ORIGINAL PAPER

Invasive fungal disease is associated with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplant: a single center, retrospective study

Hua Jin[1] · Zhiping Fan[1] · Fen Huang[1] · Yanyan Chai[1] · Li Xuan[1] · Ren Lin[1] · Na Xu[1] · Jieyu Ye[1] · Jing Sun[1] · Qifa Liu[1,2]

Abstract

Background Invasive fungal disease (IFD) and graft-versus-host disease (GVHD) are major causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the impacts of IFD on chronic GVHD remain unknown.

Methods We conducted a retrospective study of 510 patients with hematologic malignancy undergoing allo-HSCT to explore the effects of IFD on chronic GVHD.

Results The 2-year cumulative incidences of overall (limited and extensive) and extensive chronic GVHD post-transplantation were higher in patients with IFD compared with those without IFD (69.5% ± 4.2% versus 32.9% ± 2.4%, $P < .001$; 43.0% ± 5.2% versus 6.6% ± 1.4%, $P < .001$, respectively). Moreover, the patients with IFD had higher 5-year transplant-related mortality, lower 5-year overall survival and lower 5-year disease-free survival (29.8% ± 4.3% versus 9.8% ± 1.6%, $P < .001$; 50.5% ± 4.9% versus 71.3% ± 2.4%, $P < .001$ and 48.8% ± 4.7% versus 71.8% ± 2.3%, $P < .001$, respectively). Multivariable analyses demonstrated that IFD increased the risk of chronic GVHD.

Conclusion Our results suggest that IFD significantly contributes to the development of chronic GVHD after allo-HSCT.

Keywords Invasive fungal disease · Graft-versus-host disease · Allogeneic hematopoietic stem cell transplantation

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative therapy for various hematological malignancies. Infections and graft-versus-host disease (GVHD) are the main causes of morbidity and mortality after allo-HSCT. And their close relationship, especially bacterial and viral infections, is gaining more and more attentions in recent years [1–3]. Invasive fungal disease (IFD) is a common complication in the recipients of allo-HSCT. That acute and chronic GVHD increase the risk of IFD in the recipients of allo-HSCT has been widely demonstrated [4, 5]. However, the effects of IFD on GVHD have received scant attention. Sporadic studies suggested that injection of heat-killed *Candida albicans* exacerbated lung acute GVHD in mouse models and colonization of *C. albicans* exacerbated gastrointestinal acute GVHD in the recipients of allo-HSCT [6–8]. It is rarely reported whether IFD can affect the development of chronic GVHD in the patients undergoing transplantation. This large retrospective study was performed to evaluate the effects of IFD on chronic GVHD in the patients undergoing transplantation.

Methods

Study design

We conducted a single center, retrospective, and observational cohort study. All consecutive patients with

✉ Qifa Liu
liuqifa628@163.com

1 Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, China

2 Guangdong Provincial Key Laboratory of Construction and Detection in Tissue Engineering, Southern Medical University, Guangdong, China

hematological malignancies who underwent first allo-HSCT between January 2011 and December 2015 at Nanfang Hospital were analyzed in this retrospective study. This study was performed to explore the effects of IFD on chronic GVHD. The primary endpoint for the study was chronic GVHD. The secondary endpoints included relapse, transplant-related mortality (TRM), overall survival (OS), and disease-free survival (DFS).

Patients

Patients were excluded if they did not live longer than 2 weeks post-transplantation or donor lymphocyte infusion (DLI) was used for prophylactic or therapeutic purposes. With the exception of 174 patients who received DLI and six patients who died within 2 weeks post-transplantation, a total of 510 patients were included. The median follow-up for surviving patients is 47 months (range 16–67 months) after allo-HSCT. This study was performed in accordance with the Declaration of Helsinki and was approved by the local institutional review board. All patients and donors gave written informed consent to participate in the study.

HLA typing and donor source

High-resolution DNA typing for HLA-A, -B,-C, -DRB1 and -DQB1 was performed for all patients and donors. Patients received transplants from an HLA-matched sibling, a matched unrelated or a mismatched family donor according to donor availability and allo-HSCT timing. HLA-matched sibling donor (MSD) was the first choice. If MSD was unavailable, a suitable HLA-matched unrelated donor (MUD) was used as an alternative. Patients without a suitable MSD or MUD were eligible for HLA-haploidentical-related donor (HRD) transplantation [9, 10].

Conditioning and transplantation

Conditioning regimens comprised of myeloablative conditioning (MAC) or intensified conditioning regimen. The detailed conditioning regimen has been described previously [9, 11]. The selection of conditioning regimens was based on diagnosis and disease status at transplantation. Generally, the patients with high-risk genetics or with non-remission (NR) received intensified conditioning; the patients with intermediate/low-risk genetics who were in complete remission (CR) received MAC [9, 11–13]. All patients in the HRD group received a combination of peripheral blood stem cell (PBSC) and bone marrow (BM) grafts. Most patients in the MSD group and all patients in the MUD group received PBSC grafts [9].

GVHD prophylaxis

Cyclosporine A (CsA)+ a short course of methotrexate (MTX) were administered to the patients undergoing MSD transplant for GVHD prophylaxis. CsA + MTX + antithymocyte globulin (ATG) were administered to the patients undergoing MUD transplants, and CsA + MTX + ATG + mycophenolate mofetil (MMF) were administered to the patients undergoing HRD transplants for GVHD prophylaxis [9].

