Chapter 2

Incidence, outcome and risk factors for invasive aspergillosis in 663 allogeneic hematopoietic stem cell transplantation recipients. A nested case control study.

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ABSTRACT

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients are at risk for invasive aspergillosis (IA). We conducted a retrospective study to determine incidence, outcome and risk factors for IA in alloHSCT recipients.

Methods

Patients who underwent their first alloHSCT between 2004 and 2014, were included. IA was classified according to the proven or probable EORTC/MSG criteria. In addition, patients with a positive microbiological criterium but with non-specific radiological findings, were defined as having non-classifiable IA. The 12-month IA incidence and mortality were determined. Within the total cohort, a nested case-control study was performed to determine risk factors for IA.

Results

Of 663 alloHSCT recipients, 86 developed IA within the 12 months post-transplantation (1.8% proven, 7.4% probable and 3.8% non-classifiable IA). Twelve-month all-cause mortality was 45.6% in patients with IA and 26.2% in those without IA (p-value<0.01). For the nested case-control study, 99 patients with proven/probable/non-classifiable IA were compared to 198 patients without IA. Backward logistic regression showed the following independent risk factors: neutropenia (OR 16.22; p-value<0.01), lymphocytopenia (OR 5.70; p-value<0.01), reactivation of cytomegalovirus (OR 5.27; p-value=0.02), creatinine >135 µmol/l (OR 2.48; p-value=0.03), pulmonary comorbidity (OR 2.67; p-value=0.01); and use of prednisolone 1-1500 milligram (OR 4.06; p-value<0.01) and >1500 milligram (OR 45.05; p<0.01) in the 4 weeks preceding the IA. Pre-transplant IA was not identified as an independent risk factor for post-transplant IA.

Conclusion

At twelve months post-transplantation, 13.0% of the patients had developed IA and had a decreased survival compared to patients without IA.. Several independent risk factors were found, but surprisingly pre-transplant IA was not among them. A higher dose of prednisolone was associated with a higher risk for IA, suggesting a dose-effect relationship.

INTRODUCTION

Invasive aspergillosis (IA) is a common invasive fungal disease (IFD) in recipients of allogeneic hematopoietic stem cell transplantation (alloHSCT) [1-12]. The reported incidences vary between 2.3% and 15.0%, and mortality is substantial in this particular patient group [1-4,6-8,10]. Primary prophylaxis with voriconazole or posaconazole can reduce the incidence of IA in alloHSCT recipients and is recommended in those with graft-versus-host disease (GVHD) who are in need of systemic corticosteroid or other anti-GVHD therapies [13-15]. However, apart from GVHD, many other risk factors for post-transplant IA have been described, such as older age, neutropenia, non-related donor, reactivation of cytomegalovirus (CMV) and a history of pre-transplant IFD [1-4,6,9,10,12,16,17]. Also, a genetic predisposition has been identified [18]. IA is most frequently observed in the first year post-transplantation, but a substantial part of the recipients develops IA later [1,6,7]. Therefore, the best timing and duration of primary prophylaxis should be individualized. Recognizing risk factors may help in selecting those patients that are at highest risk and benefit most of primary prophylaxis. We conducted a retrospective single-center study to study the incidence, mortality and risk factors for post-transplant IA in alloHSCT recipients.

METHODS

Study design

The study was performed at Erasmus University Medical Center (Rotterdam, the Netherlands), a tertiary referral hospital with three hematology departments at two sites. We reviewed medical files of all patients who underwent their first alloHSCT between January 2004 and December 2014. Data were obtained till the end of the study (1st of April, 2015). The following information was retrieved from medical files: age, sex, underlying hematological disease, date and donor type of alloHSCT, conditioning regimen pre-transplantation, relapse of hematological disease post-alloHSCT, documentation of IFD before and after alloHSCT, acute and chronic GVHD, renal and hepatic impairment, pulmonary comorbidity, reactivation of CMV, neutropenia, lymphocytopenia, number of CD3+ T cells, use of systemic corticosteroids, European Group for Blood and Marrow Transplantation (EBMT) risk score and mortality.

