Journal of Microbiology, Immunology and Infection (2018) xx, 1–10

Available online at www.sciencedirect.com

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journal homepage: www.e-jmii.com

Original Article

Invasive mold infections in acute leukemia patients undergoing allogeneic hematopoietic stem cell transplantation

Sheng-Hsuan Chien [a,b,c], Yao-Chung Liu [b,d], Chia-Jen Liu [b,d], Po-Shen Ko [b,d], Hao-Yuan Wang [b,d], Liang-Tsai Hsiao [b,d], Tzeon-Jye Chiou [a,b], Jin-Hwang Liu [b,e], Jyh-Pyng Gau [b,d,*]

[a] *Division of Transfusion Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan*
[b] *Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan*
[c] *Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan*
[d] *Division of Hematology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan*
[e] *Division of Hematology and Oncology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei, Taiwan*

Received 4 March 2018; received in revised form 14 September 2018; accepted 18 September 2018
Available online ■ ■ ■

KEYWORDS
Acute leukemia;
Allogeneic hematopoietic stem cell transplantation;
Mold infection;
Graft-versus-host disease;
Smoking

Abstract *Background/purpose:* Patients with acute leukemia undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) are exposed to high risk of developing invasive fungal infections, and the invasive mold infections (IMIs) are becoming more and more common after transplantation. Here, we conducted a retrospective study to analyze demographics, microbiology, and risk factors for IMIs development in adult acute leukemia patients undergoing allo-HSCT.

Methods: We reviewed 245 adult acute leukemia patients undergoing allo-HSCT from January 2003 to December 2014. Clinical characteristics including age, sex, conditioning regimens, European Group for Blood and Bone marrow Transplantation (EBMT) risk score, and presence of acute graft-versus-host disease (aGVHD) or chronic GVHD (cGVHD) were collected and analyzed. Cox proportional hazard model was adopted to explore the independent risk factors for IMIs developments.

Results: Seventeen of 245 patients developed IMIs during the study period. The cumulative incidence of IMIs in this cohort was 8.7% and 16.8% at 6 and 12 months, respectively, with *Aspergillus* species being the most common pathogen. The significant risk factors predicting IMIs were unrelated donor transplantation (hazard ratio [HR] 5.11), smoking (HR 3.55), EBMT risk score > 2 (HR 4.22), and moderate to severe cGVHD (HR 3.76).

* Corresponding author. Division of Hematology, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sec 2, Shih-Pai Road, Taipei, 11217, Taiwan. Fax: +886 2 28757762.
E-mail address: jpgau@vghtpe.gov.tw (J.-P. Gau).

https://doi.org/10.1016/j.jmii.2018.09.006

Conclusions: We identified four risk factors-unrelated donor transplantation, smoking, EBMT risk score >2 and moderate to severe cGVHD to predict IMIs among acute leukemia patients undergoing allo-HSCT. This cohort study suggests early identification of high-risk patients and to provide better prevention strategies would reduce the incidence and severity of IMIs in these patients.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative therapy for adults with acute leukemia, and acute myeloid leukemia is the most common indication for allo-HSCT.[1] Patients with acute leukemia have high risk of infections and the immunity is further compromised after allo-HSCT. With advances in supportive care, extended survival of leukemia patients by transplantation, usage of immunosuppressant agents, and improved control of bacterial infection,[2] the invasive fungal infection has become more and more important in clinical care of immune compromised host,[3] accounting for the majority of morbidities and mortality after transplantations.[4] Since the introduction of fluconazole prophylaxis in the 1990s, the incidence of yeast infection gradually decreased, coinciding with emergence of invasive mold infections (IMIs), especially invasive aspergillosis.[4,5] IMI is currently the major invasive fungal infection after transplantation,[3] resulting in poor prognosis of these patients despite recent advances in diagnosis and management of this infection.[6]

Previous studies had established the risk factors for invasive fungal infections in patients with hematological malignancies or transplantation recipients, and most studies agreed that age,[7] unrelated donor transplantation,[8] persistent neutropenia,[9,10] advanced acute graft-versus-host disease (aGVHD), or chronic GVHD (cGVHD),[7,8,11,12] and prolonged usage of corticosteroid[11,12] were the major risk factors. However, in majority of these reports, the studies were conducted on patients with heterogeneous diagnoses of hematological diseases requiring allo-HSCT treatment. Little is known about the exact risk factors for IMIs in adult leukemia patients undergoing allo-HSCT, despite of the fact that acute leukemia is the most common indication for transplantation. Understanding risk factors for IMIs would allow identification of acute leukemia patients who might benefit from early preventive strategies after allo-HSCT. Here, we conducted a retrospective study to demonstrate demographics, microbiology, and risk factors for the development of IMIs among adult patients with acute leukemia receiving allo-HSCT at a tertiary medical center within a 10-year period.

