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Reduction of Invasive Fungal Infections Among Cancer Patients With Chemotherapy-Induced Neutropenia After Protective Environment Implementation May Save Costs in a Developing Country: A Quasi-Experimental Study

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Abstract

Background: Invasive fungal infections (IFI) represent a serious threat for severely immunocompromised patients. Infection control interventions, including protective environment (PE) implementation, are essential to reduce IFI incidence, mortality and burden of hospitalization, among high-risk patients. Information about the impact of these strategies in cancer patients with chemotherapy-induced neutropenia (CIN), in developing countries, is insufficient.

Objectives: To assess the impact of PE implementation on IFI incidence, consumption and cost of antifungal treatment, in a general, tertiary teaching hospital, in Southern Brazil.

Patients and Methods: We conducted a quasi-experimental study to evaluate an institutional intervention, in a hospital ward, for patients with CIN, which consisted in renovation of the ward and measures involving air-quality technologies installation, the main one being high efficiency particulate air (HEPA) filters. Simultaneously, infection control routines were implemented. Neutropenic patients, admitted to any other hospital ward, prior to the renovation, were included in the historical control group. The IFI incidence was defined, according to the criteria proposed by the European Organization for Research and Treatment of Cancer. Direct costs of antifungal drugs were recorded, for all neutropenic patients.

Results: A total of 190 and 181 hospital admissions were included in the intervention and control groups, respectively. Total IFI incidence was reduced in the PE group (7.4% vs. 18.2%; P = 0.002) and the same was observed when considering only proven and probable IFI (1.6% vs. 8.3%; P = 0.003). This benefit persisted even after adjusting for antifungal prophylaxis (OR = 0.17; 95% CI = 0.05 – 0.60). We observed a decreasing trend in molds and yeasts IFI incidence, in the intervention group. Although the final cost of antifungal agents was lower, after intervention (78347.37 USD vs. 154176.60 USD), the median cost per admission did not differ between groups (1.00 USD = 1.9 Brazilian Real, in May 2007). Considering all admissions with IFI, the median cost was significantly higher than recorded in admissions without IFI.

Conclusions: This study showed that preventive measures, including PE implementation, reduce IFI incidence in patients with CIN, admitted in a hospital in a developing country. This suggests that those strategies may overcome their costs on the long-term, by saving costs associated with fungal infections.

Keywords: Neutropenia, Mycoses, Aspergillosis, Infection Control, Ventilation, Cost Savings

1. Background

Invasive fungal infections (IFI) are major complications of severe neutropenia, especially in patients with hematologic malignancies (1). The frequency and severity of IFI has increased steadily, over the last decades, mainly due to host defense impairment, secondary to intensive cytotoxic chemotherapies, hematopoietic stem cell transplantation (HSCT), use of corticosteroids and new immunosuppressive agents (2-4). Despite progress in IFI diagnosis and antifungal therapy performance, mortality rates in patients with IFI remain high, ranging from 20% to 57% in patients with candidemia (5, 6) and reaching around 90% in patients with invasive mold infections (7, 8). The options of antifungal effective agents are limited and often results in high healthcare costs (1).

Fungal infections may be acquired from an endogenous or external source, such as the hands, water, food and air (9). The implementation of protective measures is essential to reduce the IFI incidence, among high-risk patients,

mostly caused by *Candida* and *Aspergillus* species (3, 10). Several organizations, such as the Healthcare Infection Control Practices Advisory Committee and the center for disease control and prevention (CDC) developed guidelines to provide infection control recommendations for hospitals, including a set of prevention measures, termed protective environment (PE), directed to HSCT patients (11, 12). The PE refers to isolation practices, designed to decrease the risk of exposure to environmental fungal agents, also including standard and transmission-based precautions (12).

Because inhalation seems to be the main route of mold infection, efficiently controlled ventilation systems remain a major protective strategy (13), although their efficacy was demonstrated only in non-randomized studies (14, 15). A meta-analysis, including randomized and non-randomized studies (16), using high efficiency particulate air (HEPA) filters, with or without other control measures, showed only a modest effect on invasive mold infection that did not reach statistical significance.

Although the implementation of PE is associated with substantial cost to the healthcare system (17), it may, however, result in clinical (15, 18) and economical improvements (15). Information is lacking about the impact of these strategies in reducing IFI in developing countries.

2. Objectives

The objective of our investigation was to assess the impact of PE implementation on IFI incidence, consumption and cost of antifungal drugs among cancer patients with chemotherapy-induced neutropenia (CIN), in a general, tertiary teaching hospital in Southern Brazil.

3. Patients and Methods

We conducted a quasi-experimental study to evaluate an intervention in a hospital ward that consisted in simultaneous preventive measures, involving air-quality technologies installation, including HEPA filters, positive pressure maintenance and infection control routines. Those measures were implemented according to international recommendations, for PE wards (11, 12). Before the intervention, cancer patients were admitted in hospital wards, without special infection control measures. Only allogeneic HSCT patients were allocated in a special ward, with single rooms, restricted access to visitors and extensive hand disinfection practices. The preventive measures did not include air-quality technologies or positive pressure. Because of the high rates of infection and mortality (19), our institution developed a project to implement a PE ward to admit high-risk neutropenic patients.