The study consisted of two parts. First, in the total cohort we assessed (i) the incidence of IA at 12 months post-transplantation and overall, and (ii) the incidence of IA per calendar year of transplantation and per donor type. Also, we determined (i) the all-cause mortality at 12 months post-transplantation and overall, (ii) the IA-related mortality, defined as mortality within 6 weeks of proven, probable or non-classifiable

IA diagnosis, and (iii) the non-relapse mortality (NRM). Furthermore, the incidence and timing of IFD other than IA were evaluated.

Second, a nested case-control study was performed within the total cohort to identify risk factors for developing post-transplant IA. Cases were defined as patients with proven, probable or non-classifiable IA after alloHSCT. Controls were patients without IA. The following patients were excluded: (i) patients with possible IA, (ii) patients with IFD other than IA, (iii) patients having an episode of IA before alloHSCT who continued to receive on antifungal therapy for this episode after alloHSCT. These exclusion criteria were used to ascertain as much as possible that the cases had and the controls had not developed an IA after alloHSCT. Every case was matched randomly to 2 controls using age at transplant (±5 years), year of transplant (±1 year) and follow-up time as matching variables. Matching was performed using IBM® SPSS, version 21 (plug-in "Fuzzy"). To ensure that risk factors could also be studied in controls, the follow-up time of the two matched controls was at least as long as the case. Except for lymphocytopenia and CD3+ T cell, variables that varied over time (i.e. acute or chronic GVHD, renal or hepatic impairment, reactivation of CMV, neutropenia, use of prednisolone) were assessed during the time frame of 1 month preceding the diagnosis of IA in the cases. In the rare event that renal and hepatic function had not been tested in the indicated time period, the last observation was carried forward with a maximum of 6 months. For controls, the same variables were obtained at the number of days after alloHSCT that corresponded to this time-frame in the corresponding matched case. Lymphocytopenia and CD3+ T cells were assessed 3 months or as the first encountered number preceding IA diagnosis, respectively, since these 2 variables were not determined in time frame of 1 month in the majority of the patients.

Antifungal prophylaxis with fluconazole 400 milligrams daily was given during the post-transplantation conditioning-induced neutropenia. Primary prophylaxis for IA consisted of voriconazole 200 milligrams twice daily and was prescribed to patients with GVHD who were treated with second line anti-GVHD treatment. First line treatment consisted of voriconazole in case patients were not treated with primary prophylaxis beforehand.

Definition of IFD

IFD was categorized as proven, probable or possible according to the revised European Organization for Research and Treatment of Cancer/Invasive Infectious Diseases Study Mycoses Group (EORTC/MSG) consensus criteria [19]. In addition, patients with appropriate host criteria and positive microbiological findings but with non-specific radiological features were classified as *non-classifiable* disease. Although this category is not yet included in the EORTC/MSG definitions, these patients are treated similarly to those with probable IA given their similar outcome [20]. The same diagnostic protocol, which has

been described elsewhere [21], was used in the entire time period. If multiple periods of IA were suspected for a given patient, the period with the highest IA classification was selected.

Definitions of potential risk factors

To assess renal and hepatic impairment as risk factors for IA, the highest levels of creatinine, bilirubin and alanine aminotransferase (ALAT) in the 1-month observation period were used. To grade hepatic impairment, we used and modified the Common Terminology Criteria for Adverse Events (version 4) of the National Cancer Institute [22]. Hepatic impairment was graded after the highest grade for total bilirubin or ALAT level. Pulmonary comorbidity was based on the pulmonary function testing that was performed as a standard procedure prior to alloHSCT and categorized according to the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) [23]. Neutropenia was defined as a neutrophil count of $<0.5x10^9$/l for 10 consecutive days or more and lymphocytopenia as a lymphocyte count of $<1.0x10^9$/l. Acute and chronic GVHD were defined according to the updated Glucksberg classification and National Institutes of Health Consensus, respectively [24,25]. All patients were categorized according to the EBMT risk score [26]. For the use of corticosteroids, the cumulative dose in milligrams of prednisolone was calculated in the indicated time frame.