IFD prophylaxis and treatment

All patients received antifungal prophylaxis. With the exception of patients who entered in antifungal preventive clinical trials, patients usually received fluconazole for primary antifungal prophylaxis depending on our clinical practice guideline. The secondary antifungal prophylaxis (SAP) agents were chosen based on treatment response to initial antifungal therapy [14]. Primary antifungal prophylaxis was given 5 days pre-transplantation and continued for +30 to +90 days after transplantation. SAP was given 5 days pre-transplantation until 90 days after transplantation in patients with stable IFD. In patients with active IFD, SAP was given 5 days pre-transplantation until eradication or stability of residual foci [14, 15]. If patients with acute GVHD received a second line anti-GVHD therapy after the period of primary antifungal prophylaxis, antifungal prophylaxis would be given again. Patients suspected of IFD were given antifungal treatment. The antifungal treatment was depended on the discretion of the attending physicians according to disease activity and therapeutic response. Once breakthrough IFD was diagnosed, antifungal therapy would be modified [14].

Definitions

The diagnosis of IFD was according to the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) revised definitions of 2008 [16]. Proven disease required histopathologic or microbiologic documentation of disease from biopsied tissues, and infection was considered probable if the fungus was identified from culture of bronchoalveolar lavage fluid or sputum when consistent clinical features were present. Possible IFD was defined as clinical or radiologic features suggestive of IFD but lacking mycological evidence [5, 17, 18]. Patients with proven or probable IFD were considered to be case patients. Responses to antifungal treatment were classified into complete responses (CR), partial responses (PR), stable responses, and failure of therapy [19]. Patients who received antifungal treatment and achieved CR and PR were considered as effective. Patients with possible IFD were also

considered to be case patients if antifungal treatment was effective, otherwise not. Patients with stable IFD at transplantation were those who achieved CR after antifungal treatment-no fungal lesion by CT detection, as well as negative GM or G test. Patients with active IFD at transplantation were those who achieved PR or stable responses after antifungal treatment [14]. Chronic GVHD was defined as no, limited or extensive chronic GVHD according to standard criteria [20, 21]. Relapse was defined by reappearance of blasts in the peripheral blood, recurrence of BM blasts > 5%, or development of extramedullary disease infiltrates at any site. TRM was defined as death from any cause except relapse. OS was defined as the time from transplantation to death from all causes. DFS was defined as the time from transplantation to relapse or death in remission.

Sample size and missing data

It is difficult to determinate the sample size of observational study, especially in multiple regression model settings [22]. We had used the rule of thumb recommended by Peduzzi et al. [23] and Harrell et al. [24], namely, events per variable being ten or greater under this circumstance. We considered about 12–13 significant clinical factors in developing a model. This have required us to recruit a minimum of 130 (13 × 10) participants who had events to predict the development of cGVHD. There were no missing data in this study.

Statistical analysis

A descriptive analysis of all variables was performed including median, interquartile range, minimum and maximum value for continuous variables, and numbers and frequencies for categorical variables. The probabilities of DFS and OS were calculated with the Kaplan–Meier method, and difference between groups was compared using the log-rank test [25]. Probabilities of chronic GVHD, TRM and relapse were estimated with the use of cumulative incidence curves. Probabilities of chronic GVHD were estimated by regarding TRM and relapse as the competing events, whereas probabilities of TRM were estimated by regarding relapse as the competing events. In the analysis of cumulative incidence of IFD post-transplantation, death was considered as a competing event [26]. The cumulative incidence and its standard error was reported. Difference between groups was compared using the Gray test [27]. Risk factors associated with chronic GVHD, relapse, DFS and OS were analyzed using multivariable Cox proportional hazard models with the inclusion of factors previously found to affect outcome in patients undergoing transplantation. Competing events aforementioned were also considered when exploring risk factors associated with chronic GVHD and relapse.

Post-transplantation variables (IFD and CMV DNA) were analyzed as time-dependent covariates. Those patients who developed IFD after the diagnosis of chronic GVHD were considered as non-IFD patients when evaluating the effect of IFD post-transplantation on chronic GVHD. Those patients were included in the analysis to avoid potential patient selection bias. For categorical variables, the Chi-square statistic or Fisher's exact test were used to establish differences in their distribution; the Mann–Whitney U test was used to compare continuous variables. Tests of significance were 2-sided, with a significance P level of 0.05 or less. All statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL) and R version 3.3.0 (R Development Core Team, Vienna, Austria).

