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ORIGINAL ARTICLE

Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload

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Fungal pulmonary infections (FPIs) are frequent causes of mortality in hematopoietic stem cell transplantation (HSCT) recipients. Determination of the specific risk factors may improve the prognosis. The aim of this study was to evaluate the risk factors of FPIs due to HSCT. Patient history, physical examination, chest X-rays and the consultation records of the pulmonary disease department which were a part of the routine evaluation before and at first, third, sixth, ninth and twelfth months of HSCT were retrieved in 148 adult HSCT recipients. Results of the high-resolution computed tomography, fiber-optic bronchoscopy and the microbiological data were also included. FPI was diagnosed in 22 patients (14.9%). Multivariate analysis showed that increased ferritin levels (>1000 ng/ml; OR: 3.42, 95% CI 1.03–11.42, $P=0.045$) and the development of sinusoidal obstruction syndrome (SOS; OR: 5.09, 95% CI 1.53–16.90, $P=0.008$) were significant risk factors for FPIs. The sensitivity and specificity of ferritin >1000 ng/ml for the prediction of FPIs were 67 and 70%, respectively. There was a positive correlation between the increased risk of FPIs and pretransplantation ferritin levels ($r=0.413$, $P<0.001$) and increased ferritin levels and SOS ($r=0.331$, $P<0.001$). Increased pretransplantation ferritin levels and development of SOS are predictive factors of FPIs during HSCT.

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Introduction

Pulmonary complications occur in approximately 30–60% of hematopoietic stem cell transplantation (HSCT) recipients and approximately 30% of transplant-related mortality is attributable to pulmonary complications.[1–3]

Fungal infections are a frequent cause of pneumonia after HSCT with a reported incidence of up to 70%.[4] Effective prophylaxis to bacterial and viral pathogens results in increased frequency of invasive fungal infections (IFIs), which are the leading cause of infectious morbidity and mortality. Although fluconazole prophylaxis has reduced the incidence of infections caused by susceptible microorganisms, it resulted in a subsequent increase in the resistant species.[5,6]

Pulmonary involvement is the most common localization of IFIs during the course of HSCT.[7] Unfortunately, both the diagnosis and the therapy are a clinical dilemma as the diagnostic tools used for early and reliable diagnosis are often inadequate. A variety of fungal microorganisms are responsible for several diseases in HSCT recipients. In an autopsy study, 83% of the cases that were diagnosed at autopsy were negative for fungi antemortem by bronchoalveolar lavage and cultures.[2] In addition, invasive procedures are usually not feasible due to several contraindications such as respiratory distress or thrombocytopenia and prognosis remains to be poor even with the appropriate therapy.[3] Documentation of the risk factors of fungal pulmonary infections (FPIs) during HSCT may facilitate targeted prophylactic strategies and successful management of these patients and improve prognosis.

Previously identified major risk factors for IFI among patients with HSCT are prolonged neutropenia, acute and chronic GVHD, transplantation from an unrelated donor, high-dose corticosteroid therapy and a number of environmental factors.[8–11] Although most of the patients are reported to have multiple related risk factors, it is indicated that pulmonary complications do not develop in this entire high-risk group of patients that suggest that pathogenesis and the risk factors have not been completely understood yet.[5] The aim of this study was to evaluate the risk factors of FPIs due to HSCT.

Materials and methods

Patient population

The medical records of all patients who underwent HSCT at the Department of Bone Marrow Transplantation, Gazi

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University School of Medicine from November 2003 to September 2008 were reviewed retrospectively. Among them, 36 patients with missing data, 20 patients who died at the early post transplant period due to extrapulmonary causes and 12 patients who failed to attend follow-up visits regularly were excluded from the analysis. Among patients who received more than one allogenic HSCT, data of the first transplantation were reviewed. If the patient received an allogenic transplantation followed by an autologous HSCT, pretransplantation records of the allogenic transplantation were reviewed.