The air entering the PE ward passes through a set of filters that remove particles $\geq 0.3\ \mu m$ in diameter, with 99.97% efficiency, adjusted to 34 air exchanges per hour. Air pressures, air-supply ducts and filters were checked weekly. The ward comprises 25 beds, allocated in 15 rooms, each one equipped with HEPA filters and hand hygiene facilities (hand operated sinks). The air pressure within each room is maintained positive in comparison to the hallway. To maximize appropriate pressure relationships and HEPA filtration, the doors to individual rooms were kept closed, at all times. The ward has restricted access to visitors. Additional protective conditions are the presence of a monitored anteroom, with hand hygiene facilities, closed by a double door, to maintain positive pressure in the ward and to prevent the access of non-authorized people. Extensive hand disinfection is part of the staff routines and was introduced through educational sessions. Allogeneic HSCT patients remain alone in a room and neutropenic patients that require going outside the unit for diagnostic procedures are required to wear N95 masks. During the renovation, the ward was separated from the other hospital wards, by physical barriers, to prevent dust dissemination.

The renovation started on December 2005 and the ward has been available for patient admission since May 21, 2007. The PE ward admits high-risk neutropenic patients, submitted to high dose chemotherapy, or HSTC. All neutropenic patients [leukocyte counts up to 1.000/mm^3 or absolute neutrophil counts (ANC) up to 500/mm^3] admitted on PE until September 2008 were identified by prospective surveillance and were included in the intervention group. Neutropenic patients, admitted to any hospital ward from January to December 2006, were identified in the institution's patient record system and were included in the historical control group. Patients had their clinical records reviewed, until discharge or death. Subsequent admissions of the same patient were included as separate episodes. Exclusion criteria were age less than 18 years, HIV positivity, neutropenic episodes, secondary to infection, and presence of febrile neutropenia, at the time of admission.

Data regarding the underlying disease, length and severity of neutropenic episodes, central venous catheter (CVC) use, antifungal prophylaxis use until fever, corticosteroid use, graft-versus-host disease (GVHD), IFI, antifungal use, length of hospital stay and mortality were recorded for all included patients.

Severe neutropenia was defined by ANC up to 100/mm3. Neutropenia recovery was defined as the first day on which leukocyte counts and ANC exceeded 1.000/mm^3 and 500/mm^3, respectively, for 2 consecutive days. To identify possible differences in risk characteristics between groups, patients were classified in four risk categories: 1- autologous HSTC in the actual hospital admission; 2- allogeneic HSCT in the actual hospital admission; 3- acute myeloid leukemia (AML); 4- other diseases.

The primary endpoint of the study was IFI incidence during hospitalization, defined according to the consensus criteria, proposed by the European Organization for Research and Treatment of Cancer (2008) and the National Institute of Allergy and Infectious Diseases (NIAID) Mycosis Study Group (20). Briefly, proven disease requires histopathologic or microbiologic documentation of disease, from biopsied tissues. Probable infection is considered if the fungus is identified from cultures of bronchoalveolar lavage (BAL) fluid or sputum, when consistent signs and symptoms are present, whereas pos-

sible infection requires at least one host factor criterion and one microbiologic or clinical criteria from abnormal site, consistent with infection. Invasive mold infection was considered as probable nosocomial, if the first clinical symptoms occurred > 7 days after admission.

The secondary endpoints were consumption and cost of antifungal drugs. The same price was considered for each antifungal formulation, in the periods before and after the renovation, to avoid false comparisons due to antifungal price adjustment, along the 33 months (1.00 USD = 1.9 Brazilian Real, in May 2007).

The sample size needed to detect a reduction of 50% in the risk of IFI, with power of 80% and α error of 5%, was 247 patient hospitalizations, for each group. Data were analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and a $P \leq 0.05$ was considered. Chi-square or exact tests (Fisher's or Monte Carlo's exact tests) were used in the comparison of categorical variables and Student's t test or Mann-Whitney U were applied to compare continuous variables. Adjusted odds ratio (OR) for probable or proven IFI was computed in a logistic regression model, including antifungal prophylaxis. The study was approved by the Institution's Review and Ethics Committee.

4. Results

A total of 190 and 181 hospital admissions were included in the intervention and control groups, corresponding to 128 and 124 patients, respectively. Most patients were admitted only once (71.9% in intervention and 78.2% in control group). Characteristics of the studied sample are shown in Table 1. The rate of multiple myeloma (MM) was higher in the intervention group, while acute lymphoid leukemia (ALL), non-Hodgkin's lymphoma (NHL) and solid tumors rates were significantly lower. There was a trend to higher rates of antifungal prophylaxis use in the PE group (48.9% vs. 39.8 %; P = 0.08).

