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Epidemiology, utilisation of healthcare resources and outcome of invasive fungal diseases following paediatric allogeneic haematopoietic stem cell transplantation

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Summary

Background: Epidemiology and management practices of invasive fungal diseases (IFD) after allogeneic haematopoietic stem cell transplantation (HSCT) are a subject of constant change. We investigated the contemporary incidence, diagnostics, antifungal management and outcome at a major paediatric transplant centre in Germany.

Methods: The single-centre retrospective observational study included all paediatric allogeneic HSCT patients (pts) transplanted between 2005 and 2015. Patient-related data were assessed up to 365 days post-transplant. The primary endpoint was the incidence of possible, probable and proven IFDs. Secondary endpoints included diagnostics and antifungal treatment; analysis of risk factors; and overall survival with the last follow-up in January 2017.

Results: A total of 221 first (196), second (21) or third (4) procedures were performed in 200 pts (median age: 9 years, range, 0.5-22) for leukaemia/lymphoma (149) and non-malignant disorders (72). Prophylaxis was administered in 208 HSCT procedures (94%; fluconazole, 116, mould-active agents, 92). At least one computed tomography scan of the chest was performed in 146, and at least one galactomannan antigen assay in 60 procedures. There were 15 cases of proven (candidemia, 4; aspergillosis, 4) or probable (aspergillosis, 7) IFDs, accounting for an incidence rate of 6.8%. Overall mortality at last follow-up was 30%; the occurrence of proven/probable IFDs was associated with a reduced survival probability ($P < .001$).

Conclusion: Morbidity and mortality from IFDs at our institution were consistent with data reported from other centres. Utilisation of healthcare resources for prevention, diagnosis and management of IFDs was considerable.

KEYWORDS

children, diagnostics, management, mycoses, transplantation

1 | INTRODUCTION

For more than five decades, paediatric allogeneic haematopoietic stem cell transplantation (HSCT) is providing a curative option for children and adolescents with a variety of diseases, including leukaemia, congenital or acquired bone marrow insufficiency, severe immunodeficiency, anaemia or inborn errors of metabolism.[1,2] While in malignant diseases, the leading cause of death post-transplant is relapse, allogeneic HSCT is also associated with an elevated risk of infectious morbidity and mortality. This is the result of the patient's compromised immune system to which many factors contribute, such as the underlying disease, previous chemotherapies, the type and intensity of the conditioning regimen, donor choice, stem cell source, and occurrence of acute and chronic graft-versus-host disease (GVHD).[3-5]

Bacterial, viral and fungal infections are frequently observed in children and adolescents post-transplant.[6-8] Invasive fungal disease (IFD) is a life-threatening condition. Early detection is crucial, but still difficult despite recent progress in the diagnosis of IFDs in immunocompromised hosts. The two most common paediatric IFDs are invasive candidiasis and invasive aspergillosis.[8] However, data about the epidemiology of IFDs in paediatric stem cell transplant recipients and the utilisation of healthcare resources for prevention, diagnosis and management are limited and a subject of constant change. To assess the current status in this dynamic field, we investigated the contemporary incidence, diagnostics, antifungal management and outcome at a major paediatric transplant centre in Germany over an extended period of time.

2 | METHODS

The study was a retrospective, single-centre observational cohort study of all children, adolescents and young adults who underwent allogenic HSCT between 2005 and 2015 at the Center for Bone Marrow Transplantation of the Department of Pediatric Hematology and Oncology of the University Children's Hospital Münster. The ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to: written informed consent for data collection and analysis was obtained within the consent procedure for cancer treatment, HSCT and specialised medical care approved by the local institutional review board.

The Department of Pediatric Hematology and Oncology serves a population of five million people in the Northwest of Germany. Each year, 140 to 160 unselected patients with the diagnosis of new or recurrent cancer are admitted. The haematopoietic stem cell transplant programme performs approximately 30 allogeneic and 10 autologous HSCT procedures per year and is accredited by the Joint Accreditation Committee of the International Society for Cellular Therapies and the European Society for Blood and Marrow Transplantation (JACIE).