Definitions

Engraftment was defined as the time the neutrophil count exceeds 0.5×10^9 per liter and platelet count exceeds 20×10^9 per liter for at least 3 consecutive days without transfusion.[12] IFIs were categorized as possible, probable and proven according to the standardized definitions of European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Disease Mycoses Study Group.[13] As this study is limited to FPIs, the diagnosis was based on the investigation of the specimens obtained from lung in accordance with the clinical and radiographic findings. Patients were diagnosed with sinusoidal obstruction syndrome (SOS) based on the Seattle criteria.[14]

Antimicrobial prophylaxis

All patients were admitted to HEPA-filtered single isolation rooms and low microbial diet was provided until neutrophil recovery. Antimicrobial prophylaxis, which was performed with acyclovir and fluconazole, was given from the beginning of the conditioning until day +180 post-HSCT for autologous and until discontinuation of the immunosuppressive therapy for allogenic HSCT recipients. Patients who had a history of previous active fungal infection were on secondary prophylaxis with amphotericin B (0.5 mg/kg/day). All patients received trimethoprim–sulfamethoxazole orally as prophylaxis against *Pneumocystis jiroveci* beginning from the conditioning regimen until 1 day before stem cell infusion and from neutrophil engraftment to 6-months post-HSCT for autologous and until the discontinuation of the immunosuppressive therapy for allogeneic transplantation.

Management of GVHD

CYA and MTX were the standard prophylaxis regimen for GVHD. Acute GVHD was defined and classified according to previously published criteria.[15] GVHD was treated with 1-2 mg/kg/day prednisolone. Although under corticosteroids, the patients received valacyclovir (1000 mg/day, p.o.), fluconazole (5 mg/kg/day, p.o.) and trimethoprim–sulfamethoxazole (trimethoprim 320 mg/day and sulfamethoxazole 1600 mg/day) as antimicrobial prophylaxis. Patients who were on steroid therapy were also screened weekly with blood cultures and twice weekly with serum galactomannan assay.

Pulmonary evaluation

Patient history, physical examination and chest X-rays that were a part of the routine evaluation before and at first, third, sixth, ninth and twelfth months of the HSCT were evaluated. The history of pulmonary disease, smoking history and pretransplantation consultation records of the pulmonary disease department were included in the investigation for all patients. The results of high-resolution computed tomography and fiber-optic bronchoscopy results were also investigated. The data of the microbiological analysis including direct microscopy or culture of sputum, bronchoalveolar lavage fluid or galactomannan antigen detected in plasma, serum or bronchoalveolar lavage fluid were collected.

Iron status

Serum ferritin (normal range: 10–291 ng/ml) and transferring saturation ratio (normal range: 30–45%) were measured in 1 week before transplantation per institutional practice in all patients.

Molecular tests for fungal infection

Galactomannan enzyme immune assay analysis was performed in the presence of neutropenia and twice weekly with episodes of neutropenic fever.

Age, gender, smoking history, duration between diagnosis and HSCT, Karnofsky performance status, administration of mediastinal radiotherapy, mucositis, the CD34+ cell count, duration of neutropenia, time to neutrophil and platelet engraftment, pretransplantation transferrin saturation and ferritin levels as markers of iron status, SOS, acute GVHD, history of fungal pneumonia before HSCT were the variables that were prospectively recorded in all of the patients per institutional practice and were analyzed as possible risk factors for the development of FPIs. Both FPIs and mortality were analyzed up to post transplant day 365.

Statistical analysis

SPSS for Windows 11.0.1 software was used for the statistical analysis of the results (SPSS for Windows, SPSS Inc., Chicago, IL, USA). Results are presented as mean ± s.d. and percentiles or median (range) values. The χ^2-test and Mann–Whitney *U*-test were used for comparison of the categorical and continuous variables, respectively. All unrelated variables with a *P*-value $\leqslant 0.1$ on univariate analysis were included in the logistic regression analysis. Odds ratios and 95% CIs were reported for all statistically significant variables at a *P*-value $\leqslant 0.05$. A receiver operating characteristic curve for the assessment of the predictive value of pretransplantation ferritin level on FPIs was plotted and the area under curve was calculated. Correlation was investigated with Pearson's correlation tests for parametric values. Kaplan–Meier survival analysis was performed for comparing the outcome among patients with and without FPIs. A difference was considered statistically significant when $P < 0.05$.

Results

Demographical variables

Of the 148 HSCT, 99 (67%) were allogenic and 49 (33%) were autologous transplants. Mean age at transplant was 34.7 ± 14.1 years (range: 16–68 years) and 64% of the patients were men. Ninety-eight percent of the patients received HSCT from a related donor. Mean duration between diagnosis and HSCT was 19.7 ± 23.1 months (range: 1–198 months). Median time of follow-up was 12 months (range: 1–12 months). Indications of HSCT were as follows: AML (29.1%), multiple myeloma (19.6%), Hodgkin's disease (14.2%), ALL (11.4%), severe aplastic anemia (8.8%), non-Hodgkin's lymphoma (8.1%), CML (4.7%), myelodysplastic syndrome (2.7%), primitive neuroectodermal tumor (0.7%) and thalassemia (0.7%). The conditioning regimens were BU based in 43.2%, melphalan based in 22.9%, BEAM based in 14.8% and other regimens in the remaining 19.1% of the patients.