Results

Patient and transplant characteristics

A total of 690 consecutive patients underwent an allo-HSCT over the period of the study. With the exception of 174 patients who received DLI and six patients who died within 2 weeks post-transplantation, a total of 510 patients were enrolled. The patients had a median age of 28 years (range 13–63 years), with 309 males and 201 females. The underlying diseases included acute myeloid leukemia ($n = 224$), acute lymphoid leukemia ($n = 174$), myelodysplastic syndrome ($n = 38$), chronic myeloid leukemia ($n = 52$) and lymphoma ($n = 22$). Three hundreds and eighty-six patients achieved CR and 124 patients were in NR at the time of transplantation. Two hundreds and sixty-two patients received MSD transplantation, 123 HRD and 125 MUD transplantations. Of the 510 patients, 116 patients had a history of IFD pre-transplantation, including 81 patients with stable IFD and 35 patients with active IFD at the time of transplantation. Three hundreds and ninety-four patients received primary antifungal prophylaxis, including 244 patients received fluconazole, 80 patients received voriconazole and 70 patients received itraconazole. One hundred and sixteen patients received secondary antifungal prophylaxis (45 voriconazole, 37 itraconazole, 13 caspofungin, 17 micafungin, 3 amphotericin B, 1 amphotec). Sixty patients with acute GVHD who received a second-line anti-GVHD therapy were given antifungal prophylaxis again. The patients' and transplants' characteristics of IFD group and non-IFD groups are shown in (Table 1). Compared with non-IFD group, patients in IFD group have several characteristics, including older age, NR at the time of transplantation, intensified conditioning, polyenes prophylaxis and history of IFD pre-transplant.

Table 1 Patients' and transplants' characteristics of the study cohort

Characteristic	IFD	Non IFD	P value
Overall, no. (%)	118 (23.1)	392 (76.9)	
Age (years), median (interquartile range)	30 (21–39)	28 (20–37)	**0.037**
Gender, no. (%)			0.453
Male	68 (57.6)	241 (61.5)	
Female	50 (42.4)	151 (38.5)	
Underlying disease, no. (%)			0.832
AML	50 (42.4)	174 (44.4)	
ALL	43 (36.4)	131(33.4)	
Other[a]	25 (21.2)	87 (22.2)	
Stage of underlying disease, no. (%)			**0.023**
CR	80 (67.8)	306 (78.1)	
NR	38 (32.2)	86 (21.9)	
Source of stem cell, no. (%)			0.983
PBSC	82 (69.5)	272 (69.4)	
PBSC + BM	36 (30.5)	120 (30.6)	
Transplant type, no. (%)			0.623
MSD	56 (47.4)	206 (52.6)	
HRD	31 (26.3)	92 (23.4)	
MUD	31 (26.3)	94 (24.0)	
Conditioning regimen, no. (%)			**0.012**
Myeloablative	55 (46.6)	234 (59.7)	
Intensified	63 (53.4)	158 (40.3)	
GVHD prophylaxis, no. (%)			0.988
ATG-based	58 (49.2)	193 (49.2)	
Non-ATG based	60 (50.8)	199 (50.8)	
IFD prophylaxis[b]			**0.003**
Polyenes	3 (2.5)	1 (0.3)	
Azoles	103 (87.3)	373 (95.1)	
Echinocandins	12 (10.2)	18 (4.6)	
History of IFD pre-transplant, no. (%)			**0.041**
No	83 (70.3)	311 (79.3)	
Yes	35 (29.7)	81 (20.7)	

Bold values indicate *P* < 0.05

IFD Invasive fungal disease, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CR* complete remission, *NR* nonremission, *PBSC* peripheral blood stem cell, *BM* bone marrow, *MSD* matched sibling donor, *HRD* haploidentical related donor, *MUD* matched unrelated donor, *ATG* antithymocyte globulin, *GVHD* graft-versus-host disease

[a]The other category included myelodysplastic syndrome, chronic myeloid leukemia and lymphoma

[b]The azoles category included fluconazole, voriconazole, and itraconazole. The echinocandins category included caspofungin and micafungin. The polyenes category included amphotericin B and amphotec

IFD post-transplantation

Within 1 year post-transplantation, 118 patients experienced the 143 episodes of IFD, including 35 patients with a history of IFD pre-transplantation. Of the 35 patients with a history of IFD, 25 patients had stable IFD and ten patients had active IFD at the time of transplantation. Of the 143 episode of IFD, 96 patients had one episode of IFD, 19 patients had two episodes of IFD and three patients had three episodes of IFD. The 1-year cumulative incidence of first episode of IFD was 21.0% ± 1.8%. Of the patients with one episode of IFD, IFD was proven in 28, probable in 53 and possible in 37 patients. Among these proven IFD, *Aspergillosis* was the leading IFD (20 episodes), followed by *Candida* spp. (seven episodes) and *Penicillium* (one episode). Of the 35 patients with a history of IFD pre-transplantation, seven patients developed a new episode of fungal infection, including three during the prophylaxis and four after the SAP. And 28 patients presented IFD relapse, including 19 during the prophylaxis and nine after of SAP. Of the 22 patients who developed breakthrough IFD, six patients developed breakthrough IFD during acute GVHD.

All the 118 patients who experienced IFD after transplantation received antifungal treatment. For their initial treatment of IFD, 41 patients received voriconazole, 23 received itraconazole, nine received caspofungin, seven received micafungin, ten received amphotericin B, and 28 received a combination of two agents (i.e., voriconazole and amphotericin B in 11, voriconazole and micafungin in ten, caspofungin and voriconazole in seven). After therapy, 74 patients achieved CR, 27 patients achieved PR, five patients achieved stable responses and 12 patients died of IFD.