Patients scheduled to receive allogeneic HSCT. Patients were housed in single HEPA-filtered rooms until discharge postengraftment and received penicillin, ciprofloxacin and metronidazole as antibacterial prophylaxis from admission to the first episode of fever, non-absorbable polyenes and fluconazole from admission until day + 100 as standard antifungal prophylaxis and trimethoprim/sulfamethoxazole

TABLE 1 Demographic and transplantation-related characteristics of 221 allogeneic haematopoietic stem cell transplantations in 200 patients

Pasrameter	No. (%) of Procedures or median value
Age	median 9 y (range, 0.5-22)
Sex	male 121, female 79
1st HSCT procedures	196
2nd or 3rd procedures	25
Diagnoses	
Leukaemia/lymphoma	149
Bone marrow failure	49
Primary immune/metabolic disorder	18
Non-malignant haematological disease	3
Solid tumour	2
Donor	
MUD	147
MSD	47
Mismatched donor (related/ unrelated)	27 (5/ 22)
Preparative regimen	
Myeloablative/non-myeloablative	149/ 72
Stem cell source	
Bone marrow	148
Peripheral blood stem cells	72
Cord blood	1
Neutrophil engraftment	median 22 d (range 9-50 d)
First discharge	median 34 d (range 17-194 d)
GvHD	
Acute, grade I/ II	97 (43.9%)
Acute, grade III/ IV	33 (14.9%)
Chronic (any)	39 (17.6%)
Viral reactivation/infection	
EBV	117 (52.9%)
CMV	44 (19.9%)
Adenovirus	25 (11.3%)
Bacterial bloodstream infection	
Gram-positive	44 (19.9%)
Gram-negative	25 (11.3%)

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HSCT, haematopoietic stem cell transplantation; MSD, matched sibling donor; MUD, matched unrelated donor.

postengraftment through day + 365. Ceftazidim plus gentamicin was used as initial regimen for empiric antibacterial therapy at the time of the study. Interventions for persistent or recurrent fever consisted of blood cultures, pulmonary computed tomography (CT) imaging, galactomannan-screening in blood in the case of pulmonary lesions and appropriate modification of antibacterial therapy. Modifications of the initial prophylactic antifungal regimen and/or initiation of empirical, pre-emptive or targeted antifungal treatment were not regulated per standard operating procedure but at the discretion of the respective attending physician.

For each patient, clinical, radiographic and microbiological data up to day + 365 post-transplant were extracted from the medical information system. Data collection was accomplished by a pseudonymised standardised case report form. The primary endpoint was the incidence of possible, probable and proven IFDs through day + 365 according to the 2008 revision of the criteria set forth by the European Organization for Research and Treatment of Cancer-Invasive Fungal Infections Cooperative Mycoses Study Group (EORTC/MSG).[9] Secondary endpoints included the analysis of risk factors potentially associated with proven/probable IFDs; the utilisation of pulmonary computed tomography (CT) imaging, galactomannan antigen testing and bronchoscopies; the use of systemic antifungal agents for prevention and management of suspected or proven/probable IFD; and IFD-related mortality and survival probabilities.

Data were tabulated and analysed using descriptive statistics. Risk factors potentially associated with proven/probable IFDs were analysed by regression analysis and survival probabilities through January 2017 by the Kaplan-Meier method.[10] Since no back-to-back tandem procedures were performed in this population, the number of transplantation procedures was used as denominator throughout with the exception of the analysis of survival probability.

3 | RESULTS

3.1 | Patient cohort and demographics

Between 2005 and 2015, 200 patients underwent 221 allogeneic haematopoietic stem cell transplantation procedures. Patient-specific demographic characteristics, transplant-related data, and occurrence of systemic viral reactivations and bacterial bloodstream infections are summarised in Table 1. The median age at the time of transplantation was nine years, and there was a slight preponderance of male patients (60.5%). Most patients had a haematological malignancy, had a matched unrelated donor and had received bone marrow transplants following a myeloablative preparative regimen. The median time to neutrophil engraftment was 22 days, and the median time to first discharge from the hospital was 34 days, respectively. Grade II and IV acute GVHD was diagnosed in 14.9% of the episodes, and chronic GVHD developed in 11.3%. Reflecting the overall degree of immunosuppression, reactivation of systemic viral infections occurred after the majority of episodes and *Gram*-positive or *Gram*-negative bloodstream infections in 19.9 and 11.3%, respectively (Table 1).

3.2 | Prevalence and incidence of invasive fungal diseases

Table 2 illustrates the prevalence and incidence of possible, probable and proven invasive fungal disease in relation to defined periods before and after transplant. Prior to admission for transplantation, eight probable (6; invasive pulmonary aspergillosis) or proven (2; candidemia due to *Candida albicans* and *C kefyr*, respectively) IFDs had occurred in 7 patients (cumulative incidence rate, 3,6%). One patient with probable aspergillosis died with active disease shortly after engraftment, while a second patient with probable aspergillosis died with controlled disease from unrelated causes on day + 246. A third patient with probable aspergillosis developed a second episode of probable invasive pulmonary aspergillosis beyond day + 180 but ultimately survived (Tables 2 and 3). Following admission and transplantation, within the first 365 days, 15 cases of proven/probable IFDs were diagnosed in a total of 221 transplantations, accounting for an incidence rate of 6.8% post-transplant. The cases were evenly distributed over the time periods from transplantation until neutrophil engraftment (2.8%), from neutrophil engraftment until day + 180 (1.8%) and after day + 180 (3.7%). Proven IFDs included four cases of invasive aspergillosis (*Aspergillus fumigatus* and *Aspergillus spp* in two cases each) and 4 cases of *Candida* bloodstream infections (*C dubliensis*, 2;