Frequency, timing and mortality of FPIs

Fungal pulmonary infections were found in 22 of the 148 HSCT recipients (14.9%). Of them 82% the patients with FPIs received allogenic whereas the remaining 18% received autologous HSCT ($P<0.05$). Possible and probable FPIs were present in 6 and 16 patients, respectively, according to previously published criteria.[13] None of our patients was diagnosed as proven FPI. The diagnostic criteria of the patient group are presented in Table 1. *Aspergillus* spp was the causative microorganism in 16 patients (62.5%) whereas causative fungal agent could not be isolated in the remaining 6 patients (37.5%). The median time from HSCT to the diagnosis of fungal pulmonary complication was 76 days (range: 7–360 days). Sixty-eight percent of the infections were diagnosed in the first 30 days (mean ± s.d. = 22 ± 8 days), 18% were diagnosed at 30–100 days (mean ± s.d. = 83 ± 16 days) and 14% were diagnosed after 100 days of HSCT (mean ± s.d. = 209 ± 78 days). The mortality was significantly higher among patients with FPIs compared with ones without FPI (45.5 vs 17.5%, $P=0.009$). Seventy percent of the deaths were due to pulmonary causes in the FPIs group.

Risk factors for the development of FPIs

Age, gender, smoking history, administration of mediastinal radiotherapy, mucositis, infused CD34+ cell count and the incidence of acute GVHD that was only evaluated in allogenic transplants were not different in patients with and without FPIs ($P>0.05$). In contrast, a history of previous fungal pneumonia, lower Karnofsky performance status, and longer duration of neutropenia, delayed neutrophil and platelet engraftment and incidence of SOS ($P<0.05$) were more frequent in patients with FPI. Although transferrin saturation ratios were similar, increased ferritin levels were observed in patients with FPIs (Table 2). When the pretransplantation ferritin levels were compared with FPI with the receiver operating characteristic curve, the cutoff value was found to be 1000 ng/ml (area under curve: 0.81, 95% CI 0.70–0.92, $P=0.000$) (Figure 1). According to this cutoff value, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the ferritin levels >1000 ng/ml for the prediction of the development of FPI were 68, 70, 28, 92 and 70%, respectively. Results of multivariate Cox regression analysis indicated that the two independent variables that were related with the increased risk of FPIs were the increased ferritin level (>1000 ng/ml) (OR: 3.42, 95% CI 1.03–11.42, $P=0.045$) and the development of SOS (OR: 5.09, 95% CI 1.53–16.90, $P=0.008$) (Table 3). In a further analysis, logistic regression was performed with pretransplantation levels of other acute-phase reactants including serum C-reactive protein and albumin levels. Also, ferritin >1000 ng/ml was again found to be an independent risk factor of FPIs.

Pearson's correlation analysis indicated positive correlation between the increased risk of FPIs and pretransplantation ferritin levels ($r=0.479$, $P<0.001$). In addition, positive correlation among increased ferritin levels and SOS has been documented ($r=0.340$, $P<0.001$). Kaplan–Meier survival analysis indicated that the development of FPIs is significantly related with the 365-day mortality among patients with allogenic HSCT (HR: 0.271, 95% CI: 0.135–0.545, $P<0.001$). The mortality analysis was not performed in the autologous HSCT recipients as only two patients died in this group.

Table 1 Diagnostic criteria of the possible and probable FPIs according to revised European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Disease Mycoses Study Group criteria[13]

	Possible FPIs (n = 6)	*Probable FPIs* (n = 16)
Host factor		
Allogeneic HSCT	6	12
Prolonged use of corticosteroids	—	3
Immunosuppressive therapy	—	1
Clinical criteria on CT		
Dense well-defined lesions with or without a halo sign	4	9
Cavity	—	3
Air-crescent sign	—	1
Dense well-defined lesions with and without a halo sign	2	—
Cavity and dense well-defined lesions with or without a halo sign	—	3
Microbiological criteria	None	
Positive galactomannan antigen in serum		11
Positive galactomannan antigen in BALF		3
Positive microscopy of mold in BALF		1
Positive culture of mold in BALF		1

Abbreviations: CT = computed tomography; BALF = bronchoalveolar lavage fluid; FPI = fungal pulmonary infection.