Table 1. Characteristics of Studied Groups[a,b]

	Protected Environment (n = 190)	Control (n = 181)	P Value
Male	90 ± 47.4	85 ± 47.0	0.94[c]
Age, y	46.3 ± 15.1	46.3 ± 15.0	0.97[d]
Underlying disease			0.001[e]
Acute myeloid leukemia	69 (36.3)	54 (29.8)	
Chronic myeloid leukemia	9 (4.7)	6 (3.3)	
Acute lymphoid leukemia[f]	14 (7.4)	26 (14.4)	
Chronic lymphoid leukemia	6 (3.2)	4 (2.2)	
Multiple myeloma[f]	49 (25.8)	20 (11.0)	
Hodgkin disease	5 (2.6)	10 (5.5)	
Non-Hodgkin's lymphoma[f]	21 (11.1)	33 (18.2)	
Myelodysplastic syndrome	6 (3.2)	5 (2.8)	
Other hematologic malignancies	2 (1.1)	3 (1.7)	
Aplastic anemia	6 (3.2)	6 (3.3)	
Solid tumors[f]	2 (1.1)	11 (6.1)	
Others	1 (0.5)	3 (1.7)	
Risk categories			0.19[c]
Autologous HSCT	50 (26.3)	37 (20.4)	
Allogeneic HSCT	19 (10.0)	18 (9.9)	
Acute myeloid leukemia[g]	63 (33.2)	52 (28.7)	
Other diseases	58 (30.5)	74 (40.9)	
Neutropenia causes			0.14[c]
Chemotherapy	179 (94.2)	163 (90.1)	
Others[h]	11 (5.8)	18 (9.9)	
Length of neutropenia – median days (P25-P75)	12 (8 - 17)	12 (8 - 18)	0.85[i]
Severe neutropenia	170 (89.5)	168 (92.8)	0.26[c]
Central venous catheter use	171 (90.0)	154 (85.1)	0.15[c]
Antifungal prophylaxis use	93 (48.9)	72 (39.8)	0.08[c]
Corticosteroid use	42 (22.1)	49 (27.1)	0.27[c]
GVHD	6 (3.2)	12 (6.6)	0.12[c]

[a] Abbreviations: HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host disease.
[b] Data are presented as mean ± SD or No. (%).
[c] Pearson's Chi-squared test.
[d] Student's t test.
[e] Monte Carlo's exact test.
[f] P < 0.05 (adjusted residual).
[g] not submitted to HSCT in the actual hospital admission.
[h] include underlying disease or other immunosuppressor agents.
[i] Mann-Whitney's test.

Invasive fungal infection occurred in 14 (7.4%) and 33 (18.2%) patient hospitalizations, in the PE and control groups, respectively, with a crude relative risk (RR) of 0.40 (95% CI: 0.22 – 0.73, P = 0.002) (Table 2). Considering only the proven and probable infections, the IFI incidence was significantly reduced in the PE group (1.6% vs. 8.3%), with a crude RR = 0.19 (95% CI: 0.06 – 0.65, P = 0.003). Adjusting for antifungal prophylaxis use, the OR was of 0.17 (95% CI: 0.05 – 0.60, P = 0.006). There was a decreasing trend in proven and probable mold infections in the intervention group (0.5% vs. 3.3%; P = 0.06), as well as in proven yeast infections (1.1% vs. 4.4%; P = 0.057). *Candida* spp. was the most frequent yeast and among the seven molds, four were not identified by culture (Table 3). The single mold infection in PE group was identified through galactomannan test. Characteristics of the seven patients with mold infection are shown in Table 4. Three patients (42.8%) had diagnoses of acute leukemia, four (57.1%) underwent HSTC and the mortality rate was 57.1%. Except for only one mold, all IFI in the control group were probably hospital acquired.

The median number of antifungals used per hospitalization did not differ between groups (one drug, P = 0.74). However, we recorded a lower frequency of liposomal amphotericin B (1.6% vs. 7.2%; P = 0.008) and a non-significant reduction in deoxycholate and lipid complex amphotericin B, in the intervention group (Table 5). Although not reaching statistical significance, the rates of fluconazole (both oral and intravenous formulation) use were higher in the PE group: it was used for prophylaxis in 43.0% of admissions in the PE vs. 34.8% in the control group (P = 0.10).

The final cost with antifungal agents was lower after intervention (78347.37 USD vs. 154176.60 USD). However, the median direct cost of all antifungal drugs by admission did not differ between groups (14.46 USD in PE vs. 6.11 USD, under control; P = 0.55). Considering only admissions with proven, probable or possible IFI, compared to admissions without IFI in all sample, the median costs were, respectively, 1461.03 USD (P25 = 86.84; P75 = 6720.52) and 3.37 USD (P25 = 0.0; P75 = 34.42) with a P < 0.001. For proven or probable molds IFI, the figures were 3617.29 USD (P25 = 2990.17; P75 = 10653.70) and 6.26 USD (P25 = 0.0; P75 = 41.21), P < 0.001.

The median of days of hospital stay did not differ between groups (28 days; P = 0.38). On the other hand, the median length of hospital stay in admissions with proven, probable or possible IFI identified either in intervention or control groups was significantly higher than in admissions without IFI (48 days; P25 = 29 and P75 = 66 vs. 27 days; P25 = 22 and P75 = 35; P < 0.001).