TABLE 2 Incidence of possible, probable, and proven invasive fungal disease (IFDs) among 221 allogeneic hematopoietic stem cell transplantations in 200 patients prior to and following allogeneic hematopoietic stem cell transplantation

	IFDs before transplantation		**IFDs between transplantation and neutrophil engraftment**		**IFDs between neutrophil engraftment and day + 180**		**IFDs beyond day +180**		**Total IFDs after transplantation**	
IFD category	**n**	**%**	n	%	n	%	n	%	**n**	**%**
Possible	**18**	**8.1**	23	10.4	6	2.7	0	0	**29**	**13.1**
Probable	**6**	**2.7**	2	0,9	0	0	5	2.3	**7**	**3.2**
Proven	**2**	**0.9**	3	1.4	2	0.9	3	1.4	**8**	**3.6**
Total	**26**	**11.8**	28	12,6	8	3.6	8	3.6	**44**	**19,9**
probable + proven	**8**	**3.6**	5	2,3	2	0.9	8	3.6	**15**	**6.8**

C lusitaniae,1; and *C dubliensis plus C parapsilosis*, 1). The latter were due to pan-susceptible organisms and were breakthrough infections on posaconazole suspension (1), low-dose liposomal amphotericin B (1 mg/kg;1) and therapeutic doses of caspofungin (2) Probable IFDs included 7 cases of invasive aspergillosis (three based on a positive culture (*A fumigatus*, 2; *Aspergillus flavus*, 1) and four on positive serum galactomannan, accounting for an incidence rate of proven/probable invasive aspergillosis and candidemia of 5 and 1.8%, respectively (Table 2). Univariate analysis of patient demographics, transplant characteristics and transplant-related complications revealed the presence of chronic GVHD and *Adenovirus* replication in blood as being significantly associated with the development of proven/probable IFDs (Table S1). The detailed clinical characteristics of all patients with probable and proven IFD are summarised in Table 3 (Table 3).

3.3 | Utilisation of imaging studies and microbiological diagnostics

Figure 1 depicts the number of imaging studies during the transplant period. At least one chest CT was performed in 146 of 221 transplant procedures (66%); findings compatible with pulmonary mould infection were found in one-third of the imaging studies. Imaging of the brain, the abdomen (by MRT) and the paranasal sinuses was ordered in < 10% of transplantations (Figure 1). Invasive fungal disease involving the central nervous system was documented by MRI in two cases, both in the period of long-term follow-up after discharge from the paediatric BMT unit.

At least one galactomannan assay in blood was performed in 60 of 221 transplant procedures (27%). The assay was less frequently applied in bronchoalveolar lavage fluid (9), pleural fluid (1) and cerebrospinal fluid (1). A bronchoscopy was documented in 20 cases (9%); in five cases, one or more microbiological criteria of invasive aspergillosis were detected. Blood cultures grew *Candida* in 6 cases (2.7%; two pretransplant); moulds were not detected.

3.4 | Antifungal prophylaxis and treatment

Figure 2 illustrates the flow of utilisation of antifungal prophylaxis and treatment. Prophylaxis was administered in 208 HSCT procedures (94%; fluconazole, n = 116, mould-active agents, n = 92 (posa- or voriconazole, 19; liposomal amphotericin B, 72; micafungin, 1). In 113 procedures, mould-active therapy mostly (>90%) in form of liposomal amphotericin B was initiated due to persistent fever and/or further IFD criteria (monotherapy, n = 88; modified monotherapy, n = 8; combination therapy, n = 18) (Figure 2).

3.5 | Overall mortality

Overall mortality in the 200 patients at the last follow-up, one year after the inclusion of the last patient, was 30%; in 7 instances, death was attributable to IFD (IFD-related overall mortality: 3,5%; IFD-related case fatality rate: 46.7%). The diagnosis of proven/probable IFD post-transplant was associated with a significantly reduced survival probability in comparison with patients without IFD (31.3% vs 70.1%; $P = .001$) (Figure 3) and in comparison with patients fulfilling criteria of possible IFDs (31.3% vs 67.6%; $P = .007$) (Figure 3).