Discussion

This study showed that FPIs are frequent complications of HSCT and result in poorer prognosis. Increased pretransplantation ferritin levels and the development of SOS are

Table 2 Comparison of the risk factors among patients with and without FPIs

	FPIs (+), n = 22	*FPIs (−), n = 126*	P-*value*
Age (years)	39.6 ± 14.1	33.9 ± 13.9	0.076
Gender (male/female) (*n*)	13/9	82/44	0.634
Smoking history	40%	41%	0.572
Duration between diagnosis and HSCT (days)	898 ± 1401	476 ± 459	0.895
Previous fungal pneumonia	36%	11%	**0.006**
Karnofsky performance status (mean ± s.d.)	77 ± 19	86 ± 10	**0.008**
Mediastinal radiotherapy (%)	4.5	7.1	0.545
Mucositis (mean ± s.d.)	2.5 ± 1.6	1.9 ± 1.6	0.204
Infused CD34+ cell count ($\times 10^6$ cells per kg) (mean ± s.d.)	3.9 ± 2.5	4.7 ± 2.4	0.089
Neutrophil engraftment (days) (median) (minimum−maximum)	18 (14–28)	16 (9–30)	**0.012**
Duration of neutropenia (days) (median) (minimum−maximum)	17 (11–29)	13 (5–34)	**0.016**
Platelet engraftment (days) (median) (minimum−maximum)	24 (9–40)	13 (0–62)	< **0.001**
Sinusoidal obstruction syndrome (%)	50	11.9	< **0.001**
Acute GVHD (%)	26	22	0.765
Transferrin saturation (%)	58 ± 48	35 ± 27	0.078
Ferritin (ng/ml)	2384 ± 1695	795 ± 869	< **0.001**

Abbreviation: FPI = fungal pulmonary infection.
Bold values are statistically significant.

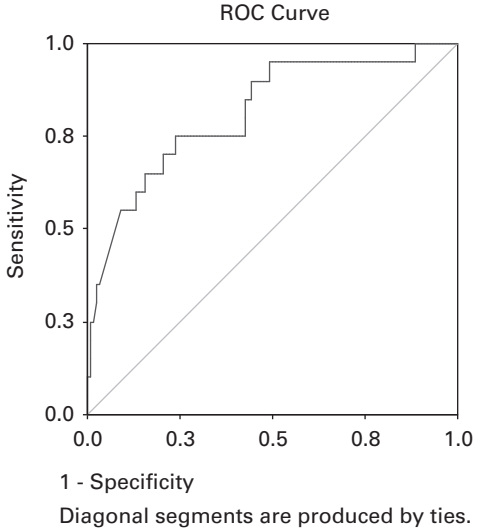

Figure 1 Comparison of the pretransplantation ferritin level and the development of fungal pulmonary infection with receiver operating characteristic (ROC) curve.

Table 3 Results of multivariate analysis for the risk factors of FPIs

Variable	*Odds ratio*	*95% confidence interval*	P-*value*
Previous fungal pneumonia	2.19	0.57–8.45	0.254
Duration of neutropenia	1.03	0.93–1.14	0.581
Karnofsky performance status	0.97	0.93–1.02	0.253
Ferritin >1000 ng/ml	3.42	1.03–11.43	**0.045**
Sinusoidal obstruction syndrome	5.09	1.53–16.90	**0.008**

Bold values are statistically significant.

independent risk factors for the development of FPIs. Ferritin levels above 1000 ng/ml could be a predictor of the development of FPIs.

The frequency rate of FPIs in our study was comparable with the previous reports that indicated an incidence of 10–15%. In addition, the spectrum of the fungal microorganisms was also similar with the previous reports that showed *Aspergillus* spp as the most common agent.[5,7,9,16] The major limitation of this study is the failure to detect a causative microorganism in 37.5% of the patients, which was attributable to the difficulties in performing bronchoscopy on some of the patients due to the several contraindications such as respiratory distress and severe thrombocytopenia rendering a significant number of our patients as possible cases of IFIs. However, several authors indicated that diagnostic yield of bronchoscopy for FPIs is low and does not have a survival benefit in HSCT recipients.[17,18]

The result of our study also indicates that delayed neutrophil engraftment and prolonged neutropenia are risk factors for FPIs, which have also been reported in several other studies.[5,19,20] Prolonged neutropenia has been shown to be the single most important predictor of IFIs and was attributed to the crucial role of neutrophils in host defense against fungal infections previously.[21] Delayed neutrophil engraftment may also be a consequence rather than a cause for FPIs as the migration of the neutrophils and the lymphocytes to the lung might result in a delay of the engraftment. Univariate analysis also indicates delayed platelet engraftment as a risk factor of FPIs, which should be interpreted as a consequence increased consumption of platelets rather than having a causal relationship with FPIs.