The effects of IFD on chronic GVHD

One hundred and seventy patients developed grades II–IV acute GVHD (38 grades IIII–IV acute GVHD) at a median time of 25 days (range 11–204 days) post transplantation. The overall cumulative incidences of grades II–IV and grades III–IV acute GVHD at 100 days post-transplantation were 30.4% ± 2.0% and 8.1% ± 1.3%, respectively. Two hundred and ten patients developed chronic GVHD (143 limited and 67 extensive) at a median time of 143 days (range 73–680 days) post-transplantation. The overall cumulative incidence of overall (limited and extensive) chronic GVHD at 2 years post-transplantation was 41.4% ± 2.2%, and the 2-year cumulative incidence of extensive chronic GVHD was 14.4% ± 1.7%. The 2-year cumulative incidence of overall chronic GVHD post-transplantation among patients with IFD and without IFD was 69.5% ± 4.2% and 32.9% ± 2.4%, respectively (*P* < .001; Fig. 1a), and that of extensive chronic GVHD was 43.0% ± 5.2% and 6.6% ± 1.4%, respectively (*P* < .001; Fig. 1b).
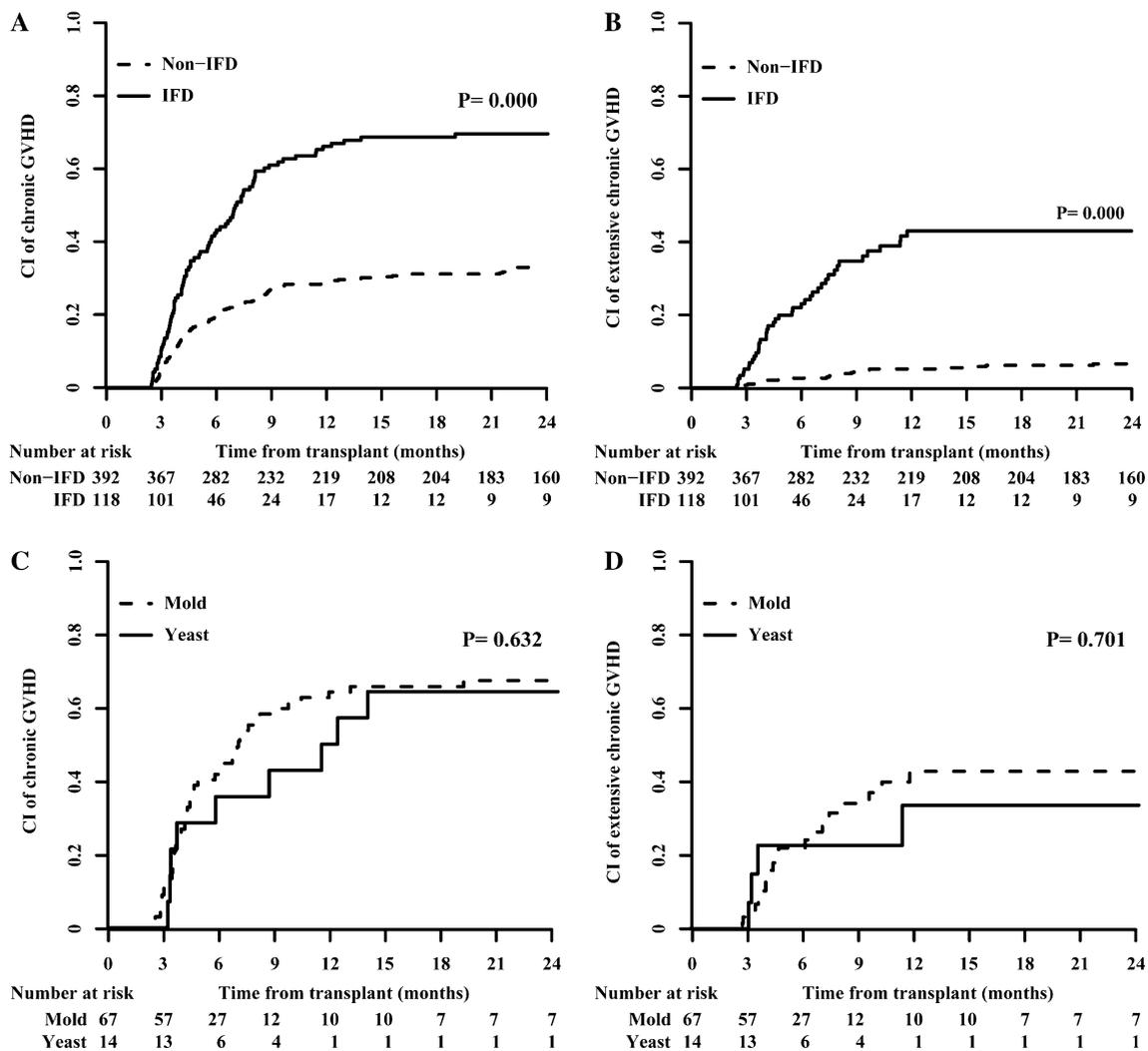
Fig. 1 Impact of IFD post-transplantation on the cumulative incidences of overall chronic GVHD (**a**) and extensive chronic GVHD (**b**). Impact of mold and yeast post-transplantation on the cumulative incidences of overall chronic GVHD (**c**) and extensive chronic GVHD (**d**)

Among the 118 patients who experienced IFD after transplantation, 82 patients developed chronic GVHD. The median time from onset of IFD to the onset of chronic GVHD was 90 days (range 17–387 days). Of 81 patients with proven and probable IFD, mold was 67 cases, yeast was 14 cases. The cumulative incidence of overall and extensive chronic GVHD between the mold and yeast groups has no differences ($P = .632$, Fig. 1c, $P = .701$, Fig. 1d). In the multivariable analysis, patient age, intensified conditioning regimen, non-ATG-based GVHD prophylaxis, IFD post-transplantation, and acute GVHD II–IV were associated with an increased cumulative incidence of chronic GVHD (Table 2). Multivariable analyses demonstrated that IFD was an independent risk factor for chronic GVHD.