Materials and methods

Study patient population

We retrospectively reviewed acute leukemia patients (age ≥ 18 years) receiving allo-HSCT between January 2003 and December 2014 in Taipei Veterans General Hospital in Taiwan. Clinical characteristics included age, sex, biological data, diseases diagnosis before transplantation, comorbidities, type of allogeneic donors, history of invasive fungal infections prior to allo-HSCT, conditioning regimens, European Group for blood and Bone marrow Transplantation (EBMT) risk scores,[13] immunosuppressant usage, and presence of aGVHD or cGVHD were collected for analysis. All patients were regularly followed till October 2015. This retrospective study of medical records was approved by the Taipei Veterans General Hospital institutional ethical committee in agreement with the Helsinki Declaration of 1975, revised in 2008.

Definition of invasive mold infection

The definition of IMIs was based on the consensus conducted by the European Organization for Research and Treatment of cancer/Invasive Fungal Infectious Disease Mycoses Study Group (EORTC/MSG).[14] Patients' clinical, pathological, microbiological, and radiological features were reviewed to clarify the evidence of IMI. Accordingly, invasive fungal infections were divided into three categories: proven, probable and possible.[14] Proven infection indicated microscopic evidence of mold infection or pathogens culture from sterile material. The diagnosis of probable IMIs was based on host factors, clinical criteria and mycological criteria. Host factors were defined as immunocompromised cases, such as patients post allo-HSCT in this study, while clinical criteria included mold infection related symptoms signs or radiographic evidence compatible with mold infection. Mycological criteria was the identification of organism by histopathological or culture from a contiguous nonsterile site. Possible infection indicates cases meeting the host factors and clinical criteria, but in the absence of mycological criteria.

Transplant details and conditioning regimens

Donor's source choices were matched sibling donors, or alternative donors, including matched unrelated donors, or haploidentical donors. Selecting sibling donors for allo-HSCT was based on low to intermediate resolution of human leukocyte antigen (HLA) typing (HLA—A, —B, —DR or —C), while high resolution of HLA typing was adopted for alternative donors selection. Myeloablative conditioning regimen included busulfan (3.2 mg/kg/day for 4 days) combined with cyclophosphamide (60 mg/kg/day for 2 days), or total body irradiation (TBI) of 12 Gy combined

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with cyclophosphamide (60 mg/kg/day for 2 days). Non-myeloablative conditioning regimen mainly indicated the fludarabine-based chemotherapy for elderly patients or multiple-comorbidity cases.

GVHD prophylaxis and immunosuppressant treatments

Standard protocol for aGVHD prophylaxis was using cyclosporine (i.v. 3.0 mg/kg/day in 2 split doses initially and titrating dosage to maintain trough plasma level at 100–250 ug/L. In addition, short-term low dose methotrexate (15 mg/m^2 on day +1 and then 10 mg/m^2 on day +3, +6 and + 11 after allo-HSCT) was also given for aGVHD prophylaxis. Recipients of unrelated donor transplants would receive additional rabbit anti-thymocyte globulin (2 mg/kg/day) for 3 days. Severity of aGVHD was evaluated according to the system of Glucksberg and Thomas.[15] Severity of the cGVHD was assessed by NIH scoring system, defining the complication as mild, moderated and severe diseases or was categorized into limited or extensive stage.[16,17] Patients experiencing more than grade II aGVHD, allo-immune related lung disease, or extensive cGVHD would usually receive methylprednisolone of 1–2 mg/kg/day. Regarding the immunosuppressant relevant to IMIs analysis, prolonged steroid use is defined as dose of prednisolone or its equivalents $\geq$0.5 mg/day and duration $\geq$30 days.[11] Since cyclosporine is routinely used for aGVHD prophylaxis in our patients, its use is considered as a risk factor for fungal infection only when being resumed after discontinuation or titrated up in management of GVHD.

Antifungal prophylaxis during transplantation

Administration of azoles or echinocandins for prophylaxis of fungal infection was a routine in this cohort. Anti-fungal agent was initiated as start of conditioning and would be maintained during whole transplantation course until engraftment. Prophylaxis for cytomegalovirus (CMV) reactivation after allo-HSCT was not a routine practice in this study. Rather, pre-emptive therapy with ganciclovir was initiated when CMV reactivation was detected by weekly surveillance using a quantitative polymerase chain reaction method. After engraftment, trimethoprim-sulfamethoxazole was prescribed for *Pneumocystis jiroveci* infection prophylaxis in parallel with immunosuppressive therapy of GVHD.