Table 2. Invasive Fungal Infections in Intervention and Control Groups

	Protected Environment, n = 190	Control, n = 181	RR (95% CI)	P Value
IFI[a]	14 (7.4)	33 (18.2)[b]	0.40 (0.22 - 0.73)	0.002[c]
Yeast[d]	2 (1.1)	8 (4.4)	0.24 (0.05 - 1.11)	0.057[e]
Molds[d]	1 (0.5)	6 (3.3)	0.16 (0.02 - 1.31)	0.062[e]
Possible IFI	11 (5.8)	18 (10.0)	0.58 (0.28 - 1.20)	0.14[c]

[a]Data are presented as No.(%).
[b]One histoplasma capsulatum infection.
[c]Pearson's chi-squared test.
[d]Proven and probable infections.
[e]Fisher's exact test.

Table 3. Fungal Distribution of Proven and Probable Invasive Infections

Infections Caused by[a]	Protected Environment, n = 190		Control, n = 181	
	Proven	Probable	Proven	Probable
Yeasts				
Candida spp.	2	0	5	0
Pichia spp.	0	0	2	0
Trichosporon spp.	0	0	1	0
Molds				
Aspergillus spp.	0	1[b]	1	0
Fusarium spp.	0	0	1	0
Rhizopus spp.	0	0	1	0
Nonspeciated spp.[c]	0	0	2	1

[a]one histoplasma capsulatum infection was identified in control group.
[b]Galactomannan positivity in serum (index ≥ 0.5 twice) without culture isolation of causative species.
[c]Histopathologic or cytopathologic evaluation, without culture isolation of causative species.

Table 4. Characteristics of Patients with Invasive Mold Infections[a]

Group, Patient n.	Age	Diagnosis	Microbiological Criteria	Fungi Detected	Level	Outcome
Control						
1	31	AML	Positive biopsy for *A. niger*	*A. niger*	Proven	Died
2	26	AA[b]	Positive biopsy for *Rhizopus* spp.	*Rhizopus* spp.	Proven	Died
3	57	NHL[c]	Positive blood culture for *Fusarium* spp.	*Fusarium* spp.	Proven	Alive
4	38	CML	Positive biopsy for hyphae.	NS	Proven	Alive
5	43	ALL	Positive biopsy for hyphae.	NS	Proven	Died
6	37	AML[c]	Positive findings of cytopathologic evaluation for hyphae from BAL specimens.	NS	Probable	Alive
Protected environment						
1	60	MDS[b]	Positive result for galactomannan in > 2 blood samples.	NS	Probable	Died

[a]Abbreviations: AA, aplastic anemia; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; ANC, absolute neutrophil count; BAL, bronchoalveolar lavage; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-hodgkin's lymphoma; NS, non-speciated.
[b]Allogeneic hematopoietic stem cell transplant in previous admissions.
[c]Autologous hematopoietic stem cell transplant.

Table 5. Frequency and Duration of Antifungal use in Intervention and Control Groups[a]

Antifungal	Frequency of Use[b]			Days of Use - Median Days (P_{25} - P_{75})		
	PE (n = 190)	Control (n = 181)	P Value	PE (n = 190)	Control (n = 181)	P Value[c]
Amphotericin B (deoxycholate)	24 (12.6)	32 (17.7)	0.17[d]	5.5 (3 - 10.5)	6 (3 - 10.7)	0.87
Amphotericin B (liposomal)	3 (1.6)	13 (7.2)	0.008[d]	7 (3 - 10)	7 (2.5 - 9.5)	0.89
Amphotericin B (lipid complex)	0	2 (1.1)	0.24[e]	-	5.5 (4 - 7)	-
Caspofungin	2 (1.1)	1 (0.6)	1.00[e]	8.5 (3 - 14)	7 (7 - 7)	1.0
Fluconazole (PO)	96 (50.5)	78 (43.1)	0.15[d]	12 (5 - 20)	11.5 (6 - 18)	0.81
Fluconazole (IV)	77 (40.5)	59 (32.6)	0.11[d]	6 (1 - 1)	5 (1 - 11)	0.99
Itraconazole	12 (6.3)	15 (8.3)	0.47[d]	12.5 (3.5 - 28.7)	21 (8 - 24)	0.96
Voriconazole (PO)	8 (4.2)	8 (4.4)	0.92[d]	15.5 (10.2 - 22.5)	13.5 (9.5 - 33)	0.87
Voriconazole (IV)	3 (1.6)	4 (2.2)	0.72[e]	2 (1 - 10)	5 (2.5 - 6.7)	0.59

[a]Abbreviations: PO, per Os; IV, intravenously.
[b]Data are presented as No. (%).
[c]Mann-Whitney's test.
[d]Pearson's chi-squared test.
[e]Fisher's exact test.

5. Discussion

This paper suggests that the implementation of a PE ward in a general, tertiary and university affiliated hospital, in Southern Brazil, reduces the incidence of IFI in patients with CIN. In the control group, the incidence was higher than in a large Italian cohort study of patients with hematologic malignances, whereas it was lower after the renovation (3). After intervention, the rate of IFI was significantly reduced (7.4% vs. 18.2%, P = 0.002), even considering only proven or probable infections (1.6% vs. 8.3%, P = 0.003). This benefit remained after adjusting to antifungal prophylaxis (OR = 0.17, 95% CI: 0.05–0.60). The GVHD is considered a main risk factor for invasive candidiasis and aspergillosis, in allogeneic HSCT patients (1, 21), and although not reaching statistical significance, it was more commonly identified in the control group (3.2% vs. 6.6%, P = 0.12). When prophylaxis and GVHD were included in the logistic regression model, GVHD was not independently associated with IFI incidence, and did not modify the PE effect. Multicolinearity with antifungal prophylaxis may explain this result. The small incidence of IFI precluded adjustment for other possible confounders, but our analysis is conservative since the intervention group is likely to be of higher risk (Table 1).