Survival

At a median follow-up of 1004 days (range 61–2041 days) post-transplantation, 347 patients survived and 163 patients died. Causes of death included relapse (93 cases), infections (36 cases, including 15 bacterial infection, 12 fungal infection, five EBV-associated post-transplant lymphoproliferative disease (PTLD) and four CMV-associated diseases), nine acute GVHD, 16 chronic GVHD, and nine others. The overall 3-year cumulative incidence of relapse was $19.3\% \pm 1.8\%$. There were no significant differences in 3-year cumulative incidence of relapse between IFD group and non-IFD group ($20.7\% \pm 3.8\%$ versus $18.9\% \pm 2.0\%$, $P = .447$; Fig. 2a). The overall 5-year estimated probability of TRM post-transplantation was $14.5\% \pm 1.6\%$. The

Table 2 Multivariable analysis of risk factors for chronic GVHD

Factors	Chronic GVHD	
	HR (95% CI)	P
Patient age (years)		
≤28 versus ≥28	1.72 (1.25–2.35)	**0.001**
Donor age (years)		
≤31 versus ≥31	1.07 (0.79–1.45)	0.673
Gender		
Male versus female	0.80 (0.59–1.07)	0.127
Underlying disease		
AML	1.00	
ALL	1.17 (0.84–1.64)	0.357
Others[a]	0.94 (0.63–1.40)	0.762
Stage of underlying disease		
CR versus NR	0.77 (0.54–1.10)	0.152
Transplant type		
MSD	1.00	
HRD	1.73 (0.90–3.35)	0.102
MUD	1.63 (0.87–3.03)	0.127
Conditioning regimen		
Myeloablative versus intensified	1.39 (1.01–1.91)	**0.045**
GVHD prophylaxis		
ATG-based versus non-ATG-based	2.38 (1.30–4.35)	**0.005**
History of IFD pre-transplant		
No versus yes	0.75 (0.52–1.07)	0.112
IFD post-transplant		
No versus yes	1.78 (1.24–2.56)	**0.002**
CMV DNA		
Negative versus positive	1.10 (0.82–1.48)	0.522
Acute GVHD		
Grades 0–I versus Grades II–IV	1.94 (1.45–2.60)	**0.000**

Bold values indicate $P < 0.05$

HR Hazard ratio, *CI* confidence interval, *GVHD* graft-versus-host disease, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CR* complete remission, *NR* nonremission, *MSD* matched sibling donor, *HRD* haploidentical related donor, *MUD* matched unrelated donor, *ATG* antithymocyte globulin, *IFD* invasive fungal disease, *CMV* cytomegalovirus

[a]The other category included myelodysplastic syndrome, chronic myeloid leukemia and lymphoma

5-year TRM post-transplantation was significantly higher in patients with IFD compared to those without IFD (29.8% ± 4.3% versus 9.8% ± 1.6%, $P < .001$, Fig. 2b). The 5-year probability of DFS for all patients was 66.4% ± 2.2%: 48.8% ± 4.7% for patients with IFD and 71.8% ± 2.3% for patients without IFD ($P < .001$, Fig. 2c). The 5-year probability of OS for all patients was 66.9% ± 2.2%: 50.5% ± 4.9% for patients with IFD and 71.3% ± 2.4% for patients without IFD, respectively ($P < .001$, Fig. 2d). The 5-year DFS and OS benefit were highly significant among patients without IFD. In the multivariable analysis, stage of underlying

disease NR, MUD transplant, and acute GVHD III–IV were risk factors associated with a higher relapse. For DFS, stage of underlying disease NR, MUD transplant, IFD post-transplantation, acute GVHD III–IV, and extensive chronic GVHD were risk factors associated with a worse DFS. For OS, stage of underlying disease NR, MUD transplant, IFD post-transplantation, CMV DNA positive, acute GVHD III–IV, and extensive chronic GVHD were risk factors associated with a worse OS (Table 3).