Study endpoints and statistical analysis

Patients' biological data, age, conditioning regimens, co-morbidities, diagnosis before transplantation and IMIs were presented as the total number (*n*) and proportion (%). Results were reported as medians and interquartile ranges (IQR) for skewed data. Mann–Whitney U tests or Fisher's exact tests was adopted to compare patients with or without IMIs and statistical testing was performed using 2-tailed tests. The cumulative incidence of IMIs was estimated accounting for the competing risk of non-mold infection related death. Univariate and multivariate analyses were calculated by Cox proportional hazard models adjusted with other co-morbidities to identify the independent predictors for IMIs. All predictors with *P* value less than 0.1 in the univariate analysis were further entered into the multivariate analysis. Collinearity diagnosis would be performed to exclude the overlapped variables and $P < 0.05$ was considered statistically significant in multivariate analysis. All analyses were performed using SPSS statistical software, version 17.0 (SPSS, Chicago, IL) and STATA 12.

Results

Patients' characteristics

A total of 245 leukemia patients receiving allo-HSCT were collected for analysis. The median follow-up time after allo-HSCT was 16.6 months (IQR: 4.83–48.1) and median age at transplantation was 42 years (IQR: 29–50) in all patients. Acute myeloid leukemia comprised 67% of indications for allo-HSCT; 4% patients had history of invasive fungal infections prior to allo-HSCT, 85% received myeloablative conditioning regimens; 84% used azoles based agents for anti-fungal prophylaxis; 56% had EBMT risk score >2; 8% had habit of smoking. Forty-two percent of patients experienced cGVHD after allo-HSCT, and 26% developed moderate to severe cGVHD. Detail information was demonstrated in Table 1.

Incidence of invasive mold infection after allo-HSCT and outcome

Seventeen of 245 patients developed IMIs with a median time to onset after allo-HSCT of 385 days (IQR: 235–530). The cumulative incidence of mold infections adjusted by competing risk in this cohort was 8.7%, 16.8%, and 23.0% at 6 months, 12 months, and 24 months, respectively (Fig. 1) *Aspergillus* species were the most common pathogen, representing 65% of mold infections, and lung was the mostly involved organ. Pathogens were mostly obtained from sputum culture, while other infection evidences were derived from detection of galactomannan in serum or bronchial lavage. All infection cases were probable. Among these seventeen mold infection patients, three patients (18%) developed grade III-IV aGVHD, while ten (59%) experienced moderate to severe cGVHD. Fourteen patients used azoles as prophylaxis agents, two used echinocandin, and one used voriconazole. Nine patients died of IMIs and the median survival after mold infection was 64 days. Three patients had co-infection with yeast and one was co-infected with Nocardia. Detail information was illustrated in Table 2.

Characteristics for adult leukemia with or without mold infection after allo-HSCT

Table 3 demonstrated the characteristics of patients with or without mold infections after allo-HSCT. The proportion of unrelated donor was relatively higher in patients with IMIs compared to those without mold infections (88% vs. 55%, $P = 0.008$). Patients with IMIs had higher EBMT risk score than those without the infection (82% vs. 54%,

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Table 1 Characteristics of adult leukemia patients receiving allogeneic hematopoietic stem cell transplantation (n = 245).

Characteristics	
Age at SCT, years	42 [IQR: 29—50]
Sex, male, *n* (%)	125 (51)
Diagnosis	
Acute myeloid leukemia, *n* (%)	164 (67)
Acute lymphoid leukemia, *n* (%)	81 (33)
Medium time from diagnosis to SCT, days	192 [IQR: 142—265]
Disease status at SCT	
Remission, *n* (%)	175 (71)
Non-remission, *n* (%)	70 (29)
Donor source	
Sibling donors, *n* (%)	104 (42)
Unrelated donors, *n* (%)	141 (58)
Peripheral blood stem cell, *n* (%)	234 (96)
Bone marrow stem cell, *n* (%)	11 (4)
HLA typing	
Full match, *n* (%)	126 (51)
Mismatch in any antigens or alleles, *n* (%)	119 (49)
Invasive fungal infection prior SCT, *n* (%)	12 (4)
Yeast, proven, *n* (%)	6 (2)
Mold, proven/probable/possible, *n* (%)	1(0.4)/1(0.4)/4(1)
Conditioning regimens	
Fludarabine based conditioning, *n* (%)	34 (14)
TBI based conditioning, *n* (%)	93 (38)
Myeloablative conditioning, *n* (%)	209 (85)
Non-myeloablative conditioning, *n* (%)	36 (15)
Anti-fungal prophylaxis	
Azoles based prophylaxis, *n* (%)	206 (84)
Echinocandins based prophylaxis, *n* (%)	39 (16)
GVHD status	
Acute GVHD, grade III-IV, *n* (%)	38 (16)
Chronic GVHD, any grade, *n* (%)	103 (42)
Moderate to serve chronic GVHD, *n* (%)	64 (26)
Immunosuppressant	
Prolonged steroid used, *n* (%)	66 (27)
Mycophenolate mofetil, *n* (%)	36 (15)
Imuran, *n* (%)	7 (3)
Tacrolimus, *n* (%)	6 (2)
Cyclosporine, *n* (%)	45 (18)
Anti-thymocyte globulin, *n* (%)	7 (3)
EBMT risk score > 2, *n* (%)	137 (56)
Repeat SCT (numbers ≥ 2)	10 (4)
Cytomegalovirus serostatus	
Recipient negative/donor negative, *n* (%)	4 (2)
Recipient positive/donor negative, *n* (%)	18 (7)
Recipient negative/donor positive, *n* (%)	10 (4)
Recipient positive/donor positive, *n* (%)	213 (87)
Cytomegalovirus reactivation, *n* (%)	130 (53)
Smoking, *n* (%)	20 (8)
Diabetic mellitus, *n* (%)	15 (6)