Statistical analysis

Statistical analysis was performed using IBM® SPSS (version 21) and Stata (version 13). Clinical characteristics were analyzed using Chi-square test or Mann-Whitney U test as appropriate. We used a backward likelihood logistic regression to determine potential risk factors for IA. A p-value of <0.05 was considered statistically significant.

RESULTS

In total, 663 patients received their first alloHSCT between January 2004 and December 2014. The median age at alloHSCT was 52.8 years (range 16.7 to 71.1). The most frequent underlying hematological disease was acute leukemia (53.5%), followed by lymphoma (11.9%), myelodysplastic syndrome (9.2%), myeloproliferative neoplasms (8.6%), plasma cell neoplasm (6.9%), chronic lymphocytic leukemia (4.7%) and other hematological disorders (5.1%). The mean follow-up was 39.8 months (range 0 to 134.5).

Proven, probable, possible or non-classifiable IA was found in 109 (16.4%) recipients after 12 months post-alloHSCT and overall in 137 (20.7%)(table 1). Over a period of 10 years, the 12-month incidences of IA ranged from 6.4% to 33.3% per alloHSCT year ($p<0.01$)(table 2). An increase in the incidence was observed in 2012 and 2013 (figure

1). The 12-month incidences in matched related, matched unrelated and cord blood alloHSCT were 11.8%, 17.4% and 27.3% (p<0.01), respectively. The mean time from alloHSCT to development of IA was 266 days with a range of 9 to 2093 days (figure 2). In the total cohort, the all-cause mortality 12 months after alloHSCT was 29.1% and the overall all-cause mortality was 45.2%. Patients with proven, probable and non-classifiable IA had a higher mortality in compared to patients without IA at 12 months (45.6% versus 26.2%; p-value<0.001) as well as in overall (63.1% versus 41.1%; p-value<0.001). The IA-related mortality was 26.3% (36/137). Figure 3 shows the cumulative incidence of NRM with relapse as competing risk of patients with IA versus those without IA (NRM at 5 years 49±5% versus 16±2%, respectively, p<0.001).

Table 1. Invasive aspergillosis (IA) after allogeneic hematopoietic stem cell transplantation (alloHSCT) per donor type.

Classification of IA	Number of patients (%) at 12 months				Number of patients (%) in entire follow-up time or end of the study			
	Total n = 663	Matched related n = 270	Matched unrelated n = 305	Cord blood n = 88	Total n = 663	Matched related n = 270	Matched unrelated n = 305	Cord blood n = 88
IA (all)	109 (16.4)	32 (11.9)	53 (17.4)	24 (27.3)	137 (20.7)	43 (15.9)	68 (22.3)	26 (29.5)
Proven	12 (1.8)	2 (0.7)	5 (1.6)	5 (5.7)	13 (2.0)	2 (0.7)	6 (2.0)	5 (5.7)
Probable	49 (7.4)	12 (4.4)	29 (9.5)	8 (9.1)	58 (8.7)	15 (5.6)	34 (11.1)	9 (10.2)
Possible	23 (3.5)	11 (4.1)	6 (2.0)	6 (6.8)	34 (5.1)	15 (5.6)	12 (3.9)	7 (8.0)
Non-classifiable	25 (3.8)	7 (2.6)	13 (4.3)	5 (5.7)	32 (4.8)	11 (4.1)	16 (5.2)	5 (5.7)
No IA	554 (83.6)	238 (88.1)	252 (82.6)	64 (72.7)	526 (79.3)	227 (84.1)	237 (77.7)	62 (70.5)

Table 2. Invasive aspergillosis (IA) after allogeneic hematopoietic stem cell transplantation (alloHSCT) per transplantation year.