4 | DISCUSSION

Allogeneic haematopoietic stem cell transplantation (HSCT) carries risks for an array of relevant and life-threatening infections. In this retrospective single-centre study, we analysed the incidence and the outcome of invasive fungal diseases (IFDs) in paediatric patients undergoing allogeneic HSCT in a large European stem cell transplant programme. The diagnosis of possible, probable and proven IFDs was based on the criteria elaborated by the European Organization for Research and Treatment of Cancer-Invasive Fungal Infections Cooperative Mycoses Study Group (EORTC/MSG) for the purpose of clinical research studies.[9] In the 11-year period between 2005 and 2015, 200 patients underwent 221 transplant procedures; all cases of IFDs from the beginning of the preparative regimen until the last follow-up in January 2017 were included in the analysis. The total incidence of possible, probable and proven IFDs was 19.9% post-transplant; probable and proven IFDs accounted for 6.8%. We did not find a difference in the numbers of probable and proven IFDs which is in contrast to a study published recently.[11] This difference may be due to the fact that the relatively higher frequency of probable IFDs was observed in the population of unselected paediatric haemato-oncological patients and not exclusively in allogeneic HSCT recipients.

Candida and *Aspergillus* species were the predominant fungal organisms, and the lower respiratory tract and the bloodstream were the main sites of infection. These data are similar to those published from other centres.[12-15] Most cases of IFDs were identified in the period beyond day + 180 post-transplant. Considering the eight cases of IFDs in the treatment phase before admission for allogeneic HSCT, a total of 23 cases of probable and proven IFDs were diagnosed in 20 patients until last follow-up post-transplant. Of note, more than half of the 15 cases diagnosed post-transplant occurred after day + 180, and univariate analysis revealed the presence of chronic GvHD as the only statistically significant predictor for probable or proven IFDs ($P = .004$). Similar findings were also reported by a contemporary paediatric study where chronic GvHD was identified as a major risk factor for IFDs after day + 100 postallogeneic HSCT.[16] The cumulative overall mortality of the entire cohort at the last follow-up was 30% with a significant difference in the survival probability between patients with probable and proven IFDs relative to patients with possible IFDs ($P = .026$) and no IFDs ($P = .001$). In seven of the 12 patients who died with a diagnosis of probable and proven IFDs, death was directly attributable to fungal disease (3.5%).

Systemic antifungal prophylaxis is strongly recommended by current paediatric guidelines for patients undergoing allogeneic HSCT, and in the absence of factors indicating a high risk for

TABLE 3 Clinical characteristics of patients with probable and proven invasive fungal diseases (IFDs)

No.	mf	Age	Year of SCT	Diagnosis	EORTC class.	Period	Fungus	Localisation	Imaging/endoscopy
1	f	22	2008	HD, 2nd relapse	probable	before SCT	*Asperg. fumigatus*	lung	CT thorax
2	m	6	2010	ALL	probable	before SCT	–	lung	CT thorax
3	m	3	2010	AML	probable	Tx-engraftment	–	lung	CT thorax
4	f	13	2011	MDS	probable	Tx-engraftment	*Asperg. flavus*	lung	CT thorax bronchosc.
5	m	15	2011	CML	probable	LTFU7	–	Lung meninges	CT thorax MRI head bronchosc.
6	f	15	2011	AML	probable	before SCT	–	lung	CT thorax
7	f	15	2013	AML, relapse	probable	LTFU	*Asperg. fumigatus*	lung	bronchosc. x-ray thorax
8	m	17	2014	ALL	probable	before SCT	–	Lung CNS	CT thorax bronchoscopy MRI head
9	m	1	2015	ALL	probable	LTFU	–	lung	CT thorax
10	m	3	2015	AML	2 episodes, probable	1) before SCT 2) LTFU	–	lung	CT thorax
11	m	5	2006	ALL, relapse	proven	Tx-engraftment	*Asperg. fumigatus*	Broviac catheter	–
12	m	14	2010	MDS	proven	engraftment-day + 180	*Asperg. ssp*	Lung CNS	CT thorax CT head
13	m	10	2010	MDS	proven	engraftment-day + 180	*Asperg. fumigatus*	diss.	CT thorax bronchosc.
14	m	16	2011	NHL	proven	LTFU	*Asperg. ssp*	lung	CT thorax
15	w	15	2013	VSAA	proven	Tx-engraftment	*Cand. dubliensis*	diss.	ultrasound spleen gastroscopy
16	w	18	2014	FA	proven	Tx-engraftment	*Cand. dubliensis*	blood	–
17	m	5	2015	AML	proven	before SCT	*Cand. albicans*	blood	–
18	m	17	2015	ALL	proven	LTFU	*Cand. lusitaniae*	blood	–
19	m	4	2015	XLP	1) probable 2) proven	1) LTFU 2) LTFU	1) *Asperg. Fumigatus* 2) *Cand. dubliensis*, *Cand. Parapsilosis*	1) lung 2) blood	CT thorax
20	m	13	2015	VSAA	1) probable 2) proven	1) before SCT 2) before SCT	*1) Asperg. spp.* 2) *Cand. Kefyr*	1) CT thorax 2) blood, spleen	CT thorax ultrasound, MRI abdomen