SCT, stem cell transplantation; IQR, interquartile range; HLA, human leukocyte antigen; TBI, total body irradiation; GVHD, graft-versus-host disease; EBMT, European Group for Blood and Bone marrow Transplantation.

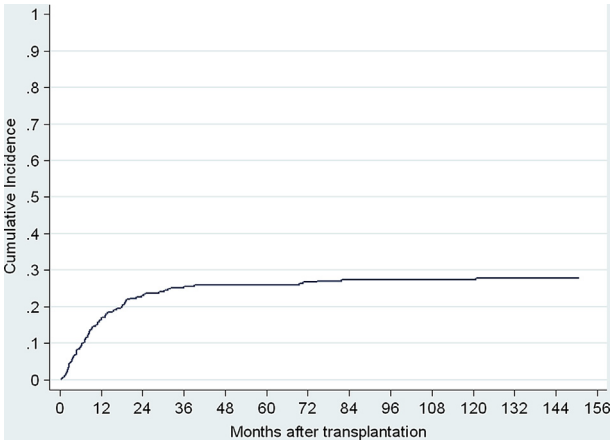

Figure 1. Cumulative incidence of invasive mold infections in adult leukemia patients after allogeneic hematopoietic stem cell transplantation.

$P = 0.023$). The moderate to severe cGVHD was significantly more in mold infection group (59% vs. 24%, $P = 0.001$). In addition, smokers (29% vs. 7%, $P = 0.001$) and prolonged steroid usage (59% vs. 25%, $P = 0.004$) were more common in patients with IMIs. Others relevant factors, such as age, sex, myeloid or lymphoid malignancy, history of invasive fungal infections prior to transplantation, Fludarabine based or myeloablative based conditioning regimens, azoles based prophylaxis, grade III-IV aGVHD, CMV reactivation, and diabetic mellitus were not significantly different between patients with or without IMIs.

Risk factors for adult leukemia with IMIs after allo-HSCT

The univariate analysis revealed the potential risk factors for IMIs development in adult leukemia patients after allo-HSCT as follows: unrelated donors (hazard ratio [HR] 6.02; 95% confidence interval [CI] 1.37—26.32, $P = 0.017$), smoking (HR 5.77; 95% CI 2.00—16.58, $P = 0.001$), EBMT risk score>2 (HR 6.08; 95% CI 1.74—21.23, $P = 0.005$), moderate to severe cGVHD (HR 2.82; 95% CI 1.07—7.41, $P = 0.036$), prolonged steroid usage (HR 2.89; 95% CI 1.10—7.60, $P = 0.031$), and imuran usage (HR 3.60; 95% CI 0.82—15.81, $P = 0.089$). We performed the collinearity diagnosis, and found high variance inflation factor (>10) in prolonged steroid usage and moderate to severe cGVHD. Thus, we preserved moderate to severe cGVHD in multivariate analysis. By multivariate analysis adjusted by age and sex, the significant risk factors for adult leukemia with IMIs development after allo-HSCT were unrelated donor transplantation (HR 5.11; 95% CI 1.05—24.83, $P = 0.043$), smoking (HR 3.55; 95% CI 1.02—12.32, $P = 0.046$), EBMT risk score > 2 (HR 4.22; 95% CI 1.11—16.06, $P = 0.034$), and moderate to severe cGVHD (HR 3.76; 95% CI 1.21—11.73, $P = 0.022$). Detail information was demonstrated in Table 4. The cumulative incidence of IMIs in smokers was significantly higher than non-smokers (log rank $P < 0.001$) and Kaplan-Maier curve was shown in Fig. 2. The characteristics in these 20 smoking patients are presented in supplemental

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Table 2 Clinical characteristics and outcomes in patients with invasive mold infections after allogeneic hematopoietic stem cell transplantation.