The benefits of PE implementation in our institution in reducing febrile neutropenia (P = 0.009), overall mortality (P = 0.001) and 30-day adjusted mortality (P = 0.02) have already been described (18).

The benefit of PE in reducing fungal infections was de-

scribed in a prospective study conducted in Porto, Portugal. The building of new individual rooms with central HEPA filtration in the hematology unit significantly reduces the rates of proven or probable IFI (1.5% vs. 0%, P < 0.001). The consumption of antifungals was reduced and the final cost with antifungal therapy was reduced by 17.4% (15).

Evidence about infection control measures, involving ventilation and air-quality technologies, is mostly restricted to assessment of the impact of PE wards on invasive mold infections incidence, among high-risk patients undergoing HSCT or intensive chemotherapy for acute leukemia (15, 22-24). The benefit of rooms with positive pressure isolation in reducing invasive aspergillosis (IA) was registered in an adult hematological intensive care unit in a university hospital in France. The incidence of IA decreased from 13.2% before environmental modification to 1.6% after modification (14). Construction and renovation are a well-known risk factor for fungal infections, mainly caused by *Aspergillus* spp. (25, 26). This is plausible because these activities had been shown to dramatically increase the amount of airborne fungal spores (25). The benefit of preventive strategies in reducing spores counts, especially during renovation or construction, is known (13, 23, 24, 27). In our study, we observed a decreasing trend of invasive mold infections in the intervention group (0.5% vs. 3.3%, P = 0.057), in agreement to rates identified in studies that evaluated similar strategies (15, 22-24). Although patients in the control group were admitted in the period of PE ward construction, several others renovations continued to be made in our institution, after PE inauguration, and the intervention group also could have been exposed to dust. Although patients admitted to the PE ward may be adequately protected, they often required transfer to other areas of the hospital, either for diagnostic or therapeutic interventions, when they are exposed to the risk of acquiring invasive mold infections. Patients that required going outside the PE unit were required to wear N95 masks. The benefit of this strategy in preventing nosocomial aspergillosis during hospital construction was already demonstrated (28) and, furthermore, we could not expect that HEPA filtering facility would completely remove the risk of infection.

No patient treated in the PE ward developed proven invasive mold infection. Only one patient presented probable Invasive Aspergillosis after the renovation, and the microbiological criteria used in diagnosis were two sequential positive results for serum galactomannan. This indirect diagnostic test was implemented in our institution on June 2007, and was available only during the intervention group admissions. Although it is not considered an ideal test, gallactomannan is more sensitive than culture and allows early diagnosis of invasive aspergillosis (29). The use of this new diagnostic tool may considerably increase the incidence of probable invasive aspergillosis (5). If we have used the same diagnostic tests in both groups, no patient would be diagnosed with invasive mold infection in the intervention group. Even without this tool, six mold infections were identified in the control group. One proven IFI was caused by *Aspergillus niger*. Two biopsies and one cytopathologic evaluation were positive for hyphae, without species identification, although, according to pathologic evaluations, those findings were suggestive of *Aspergillus* spp. Although aspergillosis causes the most common invasive mycoses in highly immunosuppressed individuals, the other molds identified in control group are actually also expected in such patients (2, 3, 30). One patient in the control group developed fusariosis, diagnosed through positive blood cultures. In contrast to aspergillosis and most other invasive mold infections, fungemia is a common manifestation of disseminated fusariosis. The principal portal of entry for *Fusarium* spp. is the airway, followed by the skin at site of tissue breakdown, and, possibly, the mucosal membranes (31). Also, we recorded one IFI caused by *Rhizopus* spp., in the control group. This infection, termed zygomycosis, has risen significantly over the past decade, mainly in high-risk patients. The major mode of disease transmission for the zygomycetes is presumed to be via inhalation of spores from environmental sources (32), although ingestion and percutaneous exposure are also important in causing these infections (33). In this scenario, preventive interventions, involving environment control measures, remain a major protective factor.

The benefit of PE in reducing rates of invasive mold infections that were probably hospital-acquired was also observed (0.5% vs. 2.8% in PE and control groups, respectively; P = 0.11). It is difficult to determine the origin of those infections. Given the fact that no consensual definition of hospital-acquired invasive mold infection exists, we adopted the criteria in accordance with most studies published in the field of nosocomial aspergillosis (23, 26, 34, 35). The respective importance of each protective measures implemented in PE ward is difficult to assess. We hypothesized that the implementation of measures, involving ventilation and air-quality technologies installation, including HEPA filters, were the main causal factor. However, other characteristics that influenced individual exposure cannot be formally excluded, including the underlying disease and undergoing treatment. Most of mold IFI occurred in the high-risk HSTC patients or with acute leukemia (Table 4) in accordance to what was expected (2-4, 36).