Iron is a physiologically essential element that has a key role in oxygen transport and electron transfer.[22] However, particularly in its form as nontransferring-bound iron, it can also convert hydrogen peroxide to toxic-free radicals, which results in tissue damage and fibrosis.[23,24] In addition, it reduces the phagocytic and chemotactic functions of neutrophils and also inhibits the antimicrobial effects of natural killer cells and macrophages.[25,26] Recent experimental data indicate that iron is an essential element for increased growth and virulence of *Aspergillus* species.[27] A number of studies showed that iron overload (IO) is related with increased risk of *mucormycosis*, invasive *aspergillosis* and transplant-related mortality.[28–30] Increased IO at the early post transplantation period is not always associated

with increased transfusion requirement.[31–33] Ineffective hematopoiesis, release of iron from BM, liver and other tissues due to cell damage secondary to chemotherapy, and increased iron absorption as a result of mucositis might as well cause IO in HSCT recipients.[23,29,34] The results of this study support the previous data regarding the association between IO and IFIs. We have shown that IO is an independent risk factor of FPIs in this study population. Although the sensitivity and specificity of ferritin >1000 ng/ml level as a predictor of FPI is not very high, the negative predictive value is found to be 92%. Thus, serum ferritin levels can be used as a predictor of FPIs among patients with HSCT.

Another important issue that should be addressed regarding the consequences of IO is SOS and its association with FPIs. The results of our study indicate positive correlation between SOS and ferritin levels. In an autopsy study, liver dysfunction caused by SOS of the liver was reported to be an independent risk factor for fungal liver infections.[35] In two other investigations, Armand *et al*.[34] and Morado *et al*.[36] found increased serum ferritin levels as a risk factor for SOS in HSCT recipients. In a recent study by Maradei *et al*.,[37] pretransplantation ferritin level >1000 ng per 100 ml was found to be an independent risk factor of SOS. A previous report of our group has also confirmed these results.[38] Thalassemia patients undergoing HSCT are prone not only to SOS but also to fungal infections, which is attributed to transfusion-associated IO.[39,40] Regular iron chelation is known to decrease transplant-associated mortality in thalassemia patients who are candidates for HSCT, suggesting that iron chelation therapy in other patients with IO might as well decrease transplant-related complications and mortality. As a matter of fact two recent studies showed a reduced incidence and severity of SOS in HSCT recipients with iron chelation therapy.[39,40] The impact of iron chelation on transplant outcome in patients with elevated pretransplant ferritin levels should be investigated with prospective randomized trials.

Although two other risk factors; lower Karnofsky performance status and a history of fungal pneumonia were found to be variables related to FPIs, no influence of age, gender, smoking history, number of CD34+ cells infused, mucositis and a history of mediastinal radiotherapy on FPI risk was found in univariate analysis. Although active fungal disease is reported not to be a contraindication to HSCT, the risk of IFIs may be expected to be higher among patients who had a history of fungal infections.[41] Recently, Zhang *et al*.[42] reported an incidence of 18.4% of IFIs among HSCT patients with a history of previous IFI. An intriguing finding of the present study is the lack of an association between acute GVHD and FPIs, which should be validated with a larger sample size. We cannot comment on chronic GVHD as it is beyond the scope of this study; however, it might explain the difference between the risk factors of IFIs and FPIs and should certainly be highlighted with further studies.

This study has the limitations of being a single-center and retrospective study, in addition to the absence of cases with proven FPIs. The distribution of proven, possible and probable FPIs might have changed with performing biopsy. However it is not certain whether it would lead to a change in therapeutic interventions.

In conclusion, this study suggests that the risk factors of FPIs are mostly similar to systemic fungal infections. We have also confirmed positive correlation between elevated ferritin levels and IFIs and SOS. Prospective studies are required to further elucidate the risk factors and to investigate measures to prevent FPIs in high-risk patients.

Conflict of interest

The authors declare no conflict of interest.

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