Discussion

It has been well demonstrated that GVHD is associated with an increased risk of IFD in allo-HSCT [5, 18, 28]. In the studies by Takahiro et al. [5] and Amzim et al. [18], grades III–IV acute GVHD increased the incidence of invasive mold infections. Marr et al. [17] reported that clinically extensive chronic GVHD was associated with increased risks of invasive aspergillosis more than 6 months after transplantation. Pagano et al. [29] also reported that risk of IFD appears particularly prominent in patients with either steroid-refractory/dependent acute GVHD or chronic GVHD, particularly if it developed as a late complication of acute GVHD. Furthermore, when uncontrolled GVHD coincides with IFD, the prognosis will be dismal, as suppression of the host defense mechanisms to control GVHD will be inevitable [30, 31]. Our results are in line with these studies that grades II–IV acute GVHD and chronic GVHD were significant risk factors for IFD (supplemental Table 1). However, the effects of IFD on GVHD have received attentions until recent years. In animal models, Hidetaka et al. showed that injection of heat-killed C albicans exacerbated acute GVHD, particularly in the lung [6]. In the patients undergoing allo-HSCT, Marr et al. [32] and Vander et al. [8] both reported that fluconazole prophylaxis resulted in a decreased incidence and severity of acute GVHD, particularly in the gut. It is rarely reported whether IFD can affect the development of chronic GVHD in the patients undergoing transplantation. In our study, we demonstrated that IFD post-transplantation was an independent risk factor for chronic GVHD. The cumulative incidences of overall and extensive chronic GVHD were significantly higher in patients with IFD compared with those without IFD. To our knowledge, this is the first human data exploring the effects of IFD on chronic GVHD.

The mechanism that bacteria and virus increase the risk of GVHD might be due to inflammation and immune activation by loss of microbial diversity and by viral replication [33, 34]. It has been reported that innate immune activation by the viral PAMP poly I:C potentiates pulmonary GVHD [35]. Antibodies against human CMV late protein UL94 has been reported to participate in the pathogenesis of scleroderma-like GVHD [33]. The mechanism that EBV

Table 3 Multivariable analysis of risk factors for relapse, DFS and OS

Factors	Relapse		DFS		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Patient age (years)						
≤ 28 versus ≥ 28	1.45 (0.93–2.28)	0.104	1.25 (0.88–1.77)	0.216	1.20 (0.84–1.71)	0.325
Donor age (years)						
≤ 31 versus ≥ 31	0.93 (0.60–1.44)	0.743	1.05 (0.75–1.46)	0.782	1.10 (0.79–1.55)	0.569
Gender						
Male versus female	0.83 (0.54–1.28)	0.398	0.78 (0.56–1.09)	0.144	0.72 (0.51–1.01)	0.059
Underlying disease						
AML	1.00		1.00		1.00	
ALL	1.21 (0.74–1.99)	0.440	1.01 (0.68–1.50)	0.952	1.03 (0.69–1.53)	0.896
Others[a]	0.62 (0.32–1.18)	0.147	0.85 (0.54–1.33)	0.469	0.80 (0.50–1.27)	0.347
Stage of underlying disease						
CR versus NR	1.97 (1.22–3.17)	**0.006**	1.54 (1.06–2.22)	**0.022**	1.53 (1.05–2.22)	**0.026**
Transplant type						
MSD	1.00		1.00		1.00	
HRD	1.32 (0.50–3.52)	0.574	1.36 (0.66–2.78)	0.404	1.34 (0.65–2.74)	0.429
MUD	2.41 (1.00–5.80)	**0.049**	2.24 (1.12–4.47)	**0.022**	2.13 (1.06–4.28)	**0.035**
Conditioning regimen						
Myeloablative versus intensified	1.04 (0.64–1.70)	0.862	1.11 (0.77–1.62)	0.569	1.09 (0.75–1.59)	0.657
GVHD prophylaxis						
ATG-based versus non-ATG-based	1.73 (0.72–4.13)	0.217	1.75 (0.89–3.43)	0.106	1.74 (0.88–3.44)	0.111
History of IFD pre-transplant						
No versus yes	1.55(0.97–2.46)	0.065	1.39 (0.96–2.00)	0.080	1.33(0.92–1.93)	0.133
IFD post-transplant						
No versus yes	0.99 (0.55–1.78)	0.984	1.98 (1.35–2.89)	**0.000**	2.15 (1.47–3.13)	**0.000**
CMV DNA						
Negative versus positive	0.73 (0.47–1.13)	0.158	1.35 (0.97–1.87)	0.077	1.47 (1.05–2.06)	**0.023**
Acute GVHD						
Grades 0–II versus Grades III–IV	2.14 (1.14–4.00)	**0.017**	2.07 (1.30–3.30)	**0.002**	1.96 (1.22–3.15)	**0.005**
Chronic GVHD						
No	1.00		1.00		1.00	
Limited	0.85 (0.48–1.53)	0.594	0.95 (0.62–1.48)	0.835	0.88 (0.58–1.35)	0.560
Extensive	0.65 (0.25–1.70)	0.378	1.72 (1.05–2.83)	**0.032**	1.62 (1.00–2.60)	**0.048**

Bold values indicate *P* < 0.05

HR Hazard ratio, *CI* confidence interval, *DFS* disease-free survival, *OS* overall survival, *IFD* invasive fungal disease, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *CR* complete remission, *NR* nonremission, *MSD* matched sibling donor, *HRD* haploidentical related donor, *MUD* matched unrelated donor, *ATG* antithymocyte globulin, *GVHD* graft-versus-host disease, *CMV* cytomegalovirus