There was a trend to lower incidence of yeast infections after the renovation, especially caused by *Candida* spp. These infections are usually acquired from the patient's own gastrointestinal or mucocutaneous flora (1), several of which being of endogenous origin (37). However, yeasts may be passed to patients by healthcare professionals (10). This assumption is based on the fact that a large proportion of medical professional carry *Candida* spp. on their hands (10) and that hand hygiene compliance is low (38). Extensive hand disinfection was part of the staff routine in the PE ward and visitors were moni-

tored about hand hygiene, when entering in the unit. This strategy could significantly minimize the risk of exposure to exogenous yeasts (1). Although we did not monitor hand hygiene compliance before and after the intervention, studies showed that strategies like hand hygiene promotion programs and availability of hand washing facilities are associated with higher compliance to hand hygiene practices and better outcomes (39, 40). Another possible explanation for the lower incidence of *Candida* infections in the PE group was the rising trend in antifungal prophylaxis with fluconazole, a preventive strategy with known impact in reducing invasive candidiasis and improving outcomes in high-risk patients (41).

During several admissions, antifungal agents were not used. There was no difference in the median number of agents used between groups, probably explained by increased antifungal prophylaxis in the PE group. The final direct cost of antifungal was reduced by approximately 50%, after the intervention. However, the median costs did not differ, maybe because few cases of proven or probable IFI occurred in the sample, which substantially increases the costs of treatment (42, 43). The rates of possible IFI were similar in the intervention and control groups (5.8% vs. 10.0%; P = 0.14). This level of probability of the diagnosis of IFI included multiple questionable cases, particularly those involving neutropenia, nonspecific pulmonary infiltrates and persistent fever, refractory to broad-spectrum antibiotics, without microbiological evidence of fungi. Most patients with this diagnosis received antifungal treatment and the cost of these cases represents an important proportion of the total cost with antifungal agents (data not shown). According to the revised definitions of IFI from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (44) the scope of category "possible" has been diminished. If these criteria were applied in clinical practice, it is probable that several patients with low chance of having invasive mycosis would not be treated. Considering the absolute reduction of proven, probable or possible IFI of 10.8%, after the intervention, an average of 10 cases of IFI could be prevented in neutropenic patients, if 100 admissions occurred in the PE ward, leading to savings of approximately 15000.00 USD (1461.03 USD minus 3.37 USD per infection). Although the median days of hospital stay did not differ between groups, admissions with proven, probable or possible IFI, in the entire sample, were associated with lengthy admission (P < 0.001). Considering that hospitalizations costs are commonly described as important in the overall healthcare costs, the impact of PE implementation in reducing IFI incidence could be associated with long-term economic benefit. Future research should evaluate the overall costs of hospital care after implementation of a PE ward, in a developing country.

The present study was limited by the quasi-experimental design and the non-contemporaneous control group. Although these types of studies can provide valuable information regarding the effectiveness of various interventions, several factors decrease the certainty of attributing improved outcomes to a specific intervention. These include difficulties in controlling for important confounding variables and the simultaneous use of multiple interventions (12). However, it was not possible to implement a randomized controlled trial because of ethical and logistical considerations. The sample size was estimated considering all IFI and was not reached. However, the studied sample was able to show a significant reduction in the primary endpoint. Because of the small incidence of events, the sample was underpowered for secondary and subgroup analysis. Since we analyzed neutropenic episodes, about 25% of patients were admitted more than once. However, exclusion of repeated patients did not modify the observed benefits. The economic analysis was limited to direct costs of antifungals and further evaluations, considering the burden of IFI, are desired.

In conclusion, this study showed that preventive measures including a PE implementation reduces IFI in neutropenic patients, especially in those with hematologic malignancies, admitted in a general, tertiary teaching hospital, in a developing country. It suggests that those strategies may overcome their costs on the long-term, by saving expenditures associated with fungal infections.

Footnotes

Authors' Contribution: Study concept and design: Paula Stoll and Leila Beltrami Moreira. Acquisition of data: Paula Stoll, Caroline Mioto Menegat Cola and Bruno Ismail Splitt. Analysis and interpretation of data, drafting of the manuscript, statistical analysis: Paula Stoll and Leila Beltrami Moreira. Study supervision: Leila Beltrami Moreira.

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References

1. Martino R, Subira M. Invasive fungal infections in hematology: new trends. *Ann Hematol.* 2002;**81**(5):233–43. doi: 10.1007/s00277-002-0466-3. [PubMed: 12029531]
2. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis.* 2002;**34**(7):909–17. doi: 10.1086/339202. [PubMed: 11880955]
3. Pagano L, Caira M, Candoni A, Offidani M, Fianchi L, Martino B, et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica.* 2006;**91**(8):1068–75. [PubMed: 16885047]
4. Martino R, Subira M, Rovira M, Solano C, Vazquez L, Sanz GF, et al. Invasive fungal infections after allogeneic peripheral blood stem cell transplantation: incidence and risk factors in 395 patients. *Br J Haematol.* 2002;**116**(2):475–82. [PubMed: 11841455]
5. Jantunen E, Salonen J, Juvonen E, Koivunen E, Siitonen T, Lehtinen T, et al. Invasive fungal infections in autologous stem cell transplant recipients: a nation-wide study of 1188 transplanted patients. *Eur J Haematol.* 2004;**73**(3):174–8. doi: 10.1111/j.1600-0609.2004.00273.x. [PubMed: 15287914]

