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MAJOR ARTICLE

Invasive Mold Infections in Allogeneic Hematopoietic Cell Transplant Recipients in 2020: Have We Made Enough Progress?

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Background. Despite progress in diagnostic, prevention, and treatment strategies, invasive mold infections (IMIs) remain the leading cause of mortality in allogeneic hematopoietic cell transplant (allo-HCT) recipients.

Methods. We describe the incidence, risk factors, and mortality of allo-HCT recipients with proven/probable IMI in a retrospective single-center 10-year (01/01/2010–01/01/2020) cohort study.

Results. Among 515 allo-HCT recipients, 48 (9.3%) patients developed 51 proven/probable IMI: invasive aspergillosis (IA; 34/51, 67%), mucormycosis (9/51, 18%), and other molds (8/51, 15%). Overall, 35/51 (68.6%) breakthrough IMIs (bIMIs) were identified: 22/35 (62.8%) IA and 13/35 (37.1%) non-IA IMI. One-year IMI cumulative incidence was 7%: 4.9% and 2.1% for IA and non-IA IMI, respectively. Fourteen (29.2 %), 10 (20.8%), and 24 (50.0%) patients were diagnosed during the first 30, 31–180, and >180 days post-HCT, respectively. Risk factors for IMI included prior allo-HCT (sub hazard ratio [SHR], 4.06; $P = .004$) and grade ≥2 acute graft-vs-host disease (aGvHD; SHR, 3.52; $P < .001$). All-cause 1-year mortality was 33% (170/515): 48% (23/48) and 31.5% (147/467) for patients with and without IMI ($P = .02$). Mortality predictors included disease relapse (hazard ratio [HR], 7.47; $P < .001$), aGvHD (HR, 1.51; $P = .001$), CMV serology–positive recipients (HR, 1.47; $P = .03$), and IMI (HR, 3.94; $P < .001$). All-cause 12-week mortality for patients with IMI was 35.4% (17/48): 31.3% (10/32) for IA and 43.8% (7/16) for non-IA IMI (log-rank $P = .47$). At 1 year post–IMI diagnosis, 70.8% (34/48) of the patients were dead.

Conclusions. IA mortality has remained relatively unchanged during the last 2 decades. More than two-thirds of allo-HCT recipients with IMI die by 1 year post–IMI diagnosis. Dedicated intensified research efforts are required to further improve clinical outcomes.

Keywords. allogeneic hematopoietic cell transplant recipients; epidemiology; invasive aspergillosis; invasive mold infections; mortality.

Despite progress in diagnostic, prevention, and treatment strategies, invasive mold infections (IMIs) remain a leading cause of mortality in allogeneic hematopoietic cell transplant (HCT) recipients [1–5]. Soon after the introduction of fluconazole as primary antifungal prophylaxis, a shift from infections due to *Candida* spp. to invasive mold infections (IMIs), particularly invasive aspergillosis (IA), was reported [1–7]. Since then, administration of primary mold-active antifungal prophylaxis has been well established [8, 9]. As a result, antifungal prophylaxis breakthrough IMIs (bIMIs), associated with dismal outcomes, have been increasingly reported [10, 11]. Contemporary data in the era of routine mold-active prophylaxis on the epidemiology and outcomes of IMI in allogeneic HCT recipients are limited [5, 12–15]. We sought to describe the incidence, risk factors, and clinical outcomes of IMI in a recent 10-year cohort of allogeneic HCT recipients.

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METHODS

Study Design

This was a retrospective observational single-center cohort study performed from January 1, 2010, through December 31, 2019. All adult (≥18-year-old) patients who received an allogeneic HCT during the study period and with a minimum

follow-up of 1 year were included in the study. The study was approved by our institutional ethics committee.

Study Objectives

The primary objective of this study was to describe the cumulative incidence of proven and probable IMI during the first year post-transplantation. The following secondary objectives were described: (i) cumulative incidence and timing of IA and non-IA IMI, (ii) risk factors associated with IMI, and (iii) all-cause mortality and mortality predictors overall and in patients with IMI.