Classification of IA	Number of patients (%) per year of alloHSCT									
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
IA										
Possible	0	0	3 (5.1)	1 (1.8)	1 (1.9)	0	2 (4.2)	3 (4.2)	3 (4.9)	8 (10.7)
Probable	6 (9.8)	1 (2.1)	1 (1.7)	3 (5.4)	1 (1.9)	2 (2.9)	4 (8.3)	5 (7.0)	6 (9.8)	13 (17.3)
Proven	2 (3.3)	0	1 (1.7)	0	1 (1.9)	3 (4.4)	0	2 (2.8)	2 (3.3)	0
Non-classifiable	1 (1.6)	2 (4.3)	1 (1.7)	4 (7.1)	1 (1.9)	2 (2.9)	1 (2.1)	2 (2.8)	3 (4.9)	4 (5.3)
No	52 (85.2)	44 (93.6)	53 (89.8)	48 (85.7)	50 (92.6)	61 (89.7)	41 (85.4)	59 (83.1)	47 (77.0)	50 (66.7)

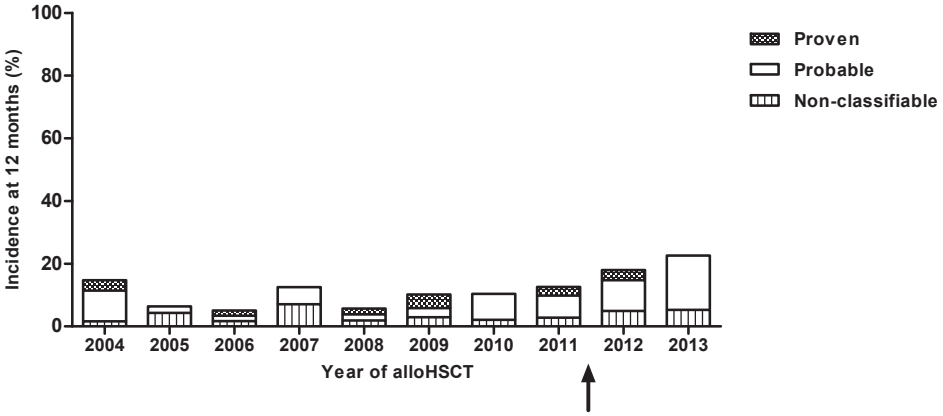

Figure 1. Incidence in percentage of invasive aspergillosis at 12 months per year of allogeneic hematopoietic stem cell transplantation. Arrow: In mid-2011, a long-lasting demolition and construction of a neighboring hospital building started at the site where patients underwent their allogeneic hematopoietic stem cell transplantation.

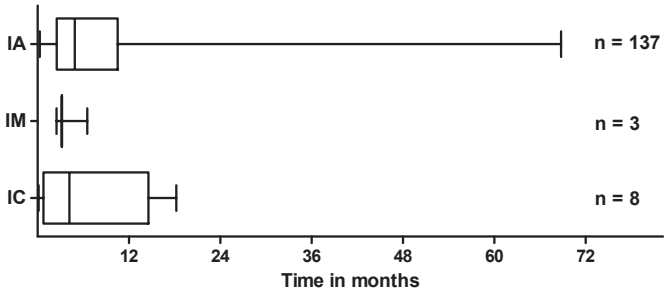

Figure 2. Time distribution from allogeneic hematopoietic stem cell transplantation to diagnosis of invasive aspergillosis (IA), invasive mucormycosis (IM) and invasive candidiasis (IC).

Aspergillus cultures were positive in 59 of the 103 patients with proven, probable or non-classifiable IA. Three cultures were isolated from lung biopsies, 1 from sinus biopsy, 1 from cerebral abscess and the remaining from respiratory specimens (sputum and/or bronchoalveolar lavage). *A. fumigatus* was found most commonly (n=53), followed by *A. terreus* (n=4), *A. flavus* (n=2) and *A. niger* (n=1). Two patients had a co-infection with an *A. fumigatus* and *A terreus.* Cultures with azole-resistant *A. fumigatus* were detected in 6 patients with proven (n=1), probable (n=3) and non-classifiable IA (n=2), and were isolated in the period of 2011 to 2014. Two of the 6 (33%) patients infected with an azole-resistant *A. fumigatus* died within 6 weeks after diagnosis.