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloic leukaemia; Asperg, Aspergillus; bronchosc, bronchoscopy; Cand, Candida; CT, computed tomography; diss, disseminated; FA, Fanconi anaemia; galactom or GM, galactomannan; GM, galactomannan antigen; HD, Hodgkin disease; LAmB, liposomal amphotericin B; LTFU, long-term follow-up; m f, male female; MDS, myelodysplastic syndrome; NHL, Non-Hodgkin-Lymphoma; PCR, polymerase chain reaction; TMP/SMX, trimethoprim + sulfamethoxazole; VSAA, very severe aplastic anaemia; XLP, X-linked lymphoproliferative disease.

invasive mould disease, fluconazole remains an evidence-based option.[17-19] In the patient cohort analysed in this study, fluconazole was the primary choice in 52.4% of all HSCT procedures; a mould-active systemic antifungal compound was administered for prophylaxis in 41.5%, and in 5.8% transplant procedures, mould-active therapy was already started before the admission for transplantation. The fluconazole dose administered at our centre at the time of the study was 6 mg/kg body weight and day and

Culture	galactom./PCR	Other	Therapy	Outcome	IFD-associated death	Survival (days)
tracheal secretion	–	–	LAmB Voriconazole Caspofungin	dead	yes	46
–	GM serum	–	LAmB	dead	no	246
–	GM serum	–	LAmB Caspofungin granulocyte transfusions	dead	yes	13
–	GM/PCR bronchial secretion	.	LAmB Voriconazole Caspofungin	alive	no	2134
–	GM bronchial secretion	–	LAmB Voriconazole	dead	no	1430
–	GM serum	–	Voriconazole LAmB Caspofungin	alive	no	1958
tracheal secretion/ bronchial brush	GM serum and bronchoalveolar fluid/pleural secretion	–	LAmB Caspofungin	dead	yes	272
–	GM serum/ bronchoalveolar fluid	–	Voriconazole LAmB	alive	no	788
–	–	ß-D-Glucan	TMP/SMX	alive	no	536
–	GM serum		LAmB Voriconazole	alive	no	441
intra-operative smear, catheter tip		direct microscopy	Voriconazole Caspofungin	alive	no	3744
–	GM serum	autopsy	LAmB Caspofungin Posaconazole	dead	yes	176
–	GM serum	direct microscopy bronchial secretion, autopsy	LAmB	dead	yes	61
–	–	biopsy lung	Voriconazole Caspofungin LAmB	dead	no	678
blood	–	eye examination. biopsy stomach	Caspofungin LAmB	Dead	yes	66
blood	–	–	Caspofungin	dead	no	19
central venous catheter	–	–	LAmB	alive	no	465
blood	–	–	LAmB Caspofungin	dead	no	272
tracheal secretion (*Cand.*, *Asperg.*), blood (*Cand.*)	–	–	LAmB Caspofungin Voriconazole	dead	yes	285
blood, central venous catheter (*Cand.*)	GM serum	–	LAmB Caspofungin Voriconazole	alive	no	636

at the lower end of the recommended dosage range (Fluconazole SPC EMA). In a study of 113 adult patients receiving stem cell transplants from matched sibling donors, a reduced dose of fluconazole with 100 mg per day did not result in an increased rate of invasive fungal infections.[20] While clinical research studies in adults investigating newer antifungal substances including micafungin, voriconazole or low-dose liposomal amphotericin B have not yet demonstrated a clear advantage over fluconazole

in situations with a low frequency of probable and proven mould infections[21-23]; however, posaconazole has been shown to significantly reduce the rate of invasive aspergillosis and IFD-related mortality in adult patients with severe GvHD.[24]

