samples. Antifungal prophylaxis using fluconazole was performed on all our patients. A high specificity of 99.6% was found by Pinel et al.[18] with positive GM antigenemia in 748 of 751 patients. Several prior studies demonstrated IA as a predictor of poor outcomes[19,20]. Among patients classified as proven/probable IA, we also observed a significant predictor of death by logistic regression analysis ($p<0.01$). This fungal infection occurs in 0.08-15% of patients with hematologic malignancies and presents mortality rates up to 80%[14,21]. Then, we suggest that the Platelia *Aspergillus* GM EIA in patient serum could be useful as a screening tool for the identification of patients at a high risk of developing IA, thus reducing the percentage of deaths.

The real incidence of resistance in *Aspergillus* sp. is relatively unknown. However, azole resistance has been increasingly reported in many other countries since the detection of first azole-resistant isolate in 1997 in the UK[22,23]. In the Netherlands, azole resistance, particularly itraconazole, has an overall prevalence of 5.3%, which ranges from 1.8 to 12.8%[24]. Similarly, itraconazole resistance has been reported in other countries, with rates ranging from 1.7 to 6%[25]. We also verified in our results that one isolate was resistant to itraconazole (**Table 2**). The *in vitro* and *in vivo* correlations of azole resistance are widely documented, with a clear association of resistant *A. fumigatus* strain isolation and lack of patient response to therapy[24].

Although non-*Aspergillus* molds are rarely found with three cases, they remain a problem in patients with hematologic disease. The rare molds (2.5%) diagnosed in our population (*Fusarium* and *Acremonium* from blood) were identified by culture, underlining the importance of cultivating every probable fungal material to achieve a diagnosis. Management of IFIs is a challenge among emerging fungal pathogens and it generally shows poor response to many antifungals[26,27]. The capability of reference method for antifungal susceptibility testing to detect emerging resistance patterns provides useful information to optimize the effectiveness of antifungal therapy. In our study, the investigation of the *in vitro* efficacy of several antifungals against different isolates showed that voriconazole and amphotericin B presented low MIC against *Fusarium* spp. In larger studies of isolates from systemic disease, voriconazole and amphotericin B have been shown to be effective against *Fusarium* spp. and *Aspergillus* spp.[28,29]. Santhanan et al.[30] verified that *Fusarium* spp. show low susceptibility to itraconazole and voriconazole.

Little information is available about the susceptibility of *Acremonium* spp. to antifungals, but they are characteristically resistant to anti-*Candida* agents, such as fluconazole and flucytosine[31]. Although breakpoint data for those organisms are lacking, MICs of amphotericin B are commonly elevated, which suggests the poor activity of this drug. *A. kiliense* seems to be less susceptible to amphotericin B, fluconazole, and itraconazole as supported by our results. Low MICs to voriconazole were found, and success was obtained after clinical treatment with this drug. Finally, voriconazole may be the best therapeutic alternative.

Although yeast infections appear to be decreasing and shift to non-*albicans Candida* spp., probably because of azoles as prophylactic agents, they remain a significant problem in hematologic malignances with poor outcome in candidemia cases[11,32]. The ratio of *C. albicans* to non-*albicans* species found in our study was 1:1 (**Table 2**). All patients were exposed to fluconazole or itraconazole as a prophylactic treatment. Therefore, the obtained results may be due to local epidemiology impact because *C. albicans*, *C. parapsilosis*, and *C. tropicalis* are the most frequently isolated species in Latin America. Moreover, according to several researchers, *C. parapsilosis* is often associated with the presence of intravascular catheters and is not influenced by exposure to fluconazole or other antifungal agents[33-35]. In our study, we found six cases of candidiasis

and three of them were lethal. Bergamasco et al.[36] found that *C. albicans* is the most frequent species, followed by *C. tropicalis* and *C. parapsilosis* in hematologic malignancy patients. They also showed that candidemia is associated with mortality. Our susceptibility data showed one *C. albicans* isolate only, with reduced susceptibility to all azoles (**Table 2**). Cross-resistance between fluconazole and voriconazole has been frequently reported in many species of *Candida*[37,38].

Considering that the crude mortality rate of IFIs was very high (6%) and early therapy may lead to improved prognosis, we suggest that efforts should be expended to reach an early fungal diagnosis in populations with hematologic disease. Similarly, *in vitro* susceptibility can be extremely useful in adjusting therapy against these infections. In summary, IFIs remain a common infection in patients with hematological malignancies being frequently disseminated and underdiagnosed ante mortem. Thus, IFIs should be monitored closely.

Conflicts of interest

The authors declare that there is no conflict of interest.

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