IFD other than IA was found in 13 patients, of whom 11 within 12-months post-transplantation. Eight patients developed invasive candidiasis (IC) due to *C. albicans* (n=4), *C. glabrata* (n=2), *C. tropicalis* (n=1) and *C. species* (n=2). One patient had a co-infection

with *C. albicans* and *C. glabrata*. Invasive mucormycosis was observed in 3 patients (all 3 *rhizomucor* species). Two patients were diagnosed with proven IFD based on a positive biopsy, but no species was determined. Nine of these 13 patients were also diagnosed with IA, of whom 5 concurrently with the other IFD.

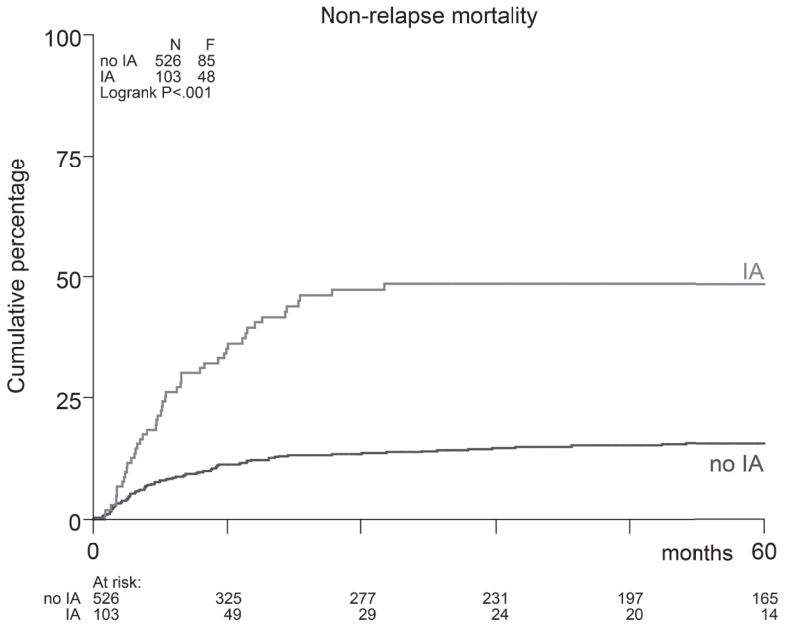

Figure 3. Non-relapse mortality in patients with proven, probable and non-classifiable invasive aspergillosis (IA) versus patients without IA.

Within the total cohort, a nested case-control study was performed to determine the risk factors for IA. Figure 4 shows the inclusion for the nested case-control study. Thirty-nine patients were excluded: 34 patients with possible IA, 4 with IFD other than IA, 1 who was already diagnosed with an IA before alloHSCT. Three more patients were excluded because of an uncertain diagnosis of IA as they recovered without antifungal treatment. Therefore, 103 potential cases and 518 potential controls were available. Matching yielded no controls for 4 cases. Consequently, 99 patients with IA (13 proven, 55 probable and 31 non-classifiable) were matched to 198 controls according to age, year of alloHSCT and follow-up duration. The clinical characteristics of the nested case-control study are shown in table 3. Cases suffered more often from renal and liver impairment, reactivation of CMV and neutropenia during the month preceding IA. Furthermore, acute or chronic GVHD and the use of systemic corticosteroids was significantly higher in the group with IA. After performing a backward logistic regression, the following risk

factors were found: neutropenia, lymphocytopenia, pulmonary comorbidity, reactivation of CMV, creatinine >135 µmol/l and use of corticosteroids (table 4). A higher cumulative dose of corticosteroids in the month preceding IA was associated with a higher risk for IA. Pre-transplant IA, acute and chronic GVHD were eliminated among others in the analysis. Patients receiving corticosteroid therapy and those with acute or chronic GVHD are a large overlapping population. Therefore, an extra analysis was performed to investigate the role of GVHD when acute and chronic GVHD were taken in one variable. Univariate analysis showed that the presence of any GVHD (acute GVHD grade II-IV or chronic moderate/severe GVHD) was associated with an odds ratio of 4.03 (95% CI 2.38 – 6.81; p<0.001). However, when any GVHD was added to the existing model, it was again eliminated (OR 0.83, 95% CI 0.33 – 2.11; p=0.70), and the same risk factors as described above remained significant.