In the majority of patients with suspected IFDs, establishing the diagnosis relies on a combination of clinical and diagnostic modalities, including the consideration of host factors, clinical and radiological criteria, and microbiological information from cultures, nucleic acid detection, microscopy and fungus-specific biomarkers in blood or affected tissues.[17] As sensitivities and specificities of the available tools are limited and IFDs may run a rapidly fatal course, antifungal therapy is often started on an empirical or pre-emptive basis.[25] Given this background of diagnostic and therapeutic uncertainty, we were also interested in analysing the utilisation of resources for prevention, diagnosis and management of IFD in our patient cohort. While the diagnosis of invasive *Candida* infections largely relies on the results of blood cultures; unfortunately, this simple and useful diagnostic tool is not reliable for the detection of most mould infections.[17,26] This is reflected by the high number of imaging studies and additional diagnostic procedures in the patients analysed in our study. In 221 transplant procedures, 116 CT scans of the lungs (66%), the most common site for invasive mould infections, were performed. In almost 50% of these imaging studies, pulmonary findings compatible with invasive mould infection (30%) or unspecific abnormal findings (16%) were reported. In addition to pulmonary imaging, 30 CT scans of the brain and the paranasal sinuses and MRI scans of the brain and the abdomen were initiated. In patients with suspicious findings in the imaging studies, there were considerable efforts for their further evaluation. These included 20 diagnostic bronchoscopies with bronchoalveolar lavage for microscopy, culture, galactomannan and, in few cases, nucleic acid detection. *Aspergillus* isolates were identified in tracheal secretions (direct microscopy or nucleic acid detection by polymerase chain reaction (PCR)), at the tip of an explanted Broviac catheter, in a lung biopsy specimen, or at autopsy. Despite the obvious difficulties in obtaining a definite diagnosis in invasive mould disease, these data confirm the necessity to evaluate all suspicious findings. Of note, in only 32.6% of all HSCT procedures, the primary antifungal prophylactic regimen was maintained throughout immunoreconstitution. The majority of patients were switched to a different regimen of systemic antifungal therapy, either on an empiric basis or based on the results of further diagnostic investigations in pre-emptive or targeted intention.

Taken together, an adequate work-up for suspected IFDs includes imaging studies, microscopy and cultures of blood and tissue, galactomannan antigen testing in blood and bronchoalveolar lavage fluid, and nucleic acid based techniques as PCR. Establishing the correct diagnosis in paediatric allogeneic HSCT recipients with

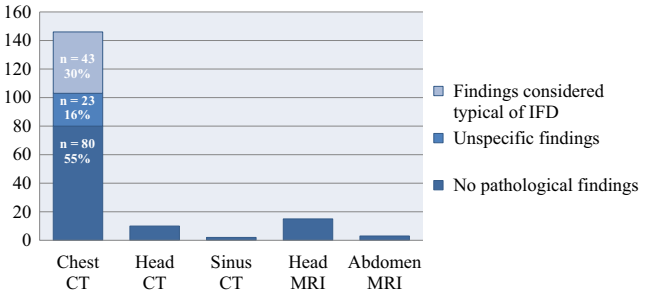

FIGURE 1 Utilisation of imaging studies and principal findings in 221 allogeneic haematopoietic stem cell transplantations in 200 patients. CT, computed tomography; MRI, magnetic resonance imaging

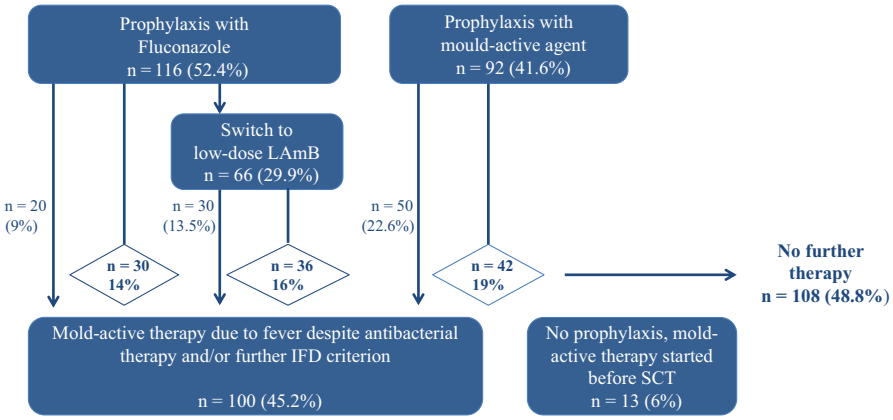

FIGURE 2 Antifungal prophylaxis and treatment in 221 allogeneic haematopoietic stem cell transplantations in 200 patients. Please note that all percentages provided in the figure refer to the total of 221 transplantation procedures. Fluconazole was given at 8 to 12 mg/kg body weight once daily; mould-active prophylaxis was used at the discretion of the respective attending physician and consisted of posaconazole suspension 12 to 18 mg/kg body weight daily in two or three divided doses (n = 7); voriconazole at the dose approved at the time of administration (n = 12); liposomal amphotericin B 1 mg/kg body weight once daily (n = 42), 1 mg/kg body weight every other day (n = 11), or 2.5 mg/kg body weight twice weekly (n = 19); and micafungin 1 mg/kg body weight once daily. Low-dose liposomal amphotericin B consisted of 1 mg/kg body weight once daily (n = 63), or 2.5 mg/kg body weight twice weekly (n = 13). Initial mould-active therapy consisted mostly (92.9%; n = 105; monotherapy 89, combination therapy 16) of liposomal amphotericin B at a dose of 3 mg/kg once daily (pl. see text). LAmB, liposomal amphotericin B; IFD, invasive fungal disease; SCT, stem cell Transplantation