[a]The other category included myelodysplastic syndrome, chronic myeloid leukemia and lymphoma

seropositivity of the donor increases the risk of chronic GVHD might be dependent on the activities of B cells stimulated by EBV infection [36]. However, it is unknown whether the mechanism of fungal infection is similar to viral and bacterial infection. The most likely possible mechanism is dependent on the expansion of Th17 cells. Recently, more and more evidence indicate that Th17 cells are closely related to augmenting acute and chronic GVHD [37, 38]. Major components of fungal cell walls, including mannan and β-D-glucan, are recognized by pattern recognition receptors Dectin-1 and Dectin-2 on host cells [39–41]. Mannan activated macrophages via Dectin-2 to induce T-cell polarization toward Th17 [6]. Moreover, both Dectin-1 and Dectin-2 are known to activate Nlrp3 inflammasome [42, 43]. Nlrp3 inflammasome activation induced an allogeneic Th17 response through caspase-1 cleavage and production of inflammatory cytokines [44]. Usually, invasive fungal infections are also accompanied by bacterial and viral infections [45]. IFD might have the potential to increase the risk of chronic GVHD via indirect effects, including bacterial and

Fig. 2 Impact of IFD post-transplantation on relapse (**a**), transplant-related mortality (**b**), disease-free survival (**c**), and overall survival (**d**)
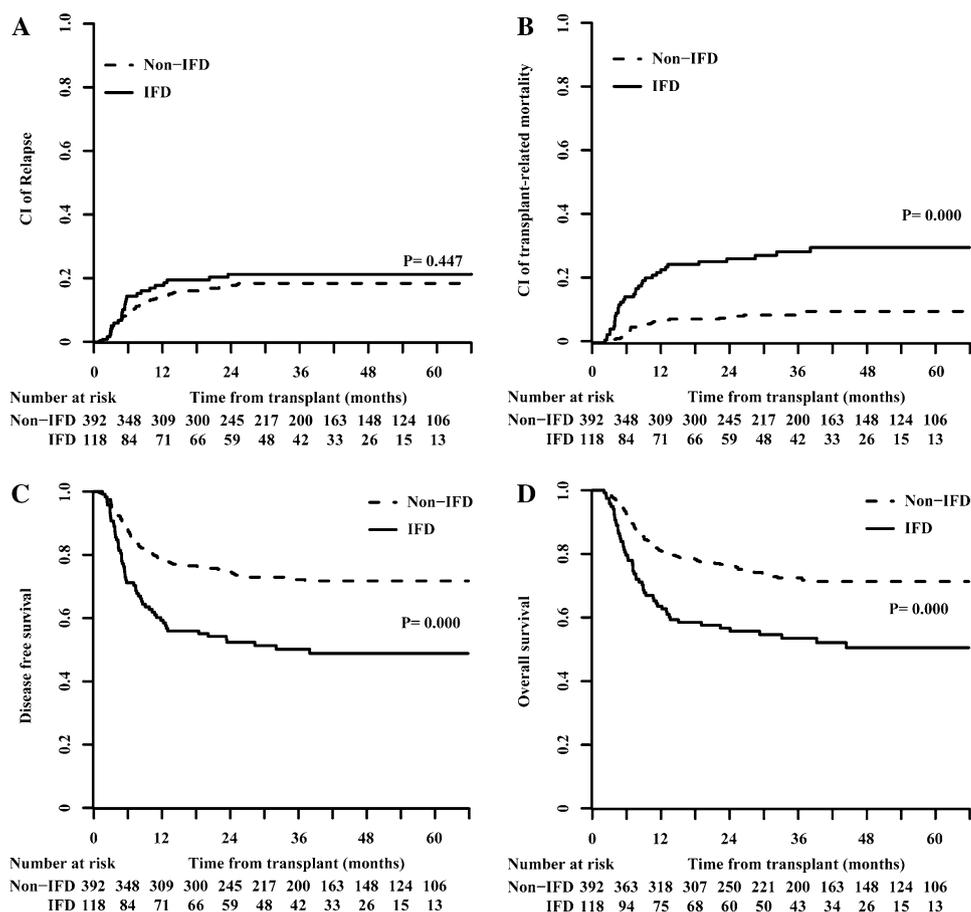
other viral infections, as are known for CMV [34, 46]. The exact mechanisms to explain how IFD augments chronic GVHD need to be further explored.

Allo-HSCT is a curative therapy for hematological malignancies, depending on its strong graft-versus-leukemia (GVL) reaction to some extent. Generally, the occurrence of GVHD is considered to be associated with the GVL reaction [13, 47]. In this report, the patients with IFD have higher incidence of GVHD, but they do not have lower incidence of relapse. The 2-year cumulative incidence of extensive chronic GVHD among patients with IFD and without IFD was $43.0\% \pm 5.2\%$ and $6.6\% \pm 1.4\%$, respectively. There were no significant differences in 3-year cumulative incidence of relapse between IFD group and non-IFD group ($20.7\% \pm 3.8\%$ versus $18.9\% \pm 2.0\%$). This finding was in agreement with recent studies showing that extensive chronic GVHD did not bring GVL effect [48]. Stern et al. [49] also reported that the strength of the GVHD/GVL correlation differs significantly between hematological malignancies. GVL effects could operate in the absence of GVHD, particularly in AML. Noga et al. [50] and Blecha et al. [51] recently reported that isolated extramedullary relapse after transplantation also occurs in patients with severe chronic GVHD. Surprisingly, we also observed that the relapse

incidence in the MUD transplant was higher than that of MSD and HRD transplants. The reason might be that most patients in the MUD transplant used the ATG for GVHD prophylaxis compared to the MSD transplants. Compared to HRD transplant, a reasonable interpretation might be due to HLA disparity.