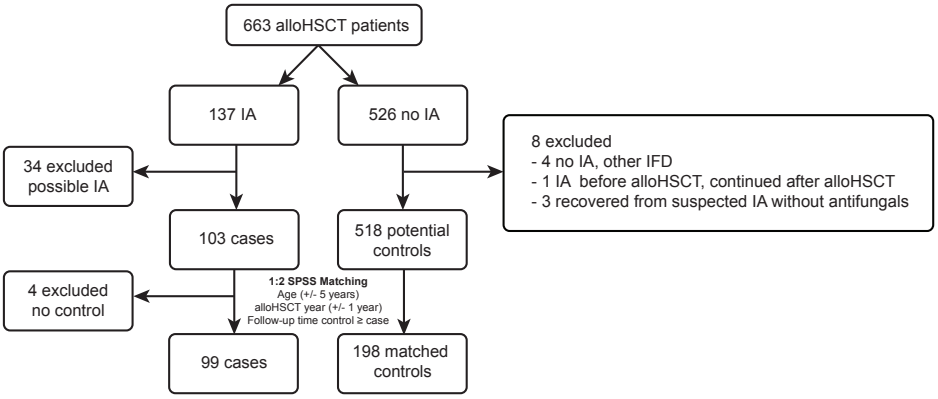

Figure 4. Inclusion for azole therapy failure and 6-week mortality analysis.
AlloHSCT, allogeneic hematopoietic stem cell transplantation. IA, invasive aspergillosis. IFD, invasive fungal disease.

Table 3. Clinical characteristics of 297 allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients included in the nested case-control study.

	Patients without IA[a] n = 198	Patients with proven, probable or non-classifiable IA[b] n = 99	p-value
Age at alloHSCT in years, median (range)	54.9 (20 – 70)	54.0 (21 – 71)	0.889
Male sex, n (%)	110 (55.6)	59 (59.6)	0.507
Underlying disease, n (%)			0.315
Acute leukemia	102 (51.5)	42 (42.4)	
Lymphoma	26 (13.1)	14 (14.1)	
Other	70 (35.4)	43 (43.4)	
Donor type, n (%)			0.071
Matched related donor	76 (38.4)	26 (26.3)	
Matched unrelated donor	99 (50.0)	55 (55.6)	
Cord blood	23 (11.6)	18 (18.2)	
Myeloablative conditioning, n (%)	33 (16.7)	17 (17.2)	0.883
Use of anti-thymocyte globulin during conditioning, n (%)	45 (22.7)	22 (22.2)	0.922
Proven, probable, non-classifiable or possible IA before alloHSCT, n (%)	28 (14.1)	14 (14.1)	1.000
Serum creatinine >135 umol/l, n (%)	38 (19.2)	37 (37.4)	0.001
Hepatic impairment, n (%)			<0.001
ALAT and bilirubine < upper normal limit	66 (33.3)	8 (8.1)	
ALAT and/or bilirubine grade I	73 (36.9)	30 (30.3)	0.262
ALAT and/or bilirubine grade II	33 (16.7)	23 (23.2)	0.173
ALAT and/or bilirubine grade III - IV	26 (13.1)	38 (38.4)	<0.001
Moderate or severe pulmonary comorbidity, n (%)	69 (34.8)	49 (49.5)	0.015
CMV reactivation, n (%)	5 (2.5)	21 (21.2)	<0.001
Neutropenia of ≥10 days, n (%)	11 (5.6)	25 (25.3)	<0.001
Lymphocytopenia, n (%)	118 (60.2)	90 (91.8)	<0.001
CD3+ T cells, median (range)	0.442 (0.005 - 5.319)	0.337 (0.000 - 2.218)	0.006
Acute GVHD, n (%)			<0.001
No acute GVHD or acute GVHD grade I	181 (91.4)	72 (72.7)	
Grade II - IV	17 (8.6)	27 (27.3)	
Chronic GVHD, n (%)			0.009
No chronic GVHD or limited chronic GVHD	175 (88.4)	76 (76.8)	
Moderate or extensive	23 (11.6)	23 (23.2)	
Use of corticosteroids (prednisolone equivalent dose in milligrams), n (%)			<0.001
0	127 (64.1)	21 (21.2)	
1 to 1500	59 (29.8)	34 (34.3)	0.393
>1500	12 (6.1)	43 (43.4)	<0.001
Insufficient data	0	1 (1.0)	-