No	Sex/age	Dx.	Organism	Onset post SCT. days	Involved organ/ Evidence of infection	Severity of aGVHD (organ/grade)	Type and severity of cGVHD (organ/ grade)	Fungal infection prophylaxis	Smoking	Survival after mold infections days[a]	Outcome
1	F/50	AML	Unidentified mold	75	Lung/Sputum culture	Skin++, grade II	–	Fluconazole	No	1	Died of mold related pneumonia
2	M/31	AML	*Aspergillus*	112	Lung/Serum galactomannan	–	–	Fluconazole	No	937	Co-infection with yeast, alive
3	F/52	AML	*Aspergillus*	142	Lung/Serum galactomannan	Skin++, liver++ Grade III	–	Voriconazole	No	1	Co-infection with yeast Died of leukemia relapse and mold infection
4	M/22	AML	*Penicillium*	205	Lung/Sputum culture	–	Eye, skin/severe	Fluconazole	No	259	Died of pericardial effusion
5	M/67	AML	*Aspergillus*	266	Lung and sinus/ Sputum culture	–	Skin, mucosa, liver/severe	Fluconazole	Yes	1	Died of mold related pneumonia
6	F/44	AML	Unidentified mold	275	Lung/Sputum culture	–	–	Fluconazole	No	216	Died of bacterial sepsis
7	M/42	AML	Unidentified mold	299	Lung/Sputum culture	–	–	Fluconazole	Yes	64	Died of leukemia relapse
8	F/30	AML	*Aspergillus*	313	Angio-invasive aspergillosis of lung/ serum galactomannan	–	–	Fluconazole	No	18	Died of mold related pneumonia, and CMV infection
9	M/50	AML	*Aspergillus*	385	Lung/Bronchial lavage galactomannan	Mucosa+ Grade I	Skin/moderate	Fluconazole	Yes	16	Co- infection with Nocardia, died of mold related pneumonia
10	F/38	ALL	*Aspergillus*	398	Lung with cavity/ Sputum culture	Skin++ Grade I	Lung, eye/severe	Fluconazole	No	172	Died of mold related pneumonia with septic shock
11	F/49	AML	*Aspergillus Penicillium*	428	Lung/Sputum culture	–	Skin, mucosa/ moderate	Fluconazole	No	2027	Died of bacterial pneumonia
12	M/48	AML	*Aspergillus*	453	Lung/Serum galactomannan	Skin+++, GI+++ Grade IV	GI/severe	Fluconazole	Yes	1	Died of mold related pneumonia
13	F/24	AML	*Aspergillus*	508	Lung/Sputum culture	Skin++, GI++ Grade III	Skin, mucosa, eye, lung/severe	Fluconazole	No	17	Died of mold related pneumonia and sepsis
14	M/20	ALL	*Aspergillus*	552	Lung/Serum galactomannan	–	GI/severe	Echinocandin	No	9	Died of hemophagocytic lymphohistiocytosis
15	F/40	ALL	*Penicillium*	553	Lung/Sputum culture	–	Eye, skin/ moderate	Echinocandin	Yes	687	Alive
16	M/31	ALL	*Penicillium*	560	Lung/Sputum culture	Skin+, Grade I	–	Fluconazole	No	169	Died of mold related pneumonia and pericardial effusion
17	F/28	AML	*Aspergillus*	668	Lung/Serum galactomannan	Skin+, GI+ Grade II	Liver, mucosa, skin/severe	Fluconazole	No	385	Co-infection with yeast related liver abscess, alive

[a] The diagnosis of mold infections confirmed immediately at or after death would be presented one day in survival column.

No, Number; Dx, diagnosis; SCT, stem cell transplantation; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; M, male; F, female; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; CMV, cytomegalovirus; GI, gastrointestinal.

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Table 3 Characteristics comparison for adult leukemia with or without mold infections after allogeneic hematopoietic stem cell transplantation.

Characteristics	Allogeneic SCT recipients ($n = 245$)		*P* value
	No mold infection ($n = 228$)	Mold infection ($n = 17$)	
Age at SCT, years	42 [IQR: 30–50]	40 [IQR: 29–50]	0.585
Sex, male, *n* (%)	117 (51)	8 (47)	0.735
Myeloid malignancy, *n* (%)	151 (66)	13 (76)	0.267
Donor source, unrelated donors, *n* (%)	126 (55)	15 (88)	0.008
HLA typing, full-match, *n* (%)	119 (52)	7 (41)	0.381
Invasive fungal infections prior SCT, *n* (%)	11 (5)	1 (6)	0.587
Fludarabine based conditioning, *n* (%)	31 (14)	3 (18)	0.641
TBI based conditioning, *n* (%)	88 (39)	5 (30)	0.452
Myeloablative conditioning, *n* (%)	196 (86)	13 (77)	0.286
EBMT risk score > 2, *n* (%)	123 (54)	14 (82)	0.023
Azoles based prophylaxis, *n* (%)	192 (84)	14 (82)	0.840
Repeat SCT (numbers ≥ 2), *n* (%)	9 (4)	1 (6)	0.697
Acute GVHD, grade III–IV, *n* (%)	35 (15)	3 (17)	0.801
Chronic GVHD, *n* (%)	93 (41)	10 (59)	0.146
Moderate to severe Chronic GVHD, *n* (%)	54 (24)	10 (59)	0.001
Immunosuppressant			
Prolonged steroid usage, *n* (%)	56 (25)	10 (59)	0.004
Mycophenolate mofetil, *n* (%)	32 (14)	4 (24)	0.288
Imuran, *n* (%)	5 (2)	2 (11)	0.078
Tacrolimus, *n* (%)	5 (2)	1 (5)	0.353
Cyclosporine, *n* (%)	42 (18)	3 (18)	1.000
Anti-thymocyte globulin, *n* (%)	7 (3)	0 (0)	1.000
Cytomegalovirus reactivation, *n* (%)	119 (52)	11 (65)	0.319
Smoking, *n* (%)	15 (7)	5 (29)	0.001
Diabetic mellitus, *n* (%)	14 (6)	1 (6)	0.961