There are several limitations to this study. First, it is a retrospective study that is subject to biases. These biases can sometimes be partially corrected by the large number of patients analyzed. Second, most of patients were diagnosed as probable IFD. The percentage of proven IFD was relatively low (28 of 118, 23.7%). The reason is mainly limited by the potential risk of an invasive biopsy. And the necropsy studies were not systematically performed, which lead to a probable underestimation of proven IFD.

Conclusions

In conclusion, our results suggest that IFD significantly contributes to the development of chronic GVHD after allo-HSCT. This finding highlights the importance of fungal management to decrease chronic GVHD and improve

survival in the recipients of allo-HSCT. However, the mechanisms of chronic GVHD induced by fungus need to be further explored.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Penack O, Holler E, van den Brink MR. Graft-versus-host disease: regulation by microbe-associated molecules and innate immune receptors. Blood. 2010;115:1865–72.
2. Cantoni N, Hirsch HH, Khanna N, Gerull S, Buser A, Bucher C, Halter J, et al. Evidence for a bidirectional relationship between cytomegalovirus replication and acute graft-versus-host disease. Biol Blood Marrow Transpl. 2010;16:1309–14.
3. Olkinuora HA, Taskinen MH, Saarinen-Pihkala UM, Vettenranta KK. Multiple viral infections post-hematopoietic stem cell transplantation are linked to the appearance of chronic GVHD among pediatric recipients of allogeneic grafts. Pediatr Transpl. 2010;14:242–8.
4. Garcia-Vidal C, Upton A, Kirby KA, Marr KA. Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection according to time after transplantation. Clin Infect Dis. 2008;47:1041–50.
5. Fukuda T, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG, Martin PJ, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood. 2003;102:827–33.
6. Uryu H, Hashimoto D, Kato K, Hayase E, Matsuoka S, Ogasawara R, Takahashi S, et al. alpha-Mannan induces
7. Th17-mediated pulmonary graft-versus-host disease in mice. Blood. 2015;125:3014–23.
7. Kontoyiannis DP. Antifungal prophylaxis in hematopoietic stem cell transplant recipients: the unfinished tale of imperfect success. Bone Marrow Transpl. 2011;46:165–73.
8. van der Velden WJ, Netea MG, de Haan AF, Huls GA, Donnelly JP, Blijlevens NM. Role of the mycobiome in human acute graft-versus-host disease. Biol Blood Marrow Transpl. 2013;19:329–32.
9. Yu S, Fan Q, Sun J, Fan Z, Zhang Y, Jiang Q, Huang F, et al. Haploidentical transplantation without in vitro T-cell depletion results in outcomes equivalent to those of contemporaneous matched sibling and unrelated donor transplantation for acute leukemia. Medicine (Baltimore). 2016;95:e2973.
10. Wang Y, Liu QF, Xu LP, Liu KY, Zhang XH, Ma X, Fan ZP, et al. Haploidentical versus identical-sibling transplant for AML in remission: a multicenter, prospective study. Blood. 2015;125:3956–62.
11. Liu QF, Fan ZP, Wu MQ, Sun J, Wu XL, Xu D, Jiang QL, et al. Allo-HSCT for acute leukemia of ambiguous lineage in adults: the comparison between standard conditioning and intensified conditioning regimens. Ann Hematol. 2013;92:679–87.
12. Xuan L, Huang F, Fan Z, Zhou H, Zhang X, Yu G, Zhang Y, et al. Effects of intensified conditioning on Epstein-Barr virus and cytomegalovirus infections in allogeneic hematopoietic stem cell transplantation for hematological malignancies. J Hematol Oncol. 2012;5:46.
13. Liu QF, Fan ZP, Zhang Y, Jiang ZJ, Wang CY, Xu D, Sun J, et al. Sequential intensified conditioning and tapering of prophylactic immunosuppressants for graft-versus-host disease in allogeneic hematopoietic stem cell transplantation for refractory leukemia. Biol Blood Marrow Transpl. 2009;15:1376–85.
14. Liu Q, Lin R, Sun J, Xiao Y, Nie D, Zhang Y, Huang F, et al. Antifungal agents for secondary prophylaxis based on response to initial antifungal therapy in allogeneic hematopoietic stem cell transplant recipients with prior pulmonary aspergillosis. Biol Blood Marrow Transpl. 2014;20:1198–203.
15. Kottaridis PD, Milligan DW, Chopra R, Chakraverty RK, Chakrabarti S, Robinson S, Peggs K, et al. In vivo CAMPATH-1H prevents GvHD following nonmyeloablative stem-cell transplantation. Cytotherapy. 2001;3:197–201.
16. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, 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. 2008;46:1813–21.
17. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood. 2002;100:4358–66.
18. Omer AK, Ziakas PD, Anagnostou T, Coughlin E, Kourkoumpetis T, McAfee SL, Dey BR, et al. Risk factors for invasive fungal disease after allogeneic hematopoietic stem cell transplantation: a single center experience. Biol Blood Marrow Transpl. 2013;19:1190–6.
19. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E, Heussel CP, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis. 2007;44:1289–97.
20. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 consensus conference on acute GVHD grading. Bone Marrow Transpl. 1995;15:825–828.
21. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, et al. Chronic graft-versus-host