Institutional Standard of Care

Between 2010 and 2014, posaconazole at 300 mg twice daily on the first day and once daily thereafter was administered to the majority of patients, starting upon completion of the conditioning regimen and until engraftment due to higher numbers of IMI between 2009 and 2011 associated with patient transfers outside the hematology units while construction work in the hospital was ongoing [16]. Since 2015 and since control of that institutional IMI outbreak, routine primary antifungal prophylaxis had consisted of fluconazole 200 mg once daily, except for patients with specific (eg, environmental) risk factors, who receive posaconazole, starting upon completion of the conditioning regimen and until engraftment. From engraftment and until day 100 post-HCT, fluconazole 400 mg once weekly is administered [17]. Patients diagnosed with an IMI before HCT receiving antifungal treatment continue their treatment through conditioning and until at least 3 months post-HCT. Patients with GvHD requiring treatment with steroids at a prednisone dose equivalent to ≥1 mg/kg/d receive prophylaxis with posaconazole until the prednisone dose is <10 mg/d [9]. Patients with moderate to severe liver function abnormalities receive antifungal prophylaxis with an echinocandin until liver function tests improve. Per institutional guidelines and limited ability to perform twice weekly, once-weekly screening with serum galactomannan enzyme immunoassay (GM-EIA) is performed for all patients until 3 months post-HCT. Investigation and treatment of neutropenic fever are performed based on institutional protocol adjusted to international consensus guidelines [18–21]. Briefly, computed tomography (CT) of the chest and/or sinuses is performed if neutropenic fever persists after 5 days of broad-spectrum antibacterial treatment and as clinically indicated. In cases with suspicious lung lesions, a bronchoscopy is performed within 24–48 hours, with the bronchoalveolar lavage (BAL) tested by fungal stain and culture and GM-EIA. Transbronchial or lung and sinus biopsies are performed as clinically indicated and when feasible. Molecular testing by a panfungal, *Aspergillus*- and *Mucorales*-specific polymerase chain reaction (PCR) on BAL and tissue samples has consistently been performed at a reference laboratory since 2016.

Data Collection

Allogeneic HCT recipients were identified through the institutional HCT database. The following variables were collected through the HCT database: (i) demographics: age, gender; (ii) HCT-related variables: underlying malignancy, conditioning regimen (myeloablative, reduced intensity), HCT donor type (matched related, matched/mismatched unrelated, or haploidentical donor), HCT source (bone marrow, peripheral blood stem cells), GvHD prophylaxis regimen, and the cytomegalovirus (CMV) serostatus of donor (D) and recipient (R); (iii) post-HCT complications: grade ≥2 acute and chronic GvHD, disease relapse, and graft loss.

A detailed chart review was performed for all patients to record antifungal prophylaxis at the time of HCT and identify patients with proven and probable IMI. To ensure that all IMI cases were captured, we compared this list with the data set provided by the mycology department with all positive cultures for molds and GM-EIA on blood, BAL, and cerebrospinal fluid and β-D-glucan in blood specimens during the study period. All identified cases were carefully reviewed by 2 investigators (R.R. and D.N.) to confirm a proven or probable IMI diagnosis based on established consensus definitions [22]. For all patients with a proven or probable IMI, the following additional data were captured: antifungal prophylaxis administration within 30 days before IMI diagnosis, diagnostic tests performed, and administered antifungal treatment.

Definitions

Invasive mold infections were defined based on international consensus guidelines [22]. A GM-EIA optical density index (ODI) ≥0.5 on 2 consecutive serum samples and/or ≥1.0 on a single serum or BAL specimen was considered for the diagnosis of probable IA. Only proven and probable IMI were included. The day of IMI diagnosis was the day on which the first diagnostic test was performed. For patients who received >1 allogeneic HCT, the last HCT was considered. When a patient was diagnosed with an IMI between 2 HCTs, the HCT before IMI was considered, and follow-up was continued until the day of the next HCT, at which time the patient was censored. Breakthrough IMIs (bIMIs) were defined as an IMI diagnosed after a minimum of 7 days of mold-active antifungal prophylaxis [10, 23]. Timing of IMI was analyzed in 3 periods: very early (from HCT to day 30 post-HCT), early (from days 31 to 180 post-HCT), and late (after day 180 post-HCT), considering the associated pathophysiology and risk factors. Acute and chronic GvHD scoring was based on established guidelines [24].