IQR, interquartile range; SCT, stem cell transplantation; HLA, human leukocyte antigen; TBI, total body irradiation; EBMT, European Group for Blood and Bone marrow Transplantation; GVHD, graft-versus-host disease.

Table 1. Regarding age, sex, myeloid or lymphoid malignancy, Fludarabine based or myeloablative based conditioning regimens, azoles based prophylaxis, immunosuppressant except prolonged steroid, grade III-IV aGVHD, CMV reactivation, and diabetic mellitus were not significant predictors for IMIs development after allo-HSCT.

Discussion

This cohort study in a single institute showed that the incidence of IMIs after allo-HSCT at 6 months and 12 months was 8.7% and 16.8%, respectively. The results are similar to that reported by Fred Hutchison Cancer Research Center, in which the incidence at 12 months was around 12% during 1998–2002 periods.[7] In an earlier study, Baddley et al. also reported the incidence at 6 months and 12 months was 11% and 15%, respectively, during the period of 1997–1998.[11] In addition, our study revealed the medium onset time of IMIs post allo-HSCT was very late, probably related to the development of cGVHD and disease relapse. By usual definition, the IMIs after transplantation designated as "early" if diagnosed <40 days, "late" if diagnosed 40–100 days and "very late" if diagnosed >100 days after transplant.[7] This shift from early to late IMIs after transplantation have been observed since 1990[5,18] despite improvement in early diagnosis of invasive fungal infection. Grow et al. had reported approximately 40% IMIs developed in late, and 38% in very late.[18] The Prospective Antifungal Therapy Alliance Registry also reported the median time for invasive aspergillosis developments after HSCT was 82 days.[19] Furthermore, an updated prospective, multicenter cohort study of allo-HSCT conducted from 2006 to 2011 revealed the median time for the occurrence of invasive fungal infection was 142 days.[20] In these updated series and latest studies, onset of IMIs becomes more and more late. Our data are in line with this trend with very late development of IMIs, the medium onset time being 385 days. The causes of this phenomenon are probably attributable to the increasingly common use of peripheral blood stem cell source and non-myeloablative conditioning in the last 2 decades, which resulted in shortened duration of post-transplant cytopenia and increased incidence of cGVHD. In these 17 IMIs, 2 patients with no cGVHD developed IMIs within 120 days post transplantation, 10 patients had previous or ongoing moderate to severe cGVHD, and another 2 patients with no cGVHD developed IMIs after disease relapsed. Hence, IMIs in our cohort occurred mainly in patients with cGVHD or disease relapse, leading to very late development of IMIs.

Invasive aspergillosis is the most common IMIs, and it accounts for more than half of mold infections in patients with allo-HSCT in previous reports.[5,7,11,12,21,22] Similar results were observed in our study, and the most commonly involved body sites by *Aspergillus* were lung and paranasal sinus.[8,21,23] It usually caused severe respiratory dysfunction and multiple morbidities. Aspergillosis remains the major

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Table 4 Risk factors for invasive mold infections in adult leukemia after allogeneic hematopoietic stem cell transplantation.