Table 3. Clinical characteristics of 297 allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients included in the nested case-control study. (*continued*)

	Patients without IA[a] n = 198	Patients with proven, probable or non-classifiable IA[b] n = 99	p-value
EBMT risk score, n (%)			0.122
0 to 2	52 (26.3)	18 (18.2)	
3 to 7	146 (73.7)	81 (81.8)	

[a] No IA was defined as no proven IA, no probable IA, no non-classifiable IA or no possible invasive fungal disease.

[b] IA, invasive aspergillosis. Proven and probable IA was defined according to the revised European Organization for Research and Treatment of Cancer/Invasive Infectious Diseases Study Mycoses Group (EORTC/MSG) criteria. Non-classifiable is defined as a patient with EORTC/MSG host and microbiological criteria fulfilled and a pulmonary infiltrate without a halo or air-crescent or well-defined nodule.

Table 4. Risk factors for developing invasive aspergillosis after allogeneic hematopoietic stem cell transplantation according to backward likelihood logistic regression analysis.

Variable	Odds ratio (95% CI)	p-value
Neutropenia	16.26 (5.64 - 46.71)	0.000
Lymphocytopenia	5.70 (1.88 - 17.31)	0.002
Reactivation of cytomegalovirus	5.27 (1.36 - 20.36)	0.016
Creatinine >135 µmol/l	2.48 (1.10 - 5.57)	0.028
Moderate / severe pulmonary comorbiditiy	2.67 (1.22 - 5.85)	0.014
Use of prednisolone		
1 to 1500 milligrams	4.06 (1.62 - 10.16)	0.003
>1500 milligrams	45.05 (14.35 - 141.42)	0.000

DISCUSSION

In this retrospective study of 663 alloHSCT recipients, we found an incidence of proven, probable, non-classifiable IA of 13.0% within the 12-months after transplantation and 15.5% in the entire follow-up time available. As expected, all-cause mortality was significantly higher in patients with than without IA. Moreover, the independent risk factors for IA we observed were neutropenia, lymphocytopenia, pulmonary comorbidity, CMV reactivation, renal impairment and the use of systemic corticosteroids. A higher corticosteroid use was associated with a higher risk of IA.

The incidence, mortality and risk factors of IA were investigated in recipients who received their alloHSCT in our hospital over a decade. Only the period with the highest IA classification per patient was registered. Therefore, the true incidence of IA may be underestimated as some patients were treated with antifungal therapy more than once. Previous studies reported mostly on the incidences of proven and probable IA. In the

current study, we found a 12-month and overall incidence of proven and probable IA of 9.2% and 10.7% respectively. These observed incidences fall within the previously reported incidences of 12-month (2.3% to 7.4%) and overall (2.5% to 15%) risk respectively [1-4,6-8,10]. A significant increased incidence was found among recipients of alternative donor transplants. This observation was also seen in other studies [3,5,8]. However, we did not find donor type to be an independent risk factor for developing IA in the nested case-control study. This may have to do with the smaller sample size of the nested case-control group. Another interesting finding was that 20.4% of all the infections occurred after the first year of transplantation, indicating a continued risk in certain alloHSCT recipients, which has been previously described as well [1,6,11].

In 2012 and 2013, there was a significant increase in the incidence of IA. There may be different reasons for this observation. First, a long-lasting demolition and renovation of a neighboring hospital building was initiated from 2011 onwards at one of the two sites where all alloHSCT patients were transplanted and followed after alloHSCT. Hospital constructions or demolitions have been associated with increased IA in patients with underlying hematological disease [27,28]. Second, azole resistance has emerged over the past decade [29]. A recent study found 16.2% resistance against voriconazole in cultures of *A. fumigatus* that were isolated between 2011 to 2013 from high-risk patients of a Dutch tertiary referral hospital [30]. In our total cohort, 6 recipients had probable or non-classifiable IA due to azole-resistant *A. fumigatus*. As fungal cultures often remain negative and because antifungal susceptibility testing was not a standard procedure in the first half of the observation period, the actual number of patients infected with azole-resistant *A. fumigatus* may have been higher. As primary prophylaxis with triazoles will not prevent azole-resistant IA, the emergence of azole resistance may have contributed to the increased incidence in IA as well. Formerly, primary prophylaxis was prescribed in our center to alloHSCT recipients with GVHD who were treated with second line anti-GVHD treatment. More recently, it was extended to patients who are treated with high-dose systemic corticosteroid therapy.