Statistical Analysis

Patient and donor characteristics were described as count and percentage for qualitative data and as median and interquartile range (IQR) for quantitative data. Categorical and continuous variables were compared with the Fisher exact test and a 2-tailed

Student *t* test, as appropriate. We estimated the cumulative incidence of proven/probable IMI using competing risk analysis (competing event = death). A risk factor analysis for IMI was performed using a competing risk regression model according to the method of Fine and Gray, including relapse, acute GVHD, and chronic GVHD as time-varying covariates [25]. Survival functions (overall survival and survival after a diagnosis of IMI) were estimated according to the Kaplan-Meier method, and risk factors were assessed using Cox regression models, as there was no competing risk consideration in this case. The log-rank test was used to compare survival distribution between groups. Statistical significance was assessed at a 2-sided .05 level for all analyses. Data were analyzed using STATA 16.0 (StataCorp, College Station, TX, USA) and R-4.0.2 (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

RESULTS

A total of 515 patients underwent at least 1 allogeneic HCT during the study period and were included in this cohort with a median follow-up time (IQR) of 696 (258–1640) days post-HCT (Table 1). Twenty-eight (5.4%) patients had received at least 1 allogeneic HCT prior. Seventeen (3.3%) patients were diagnosed with a proven or probable IMI before their HCT. Antifungal prophylaxis on the day of HCT included fluconazole (244, 47.4%), mold-active azoles (181, 35.1%: posaconazole 123 [23.8%], voriconazole 55 [10.7%], and isavuconazole 3 [0.6%]), echinocandins (67, 13.0%), and liposomal amphotericin-B (23, 4.5%).

Incidence and Risk Factors of Proven/Probable IMI

The cumulative incidence of IMI overall, IA, and non-IA IMI by 1 year post-HCT was 7%, 4.9%, and 2.1%, respectively (Figure 1A). The median time post-HCT to IMI diagnosis (IQR) was 177 (3–1299) days: 166 (3–1365) days for IA and 218 (1–727) days for non-IA IMI ($P = .46$). The following variables were identified as significant risk factors for IMI (Supplementary Table 1): prior allogeneic HCT (sub hazard ratio [SHR], 4.06; 95% CI, 1.58–10.4; $P = .004$) and acute GvHD (SHR, 3.52; 95% CI, 1.74–7.14; $P < .001$). The cumulative 1-year post-HCT incidence of IMI based on a prior allogeneic HCT is presented in Figure 1B.

IMI Characteristics, Diagnosis, and Timing

There were 48 patients with 51 IMI (Table 2): 45 (93.8%) patients had 1 IMI due to 1 pathogen, 1 (2.0%) patient had 2 separate episodes of IMI with 2 different pathogens, and 2 (4.2%) patients had an IMI with 2 different pathogens concomitantly identified. The most frequently observed IMI was IA (34/51, 66.7%), followed by mucormycosis (9, 17.6%) and IMI due to other molds (8, 15.7%). Ten (19.6%) and 41 (80.4%) IMIs were proven and probable, respectively. The vast majority (40, 78.4%) of infections involved 1 site, while 11 (21.6%) IMIs affected >1 site. A culture-positive diagnosis was obtained in 27 of 43 (62.8%) patients who had a culture performed. A positive serum GM-EIA (ODI ≥0.5) was observed in 25 (59.5%) of 42 samples tested, with a median value of the first GM-EIA test performed per patient (IQR) of 0.97 (0.57–3.51). A BAL GMA-EIA was performed in 32 of 48 (66.7%) patients. The BAL GM-EIA ODI was ≥0.5 and ≥1.0 in 12 (37.5%) and 9 (28.1%) of 32 samples tested, respectively. The median value of the BAL GM-EIA test performed per patient (IQR) was 1.66 (0.53–6.7). A β-D-glucan test was performed in 8 of 48 (16.7%) patients and was positive (≥90 pg/mL) in 6/8 (75%) cases at a median (IQR) of 155.5 (97–500) pg/mL. A PCR on BAL was performed in 24 of 48 (50.0%) patients, and 9/24 (37.5%) tests were positive, with a median value (IQR) of 1780 (1000–10 100) copies/mL.

The distribution of HCT, IMI, and antifungal prophylaxis administered per year during the study period is presented in Figure 2. Although the number of allogeneic HCTs performed increased in the last 5 years of the study period (307/515, 59.6%) compared with the first 5 years (208/515, 40.4%), the number of patients diagnosed with IMI remained relatively stable (23 vs 25). Compared with the first 5 years of the study, primary antifungal prophylaxis at the time of HCT significantly changed in the last 5 years: Fluconazole and echinocandin use increased by 21.9% and 9.7% ($P < .001$), respectively, while administration of mold-active azoles decreased by 26.9% ($P = .001$). Fourteen (29.2 %), 10 (20.8%), and 24 (50.0%) patients were diagnosed with IMI during the first 30 days, between 31 and 180 days, and after 180 days post-HCT, respectively (Supplementary Figure 1).