Predictive variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age > 30 years	0.956 (0.337–2.715)	0.933	0.673 (0.197–2.296)	0.527
Sex, Male	0.955 (0.368–2.478)	0.925	0.848 (0.295–2.438)	0.760
Myeloid malignancy	1.970 (0.642–6.048)	0.236		
HLA mismatch	1.471 (0.559–3.871)	0.434		
Invasive fungal infections prior SCT	1.399 (0.185–10.560)	0.745		
Unrelated donor transplantation	6.020 (1.376–26.32)	0.017	5.114 (1.053–24.839)	0.043
Fludarabine based conditioning	1.814 (0.520–6.328)	0.350		
TBI based conditioning	1.591 (0.560–4.519)	0.383		
Myeloablative conditioning	0.439 (0.143–1.348)	0.150		
Repeat SCT (numbers ≥ 2)	3.568 (0.471–27.04)	0.218		
Diabetic mellitus	0.976 (0.129–7.378)	0.981		
Smoking	5.771 (2.008–16.584)	0.001	3.554 (1.025–12.320)	0.046
EBMT risk score > 2	6.085 (1.744–21.234)	0.005	4.225 (1.112–16.063)	0.034
Azoles based prophylaxis	0.876 (0.252–3.053)	0.836		
Acute GVHD, grade III-IV	2.687 (0.764–9.452)	0.124		
Moderate to severe chronic GVHD	2.820 (1.073–7.414)	0.036	3.769 (1.211–11.730)	0.022
Prolonged steroid usage	2.891 (1.100–7.600)	0.031		
Mycophenolate mofetil	1.698 (0.553–5.214)	0.355		
Imuran	3.606 (0.823–15.81)	0.089	1.321 (0.233–7.472)	0.753
Tacrolimus	1.996 (0.264–15.073)	0.503		
Cyclosporine	1.293 (0.371–4.503)	0.687		
Cytomegalovirus reactivation	1.698 (0.627–4.598)	0.297		

HR, hazard ratio; CI, confidence interval; HLA, human leukocyte antigen; SCT, stem cell transplantation; TBI, total body irradiation; EBMT, European Group for Blood and Marrow Transplantation; GVHD, graft-versus-host disease.

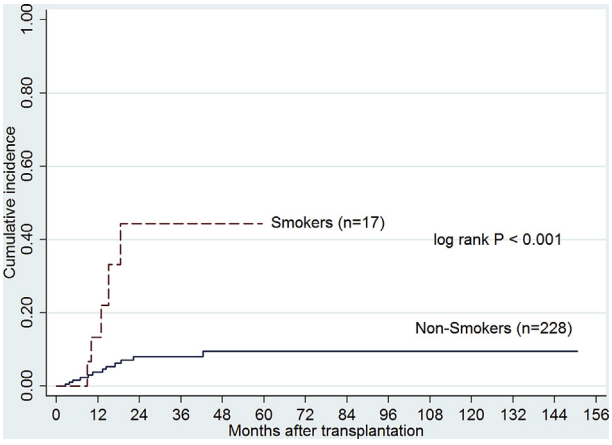

Figure 2. Cumulative incidence of invasive mold infections in smokers and non-smokers.

cause of mortality and morbidities for patients receiving allo-HSCT despite advanced diagnostic tools and antibiotic treatment. Early detection of invasive aspergillosis by image, such as cavitation, or nodules in chest computed tomography[24] provides an important clue for prompt early treatment. Besides, galactomannan assay is an emerging tool to detect *Aspergillus* infection[25] with a sensitivity and specificity of 92% and 95%, respectively for the infection.[26] EORTC/MSG had adopted the galactomannan assay as one criterion for diagnosis of probable invasive aspergillosis infection in the revised consensus published in 2008.[14,27]

Here, our study used the galactomannan assay to detect probable IMIs for adult leukemia patients post allo-HSCT. Approximately 63% (7/11) of invasive aspergillosis was detected by galactomannan assay in our study, indicating its role in diagnosing mold infection in leukemia patients after transplantation. In addition to *Aspergillus*, the second common pathogens among IMIs are variable by studies. According to Baddley et al. report, in addition to twelve patients with *Aspergillus*, the other three patients were infected by *Microascus cinereus*, *Scedosporium apiospermum*, and *Mucor* species.[11] An European study revealed that the *Zygomycetes* and *Fusarium* species accounted for 4% of cases and the other remaining IMIs resulted from *Scedosporium*, *Acremonium*, *Penicillium*, and *Cladosporium* species.[21] Another study also reported *Aspergillus* species was the most common pathogen followed by *Fusarium* species, *Zygomycetes*, *Scedosporium* species, and *Acremonium* species.[7] However, the data in our study was quite different with *Penicillium* being the second common pathogen of IMIs for leukemia patients after allo-HSCT in Asian population. This difference may be attributed from endemically mycological variation and *Penicillium* is more common in Southeast Asian, including Taiwan.[28,29]

We noticed unrelated donor transplantation, moderate and severe cGVHD, EBMT risk score >2 and smoking are significant risk factors for IMIs development after allo-HSCT. Extensive cGVHD or moderate to severe cGVHD had been identified as an important risk factor for IMIs in many studies.[7,8,11,12] The prolonged use of immunosuppressant and long-term steroid for cGVHD would lead to IMIs development.[11] Frequent surveillance should be done for