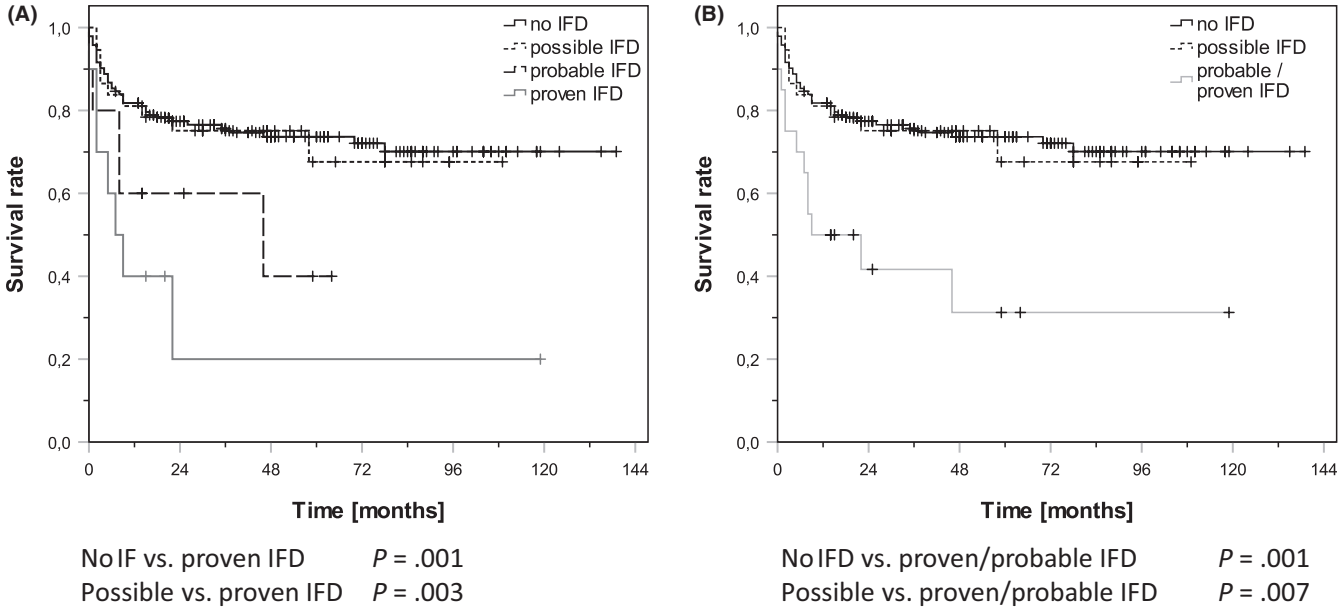

FIGURE 3 Survival probabilities among 200 patients undergoing 221 allogeneic haematopoietic stem cell transplantations. A, Comparison of patients with possible, probable, and proven invasive fungal disease relative to patients without invasive fungal diseases B, Comparison of patients with proven/probable invasive fungal diseases relative to those with possible invasive fungal diseases and no invasive fungal diseases. IFD, invasive fungal disease

prolonged fever and suspected IFDs is crucial for the overall management of these patients and justifies the considerable utilisation of resources for diagnosis and antifungal chemotherapy. Despite the existence of three classes of antifungal agents with potent activity against the majority of the relevant fungal pathogens, probable and proven IFDs continue to be a relevant cause for morbidity and have a significant impact on overall survival of paediatric allogeneic HSCT recipients.

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CONFLICT OF INTEREST

AHG has received research grants from Gilead, Merck, Sharp & Dohme, and Pfizer; is or has been a consultant to Amplyx, Astellas, Basilea, F2G, Gilead, Merck, Sharp & Dohme, and Pfizer; and served at the speakers´ bureau of Astellas, Basilea, Gilead, Merck, Sharp & Dohme, Pfizer and Schering-Plough. All other authors: no potential conflicts of interest to be declared.

AUTHOR CONTRIBUTIONS

Author contributions: CL, KE and AHG conceived the idea and the design of the study; CL and DM collected the data; CL, KE and AHG analysed the data and led the writing; MA, BF, DM, BB and CR revised the manuscript draft for important intellectual content.