bIMI

Thirty-five (of 51, 68.6%) cases were identified as bIMI, including 22/34 (64.7%) and 13/17 (76.5%) IA and non-IA IMI. Breakthrough IMIs occurred through prophylaxis with mold-active azoles (26/35, 74.3%), echinocandins (7/35, 20%), and liposomal amphotericin-B (2/35, 5.7%). Antifungal prophylaxis was administered for a median (IQR) of 30 (7–230) days before bIMI: 31 (8.5–280.5) and 29 (7–230) days before IA and non-IA, respectively. There was no significant difference in the proportion of bIMIs diagnosed in the last 5 (24/35, 68.6%) compared with the first 5 (11/16, 68.8%) years of the study ($P = 1.00$).

Mortality and Mortality Predictors

All-cause 1-year mortality post-HCT was 33% (170/515): 48% (23/48) and 31.5% (147/467) for patients with and without IMI, respectively ($P = .02$). The following variables were identified as significant mortality predictors by 1 year post-HCT (Supplementary Table 2): disease relapse (HR, 7.96; 95% CI, 5.73–11.05; $P < .001$), IMI (HR, 3.94; 95% CI, 2.46–6.31; $P < .001$), acute GvHD (HR, 1.77; 95% CI, 1.28–2.44; $P = .001$), and CMV positivity (HR, 1.47; 95% CI, 1.05–2.07; $P = .03$).

Among patients with IMI, all-cause mortality by 42 days after IMI diagnosis was 25% (12/48): 25.0% (8/32) and 25.0%

Table 1. Characteristics for 515 Allogeneic Hematopoietic Cell Transplant Recipients With and Without an Invasive Mold Infection

	All Patients (n = 515), No. (%)	IMI (n = 48), No. (%)	No IMI (n = 467), No. (%)	P[a]
Demographics				
Age, median (IQR), y	54 (43,62)	56 (38,60.5)	54 (43,62)	.58
Gender, male	316 (61.4)	29 (60.4)	287 (61.5)	.88
Underlying disease				.73
AML	239 (46.4)	25 (52.1)	214 (45.8)	.45
Lymphoma/CLL/MM[b]	90 (17.5)	7 (14.6)	83 (17.8)	.69
MDS[c]	90 (17.5)	7 (14.6)	83 (17.8)	.69
ALL	49 (9.5)	3 (6.2)	46 (9.8)	.61
MPN	22 (4.3)	4 (8.3)	18 (3.9)	.14
CML	14 (2.7)	1 (2.1)	13 (2.8)	1.00
Other[d]	11 (2.1)	1 (2.1)	10 (2.1)	1.00
Prior allogeneic HCT	28 (5.4)	6 (12.5)	22 (4.7)	.04
CMV D/R status				.22
D+R+	230 (44.7)	19 (39.6)	211 (45.2)	.54
D-R-	144 (28.0)	10 (20.8)	134 (28.7)	.31
D-R+	94 (18.2)	13 (27.1)	81 (17.3)	.12
D+R-	47 (9.1)	6 (12.5)	41 (8.8)	.43
HCT-associated variables				
Year of HCT				.28
2010–2014	207 (40.2)	23 (47.9)	184 (39.4)	
2015–2019	308 (59.8)	25 (52.1)	283 (60.6)	
Conditioning regimen				.24
Myeloablative	147 (28.5)	10 (20.8)	137 (29.3)	
Reduced intensity	368 (71.5)	38 (79.2)	330 (70.7)	
Donor				.38
MUD	236 (45.8)	25 (52.1)	211 (45.2)	.37
MRD	160 (31.1)	10 (20.8)	150 (32.1)	.14
Haplo-identical	75 (14.6)	9 (18.8)	66 (14.1)	.39
MMUD	44 (8.5)	4 (8.3)	40 (8.6)	1.00
HCT source				.27
BM	69 (13.4)	9 (18.8)	60 (12.8)	
PBSC	446 (86.6)	39 (81.2)	407 (87.2)	
GvHD prevention[e]				
Cyclosporin-A	360 (69.9)	34 (70.8)	326 (69.8)	1.00
MMF	296 (57.5)	31 (64.6)	265 (56.8)	.36
Methotrexate	203 (39.4)	14 (29.2)	189 (40.5)	.16
Tacrolimus	157 (30.5)	14 (29.1)	143 (30.6)	1.00
Cyclophosphamide	89 (17.3)	10 (20.8)	79 (16.9)	.55
Sirolimus	3 (0.6)	1 (2.1)	2 (0.4)	.26
Partial T-cell depletion	133 (25.8)	11 (22.9)	122 (26.1)	.73
Engraftment day, median (IQR), d[f]	17 (14, 20)	16 (14, 21)	17 (14, 20)	.20
GvHD-associated variables[g]				
Acute GvHD grade ≥2	246 (47.8)	31 (64.6)	215 (46.0)	.02
Day post-HCT, median (IQR)	44.5 (26, 115)	37 (18, 67)	46 (28, 118)	.18
Chronic GvHD	111 (21.6)	11 (22.9)	100 (21.4)	.85
Day post-HCT, median (IQR)	249 (179, 371)	264 (145, 410)	248 (184.5, 370.5)	.51
Donor lymphocyte infusion	110 (21.4)	8 (16.7)	102 (21.8)	.47
IMI before HCT	17 (3.3)	4 (8.3)	13 (2.8)	.06
Antifungal prophylaxis at HCT[h]				
Fluconazole	244 (47.4)	20 (41.7)	224 (48.0)	.45
Mold-active azole[i]	181 (35.1)	17 (35.4)	164 (35.1)	1.00
Echinocandin	67 (13.0)	7 (14.6)	60 (12.8)	.66
Lipid-formulation AMB	23 (4.5)	4 (8.3)	19 (4.1)	.26