6. Velasco E, Bigni R. A prospective cohort study evaluating the prognostic impact of clinical characteristics and comorbid conditions of hospitalized adult and pediatric cancer patients with candidemia. *Eur J Clin Microbiol Infect Dis.* 2008;**27**(11):1071–8. doi: 10.1007/s10096-008-0546-y. [PubMed: 18548295]
7. Jantunen E, Ruutu P, Niskanen L, Volin L, Parkkali T, Koukila-Kahkola P, et al. Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant.* 1997;**19**(8):801–8. doi: 10.1038/sj.bmt.1700737. [PubMed: 9134172]
8. Tablan OC, Anderson LJ, Besser R, Bridges C, Hajjeh R, et al. Guidelines for preventing healthcare-associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep.* 2004;**53**(RR-3):1–36. [PubMed: 15048056]
9. Fenelon LE. Protective isolation: who needs it? *J Hosp Infect.* 1995;**30 Suppl**:218–22. [PubMed: 7560953]
10. Maertens J, Vrebos M, Boogaerts M. Assessing risk factors for systemic fungal infections. *Eur J Cancer Care (Engl).* 2001;**10**(1):56–62. [PubMed: 11827268]
11. Centers for Disease Control and Prevention , Infectious Disease Society of America , American Society of Blood , Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *MMWR Recomm Rep.* 2000;**49**(RR-10):1–125. [PubMed: 11718124]
12. Siegel JD, Rhinehart E, Jackson M, Chiarello L, Health Care Infection Control Practices Advisory C. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. *Am J Infect Control.* 2007;**35**(10 Suppl 2):S65–164. doi: 10.1016/j.ajic.2007.10.007. [PubMed: 18068815]
13. Humphreys H. Positive-pressure isolation and the prevention of invasive aspergillosis. What is the evidence? *J Hosp Infect.* 2004;**56**(2):93–100. doi: 10.1016/j.jhin.2003.10.011. [PubMed: 15019219]
14. Benet T, Nicolle MC, Thiebaut A, Piens MA, Nicolini FE, Thomas X, et al. Reduction of invasive aspergillosis incidence among immunocompromised patients after control of environmental exposure. *Clin Infect Dis.* 2007;**45**(6):682–6. doi: 10.1086/521378. [PubMed: 17712750]
15. Araujo R, Carneiro A, Costa-Oliveira S, Pina-Vaz C, Rodrigues AG, Guimaraes JE. Fungal infections after haematology unit renovation: evidence of clinical, environmental and economical impact. *Eur J Haematol.* 2008;**80**(5):436–43. doi: 10.1111/j.1600-0609.2008.01034.x. [PubMed: 18194476]
16. Schlesinge A, Paul M, Gafter-Gvili A, Rubinovitch B, Leibovici L. Infection-control interventions for cancer patients after chemotherapy: a systematic review and meta-analysis. *Lancet Infect Dis.* 2009;**9**(2):97–107. doi: 10.1016/S1473-3099(08)70284-6. [PubMed: 19095499]
17. Sehulster L, Chinn RY. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep.* 2003;**52**(RR-10):1–42. [PubMed: 12836624]
18. Stoll P, Silla LM, Cola CM, Splitt BI, Moreira LB. Effectiveness of a Protective Environment implementation for cancer patients with chemotherapy-induced neutropenia on fever and mortality incidence. *Am J Infect Control.* 2013;**41**(4):357–9. doi: 10.1016/j.ajic.2012.05.018. [PubMed: 23102987]
19. Zuckermann J, Moreira LB, Stoll P, Moreira LM, Kuchenbecker RS, Polanczyk CA. Compliance with a critical pathway for the management of febrile neutropenia and impact on clinical outcomes. *Ann Hematol.* 2008;**87**(2):139–45. doi: 10.1007/s00277-007-0390-7. [PubMed: 17938926]
20. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis.* 2002;**34**(1):7–14. doi: 10.1086/323335. [PubMed: 11731939]
21. Einsele H, Bertz H, Beyer J, Kiehl MG, Runde V, Kolb HJ, et al. Infectious complications after allogeneic stem cell transplantation: epidemiology and interventional therapy strategies–guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Ann Hematol.* 2003;**82 Suppl 2**:S175–85. doi: 10.1007/s00277-003-0772-4. [PubMed: 13680165]
22. Sherertz RJ, Belani A, Kramer BS, Elfenbein GJ, Weiner RS, Sullivan ML, et al. Impact of air filtration on nosocomial Aspergillus infections. Unique risk of bone marrow transplant recipients. *Am J Med.* 1987;**83**(4):709–18. [PubMed: 3314494]
23. Loo VG, Bertrand C, Dixon C, Vitye D, DeSalis B, McLean AP, et al. Control of construction-associated nosocomial aspergillosis in an antiquated hematology unit. *Infect Control Hosp Epidemiol.* 1996;**17**(6):360–4. [PubMed: 8805066]
24. Oren I, Haddad N, Finkelstein R, Rowe JM. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. *Am J Hematol.* 2001;**66**(4):257–62. doi: 10.1002/ajh.1054. [PubMed: 11279636]
25. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak settings. *J Hosp Infect.* 2006;**63**(3):246–54. doi: 10.1016/j.jhin.2006.02.014. [PubMed: 16713019]
26. Alberti C, Bouakline A, Ribaud P, Lacroix C, Rousselot P, Leblanc T, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. *J Hosp Infect.* 2001;**48**(3):198–206. doi: 10.1053/jhin.2001.0998. [PubMed: 11439007]
27. Hahn T, Cummings KM, Michalek AM, Lipman BJ, Segal BH, McCarthy PL. Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies. *Infect Control Hosp Epidemiol.* 2002;**23**(9):525–31. doi: 10.1086/502101. [PubMed: 12269451]
28. Raad I, Hanna H, Osting C, Hachem R, Umphrey J, Tarrand J, et al. Masking of neutropenic patients on transport from hospital rooms is associated with a decrease in nosocomial aspergillosis during construction. *Infect Control Hosp Epidemiol.* 2002;**23**(1):41–3. doi: 10.1086/501967. [PubMed: 11868892]
29. Aquino VR, Goldani LZ, Pasqualotto AC. Update on the contribution of galactomannan for the diagnosis of invasive aspergillosis. *Mycopathologia.* 2007;**163**(4):191–202. doi: 10.1007/s11046-007-9010-2. [PubMed: 17410480]
30. Donowitz GR, Maki DG, Crnich CJ, Pappas PG, Rolston KV. Infections in the neutropenic patient–new views of an old problem. *Hematology Am Soc Hematol Educ Program.* 2001:113–39. [PubMed: 11722981]
31. Nucci M, Anaissie E. Emerging fungi. *Infect Dis Clin North Am.* 2006;**20**(3):563–79. doi: 10.1016/j.idc.2006.06.002. [PubMed: 16984869]
32. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev.* 2000;**13**(2):236–301. [PubMed: 10756000]
33. Chayakulkeeree M, Ghannoum MA, Perfect JR. Zygomycosis: the re-emerging fungal infection. *Eur J Clin Microbiol Infect Dis.* 2006;**25**(4):215–29. doi: 10.1007/s10096-006-0107-1. [PubMed: 16568297]
34. Pegues CF, Daar ES, Murthy AR. The epidemiology of invasive pulmonary aspergillosis at a large teaching hospital. *Infect Control Hosp Epidemiol.* 2001;**22**(6):370–4. doi: 10.1086/501915. [PubMed: 11519915]
35. Thio CL, Smith D, Merz WG, Streifel AJ, Bova G, Gay L, et al. Refinements of environmental assessment during an outbreak investigation of invasive aspergillosis in a leukemia and bone marrow transplant unit. *Infect Control Hosp Epidemiol.* 2000;**21**(1):18–23. doi: 10.1086/501691. [PubMed: 10656349]
36. Reich G, Mapara MY, Reichardt P, Dorken B, Maschmeyer G. Infectious complications after high-dose chemotherapy and autologous stem cell transplantation: comparison between patients with lymphoma or multiple myeloma and patients with solid tumors. *Bone Marrow Transplant.* 2001;**27**(5):525–9. doi: 10.1038/sj.bmt.1702822. [PubMed: 11313687]
37. Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? *Clin Infect Dis.* 2001;**33**(12):1959–67. doi: 10.1086/323759. [PubMed: 11702290]
38. Pittet D, Mourouga P, Perneger TV. Compliance with handwashing in a teaching hospital. Infection Control Program. *Ann Intern Med.* 1999;**130**(2):126–30. [PubMed: 10068358]
39. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Tou-