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REFERENCES

1. Majhail NS, Farnia SH, Carpenter PA, et al. Indications for autologous and allogeneic hematopoietic cell transplantation: Guidelines from the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2015;21:1863-1869.
2. Shaw PJ, Kan F, Ahn KW, et al. Outcomes of pediatric bone marrow transplantation for leukemia and myelodysplasia using matched sibling, mismatched related, or matched unrelated donors. *Blood*. 2010;116:4007-4015.
3. Peters C, Schrappe M, von Stackelberg A, et al. Stem-cell transplantation in children with acute lymphoblastic leukemia: a prospective international multicenter trial comparing sibling donors with matched unrelated donors-The ALL-SCT-BFM-2003 trial. *J Clin Oncol*. 2015;33:1265-1274.
4. Takahashi T, Pereda MA, Bala N, Nagarajan S. In-hospital mortality of hematopoietic stem cell transplantation among children with nonmalignancies: a nationwide study in the United States from 2000 to 2012. *Pediatr Blood Cancer*. 2019;66(6):e27626.
5. Satwani P, Freedman JL, Chaudury S, et al. A multicenter study of bacterial blood stream infections in pediatric allogeneic hematopoietic cell transplantation recipients: the role of acute gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant*. 2017;23:642-647.
6. Ustun C, Young JH, Papanicolaou GA, et al. Bacterial blood stream infections (BSIs), particularly post-engraftment BSIs, are associated with increased mortality after allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant*. 2019;54:1254-1265.
7. Rustia E, Violago L, Jin Z, et al. Risk factors and utility of a risk-based algorithm for monitoring cytomegalovirus, epstein-barr-virus, and adenovirus infections in pediatric recipients after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:1646-1653.

8. Pana ZD, Roilides E, Warris A, Groll AH, Zaoutis T. Epidemiology of invasive fungal disease in children. *J Pediatr Infect Dis Soc*. 2017;6:S3-S11.
9. de Pauw B, Walsh TJ, Donnelly JP, 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;46:1813-1821.
10. Kaplan EL, Meier P. Individual nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
11. Cesaro S, Tridello G, Castagnola E, et al. Retrospective study on the incidence and outcome of proven and probable invasive fungal infections in high-risk pediatric onco-hematological patients. *Eur J Haematol*. 2017;99:240-248.
12. Hovi L, Saarinen-Pihkala UM, Vettenranta K, Saxen H. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. *Bone Marrow Transplant*. 2000;26:999-1004.
13. Simms-Waldrip T, Rosen G, Nielsen-Saines K, Ikeda A, Brown B, Moore T. Invasive fungal infections in pediatric hematopoietic stem cell transplant patients. *Infec Dis*. 2015;47:218-224.
14. Mor M, Gilad G, Kornreich L, Fisher S, Yaniv I, Levy I. Invasive fungal infections in pediatric oncology. *Pediatr Blood Cancer*. 2011;56:1092-1097.
15. Hazar V, Karasu GT, Uygun V, et al. Risks and outcomes of invasive fungal infections in pediatric allogeneic hematopoietic stem cell transplant recipients receiving fluconazole prophylaxis: a multicenter cohort study by the Turkish Pediatric Bone Marrow Transplantation Study Group. *Med Mycol*. 2019;57:161-170.
16. Choi JK, Cho SY, Yoon SS, et al. Epidemiology and risk factors for invasive fungal diseases among allogeneic hematopoietic stem cell transplant recipients in Korea: Results of "RISK" Study. *Biol Blood Marrow Transplant*. 2017;23:1773-1779.
17. Groll AH, Castagnola E, Cesaro S, et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *Lancet Oncol*. 2014;15(8):e327-e340.
18. Science M, Robinson PD, MacDonald T, Rassekh SR, Dupuis LL, Sung L. Guideline for primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic stem cell transplant recipients. *Pediatr Blood Cancer*. 2014;61:393-400.
19. Christen D, Sohlbach K, Metzelder SK, et al. Outcome of non-mold effective anti-fungal prophylaxis in patients at high-risk for invasive fungal infections after allogeneic stem cell transplantation. *Leuk Lymphoma*. 2019;60:2056-2061.
20. Sarina B, Mariotti J, Bramanti S, et al. A reduced dose of fluconazole as primary antifungal prophylaxis is not associated with increased risk of invasive fungal infections after allogeneic stem cell transplantation from a HLA identical sibling. *Transpl Infect Dis*. 2018;20(4):e12906.
21. Park S, Kim K, Jang JH, et al. Randomized trial of micafungin versus fluconazole as prophylaxis against invasive fungal infections in hematopoietic stem cell transplant recipients. *J Infect*. 2016;73:496-505.
22. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood*. 2010;116:5111-5118.
23. Wolff SN, Fay J, Stevens D, et al. Fluconazole vs low-dose amphotericin B for the prevention of fungal infections in patients undergoing bone marrow transplantation: a study of the North American Marrow Transplant Group. *Bone Marrow Transplant*. 2000;25:853-859.
24. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med*. 2007;356:335-347.
25. Tissot F, Agrawal S, Pagano L, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica*. 2017;102:433-444.
26. Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. *Bone Marrow Transplant*. 2012;47(6):846-854.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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