Abbreviations: ALL, acute lymphoblastic leukemia; AMB, amphotericin B; AML, acute myeloid leukemia; BM, bone marrow; CLL/MM, chronic lymphoblastic leukemia/multiple myeloma; CML, chronic myeloid leukemia; CMV, cytomegalovirus; D-, donor-negative; D+, donor-positive; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplant; IMI, invasive mold infection; IQR, interquartile range; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MMUD, mismatched unrelated donor; MPN, myeloproliferative neoplasia; MRD, matched related; MUD, matched unrelated donor; PBSC, peripheral blood stem cells; R-, recipient-negative; R+, recipient-positive.

[a] *P* value was calculated with the Fisher exact for categorical variables and the *t* test for comparison of continuous variables between patients with and without IMI.

[b] This category included lymphoma (55, 10.7%), multiple myeloma (27, 5.2%), and chronic lymphoblastic leukemia (8, 1.6%).

[c] MPN included myeloproliferative syndromes.

[d] Other underlying diseases included aplastic anemia (9, 1.7%), hemoglobinopathy (1, 0.2%), and inborn error (1, 0.2%).

[e] Patients received >1 type of GvHD prophylaxis.

[f] A total of 8 patients did not engraft.

[g] Data on acute and chronic GvHD were reported for all patients.

[h] Prophylaxis included both intended primary antifungal prophylaxis at the time of HCT and antifungal treatment administered for a diagnosis of proven/probable/possible IFI before the HCT.

[i] Mold-active azoles included posaconazole (123, 23.8%), voriconazole (55, 10.7%), and isavuconazole (3, 0.6%).