Please cite this article in press as: Chien S-H, et al., Invasive mold infections in acute leukemia patients undergoing allogeneic hematopoietic stem cell transplantation, Journal of Microbiology, Immunology and Infection (2018), https://doi.org/10.1016/j.jmii.2018.09.006

patients with cGVHD and anti-fungal prophylaxis may be indicated for patients receiving long-term immunosuppressant and steroid therapy. Besides, pre-transplant assessment is also important to predict outcome of patients with allo-HSCT. Here, we identify two pre-transplant risk factors for IMIs development—EBMT risk sore >2 and smoking. The EBMT risk score is determined by age of patient, disease stage, time interval from diagnosis to transplant, donor recipient sex combination and donor types.[13] This score was initially created by Chronic Myeloid Leukemia Working Party of the European Group for Blood and Marrow Transplantation in 1998.[30] It had been modified and validated in various hematological diseases in the following fifteen years. Non-relapse mortality and poor overall survival are correspondingly associated with high EBMT risk score.[13,31–34] Our study is the first report to validate the value of EBMT risk score in predicting IMIs development after transplantation. The risk score is relatively simple and easy to use, providing rapid assessment before transplantation.

Another important pre-transplant risk for IMIs development in our study is smoking. As early as 1971, a report revealed the cigarettes were contaminated with various fungi, while the *Aspergillus* was the most prominent fungus.[35] Another recent study collecting 98 cigarettes from 14 different commercial brands also documented *Aspergillus fumigatus* as the most common isolated mold organism.[36] This study also found that tobacco was heavily contaminated with fungal spores, and approximately 270 viable fungal spores may be present in a single cigarette.[36] Besides, smoking also have immunosuppressive effects on systemic immunity with skewed both innate and adaptive immune response.[37] It damages lung surfactant proteins, causes the structural and function changes in the respiratory ciliary epithelium, and inhibits immune cells, such as neutrophils, alveolar macrophages, lymphocyte and natural killer cells.[38] Therefore, smoking may lead to fungi spores being directly inhaled into lungs, has negative effects on host respiratory defense mechanism as well as immune system, resulting in severe complication in immunocompromised patients, such as those experiencing allo-HSCT. Many previous studies have noted that exposure to marijuana or cigarette was associated with *Aspergillus*,[39] and an updated study also found acute myeloid leukemia or myelodysplastic syndrome patients with smoking had 9-fold increased risk for development of invasive fusariosis after transplantation.[40] Therefore, we suggest all patients should quit smoking as treatments start and transplantation is planned.

Our study shares the inherent limitations of retrospective cohort study. First, this is a single institute, retrospective cohort study, limited by relatively small number. Second, all IMIs in our study were probable cases because most patients with IMIs suffered from critically respiratory distress and invasive procedures for proving mold infection were dangerous and difficult to perform. Accordingly, we adopted the galactomannan assay instead of conventional biopsy. Although evidence level of this serum analysis is only probable according to EORTC/MSG, it's relatively safe, non-invasive and provides results rapidly. However, it requires further study for validation of galactomannan assay and marker guided pre-emptive antifungal therapy in transplantation patients. Third, around one quarter of infections was un-identified mold due to limited sample obtained and problems of culture technique. Although these un-identified molds could not offer further mycology information, the conclusion of *Aspergillus* most common, followed by *Penicillium* still remains unchanged. More rapid and precise molecular diagnosis for mold infection in transplantation needs to be developed in the future. Forth, although smoking is identified as a risk factor for IMIs development in multivariate analysis, only 20 of 245 patients had smoking. Relatively small number cases may limit the accuracy of this analysis. Finally, immunosuppressant may have influence on IMIs, but it is difficult to analyze this factor owning to variable treatments during disease course, particularly in a retrospective study.

In conclusion, our study updates the mycology and clinical features of mold infections in adult leukemia patients undergoing current allo-HSCT care. In Southern Asian population, *Aspergillus* remains the most common pathogen, followed by *Penicillium*. Extensive cGVHD as well as using unrelated donors' stem cell are significant risk factors for IMIs development, while EBMT risk score >2 as well as smoking are important pre-transplant risk factors to predict development of IMIs post transplantation. To identify the risk factors of IMIs and persuade the patient to quit smoking before transplantation is critically important. In addition to quitting smoking, adoption of appropriate prophylaxis strategies against IMIs for high risk patient needs further study in the future.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgment

This work was partially supported by grants from the Taipei Veterans General Hospital-National Yang-Ming University Excellent Physician Scientists Cultivation Program, No. 105-V-B-113, Taipei Veterans General Hospital (grant no. V106C-193), the Taiwan Clinical Oncology Research Foundation, and the Chong Hin Loon Memorial Cancer and Biotherapy Research Center. The funding sources had no role in the study design or conduct, or in the decision to submit it for publication. We thank Hsin-Yi Huang from the Biostatistics Task Force, Taipei Veterans General Hospital, for the statistical assistance.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jmii.2018.09.006.

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