veneau S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. *Lancet.* 2000;**356**(9238):1307–12. [PubMed: 11073019]
40. Trick WE, Vernon MO, Welbel SF, Demarais P, Hayden MK, Weinstein RA, et al. Multicenter intervention program to increase adherence to hand hygiene recommendations and glove use and to reduce the incidence of antimicrobial resistance. *Infect Control Hosp Epidemiol.* 2007;**28**(1):42–9. doi: 10.1086/510809. [PubMed: 17230386]
41. Robenshtok E, Gafter-Gvili A, Goldberg E, Weinberger M, Yeshurun M, Leibovici L, et al. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: systematic review and meta-analysis. *J Clin Oncol.* 2007;**25**(34):5471–89. doi: 10.1200/JCO.2007.12.3851. [PubMed: 17909198]
42. Dasbach EJ, Davies GM, Teutsch SM. Burden of aspergillosis-related hospitalizations in the United States. *Clin Infect Dis.* 2000;**31**(6):1524–8. doi: 10.1086/317487. [PubMed: 11096031]
43. Slavin M, Fastenau J, Sukarom I, Mavros P, Crowley S, Gerth WC. Burden of hospitalization of patients with Candida and Aspergillus infections in Australia. *Int J Infect Dis.* 2004;**8**(2):111–20. [PubMed: 14732329]
44. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis.*