Patients with proven, probable or non-classifiable IA had a significantly higher mortality compared to patients without IA in the total cohort. In the total cohort, we found an IA-related mortality (=6-week mortality after diagnosis of IA) of 26.3%. Two other studies have described the mortality 6 weeks after diagnosis of IA: Neofytos et al. found a comparable 6-week mortality of 21.5%, but the 6-week mortality observed by Nucci et al. was substantially higher (63%) [8,11]. However, the population studied by these authors has been a more heterogeneous group of patients, including recipients of autologous hematopoietic stem cell transplantation.

To investigate potential risk factors for IA, we performed a nested case-control study within the total patient cohort. Except for lymphocytopenia and CD3+ T cells, all variables that changed over time were assessed in the month before diagnosis of IA in cases and

in the same time-frame post-alloHSCT in the controls. This design was chosen to ensure that the identified time-varying risk factors really contributed to IA. It is similar to the study design of Corzo-León et al. [1], except that our cases also consisted of recipients with non-classifiable IA in addition to those with proven and probable IA. We think it is correct to include non-classifiable IA cases as well because they have the same outcome and are treated in the same way as patients with probable or proven IA [20]. Neutropenia, lymphocytopenia, pulmonary comorbidity, reactivation of CMV, renal impairment and use of prednisolone were found to be independent risk factors. Moreover, the use of a higher dose of prednisolone was associated with a higher risk for developing IA, suggesting that the effect of prednisolone on IA is dose dependent. Neutropenia, CMV reactivation and corticosteroid use have been previously described as important risk factors for IFD [2,6,12,16,17]. However, this study is the first to identify renal impairment as an independent risk factor for IA [1,11,16]. Remarkably, and in contrast to some of the other studies, the diagnoses of pre-transplantation IA or acute/chronic GVHD were not found to be independent risk factors [1-3,6,9,10,12,16]. Perhaps pre-transplant IA was not found to be risk factor, because patients with pre-transplant IA are often treated with secondary prophylaxis during alloHSCT. Also, selection bias may partially explain this finding because a subset of patients in need for an alloHSCT with uncontrolled IA may no longer be eligible for the transplantation. GVHD was analyzed as acute and chronic GVHD and in an additional analysis as one single variable, but was not found to be a risk factor. As patients with acute or chronic GVHD are almost always treated with corticosteroids, these variables are strongly associated. GVHD did not remain significant in the final model presumably because of the stronger effects of corticosteroids. The risk further increased when the cumulative dose of corticosteroids increased.

The major limitation of this study was its single-center and retrospective design. Therefore, the analysis of potential risk factors was limited to the available variables and population size. Ideally, a multi-center prospective study should be performed to determine the incidence, mortality, risk factors and the impact of primary prophylaxis. Especially the efficacy of primary prophylaxis should be evaluated in due time in the light of increasing azole resistance. However, this would have its own challenges as the policies of primary prophylaxis after alloHSCT differ among the Dutch transplantation hospitals and may change if the incidence of azole resistance continues to increase over the coming years.

In conclusion, an incidence of proven, probable and non-classifiable IA was found of 13.0% at 12-months post-transplantation and 15.5% in overall. Mortality was significantly higher in patients with IA than those without. Different independent risk factors were found: neutropenia, lymphocytopenia, pulmonary comorbidity, reactivation of CMV, renal impairment and use of prednisolone. The results are important as it may help

clinicians in starting or adjusting antifungal prophylaxis in the appropriate patient at the correct time.

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