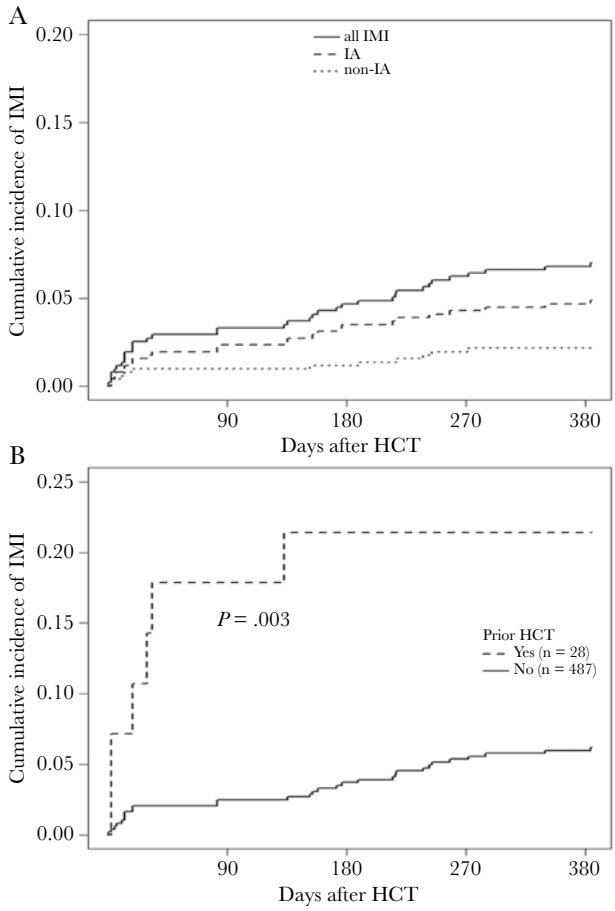

Figure 1. A, Cumulative incidence of proven or probable invasive mold infection (IMI), invasive aspergillosis (IA), and non-IA IMI during the first year after an allogeneic hematopoietic cell transplant (HCT). B, Cumulative incidence of proven or probable IMI during the first year after an allogeneic HCT based on whether patients had a prior allogeneic HCT. For 3 patients with >1 IMI, incidence was assessed based on non-IA IMI.

(4/16) for patients with IA and non-IA IMI, respectively (log-rank P = .90). All-cause mortality by 84 days after a diagnosis of IMI was 35.4% (17/48): 31.3% (10/32) and 43.8% (7/16) for patients with IA and non-IA IMI, respectively (log-rank P = .47) (Figure 3A). At 1 year post–IMI diagnosis, all-cause mortality was 70.8% (34/48): 68.8% (22/32) and 75.0% (12/16) for patients with IA and non-IA IMI, respectively (log-rank P = .75) (Figure 3B). There was a trend for higher all-cause 84-day mortality among patients with early (between 31 and 180 days post-HCT) IMI (6/10, 60%) when compared with patients with very early (≤30 days post-HCT; 4/14, 28.6%) and late (>180 days post-HCT; 7/24, 29.2%) IMI diagnosis (log-rank P = .08) (Figure 3C). All-cause mortality at 1 year post–IMI diagnosis was higher in patients with early (10/10, 100%) when compared with patients with very early (8/14, 57%) and late (16/24, 66.7%) IMI diagnosis (log-rank P = .002) (Figure 3D). Among patients with non-IA IMI, there was a trend for higher 84-day (log-rank P = .07) and 1-year survival (log-rank P = .11) in patients who underwent surgical resection of their infection (Figure 3E, F). There were no significant differences observed in 1-year post–IMI diagnosis all-cause mortality based on (a) certainty of IMI diagnosis (proven vs probable) and (b) primary vs bIMI (Supplementary Figure 2a and b).

DISCUSSION

This single-center retrospective cohort study offers important insights on the epidemiology, timing, and clinical outcomes of IMI in allogeneic HCT recipients. We report a relatively low incidence of IMI, in the range of 5%, consistent with recent clinical data [14, 26]. Invasive aspergillosis remains the most prevalent IMI, followed by mucormycosis. Breakthrough IMIs were frequently encountered, representing >50% of cases in this series and reflecting changes in the IMI epidemiology, as recently reported [10, 14, 23]. Almost one-third of patients with IA were dead by 12 weeks, similar to rates reported by the PATH Alliance Registry more than a decade ago [27]. High mortality could be, in part, attributed to the high number of bIMIs included in our study, infections historically associated with worse clinical outcomes [10]. However, we did not observe any significant difference in mortality in patients with and without bIMI. Our and other data suggest that 6- and 12-week survival in patients with IA has remained in the same range since the pivotal clinical trial comparing voriconazole with conventional amphotericin-B in 2002 [26–31]. Furthermore, a prospective placebo-controlled clinical trial failed to show an additional survival benefit in patients with a hematologic malignancy and/or allogeneic HCT recipients with IA treated with a combination of voriconazole with anidulafungin vs monotherapy with voriconazole [29]. The fact that IA survival has remained stagnant over the span of 2 decades, despite the ample availability of effective diagnostic and therapeutic modalities in the meantime, has not been adequately investigated. This could be attributed to a combined effect of the limitations associated with the currently available treatments and/or a trend for sicker hosts more recently. As HCT practices evolve and older, high-risk patients are considered more frequently for an allogeneic HCT, it is likely that the potential benefits of improved clinical care and currently available antifungal treatments are outweighed by patient comorbidities. However, data from the European Bone Marrow Transplant (EBMT) cohort show improved survival in patients transplanted after 2010 [32]. With azoles representing the major tool for the treatment of IA, one has to wonder if a certain plateau in the potential effect of this class might have been reached—particularly when considering the multiple associated toxicities, drug interactions, and therapeutic drug monitoring inconsistencies frequently leading to discontinuation of those agents [33, 34]. Improving survival beyond the limit of 70%–75% in allogeneic HCT recipients with IA represents an important challenge in this new decade.

Table 2. Detailed Description of Invasive Mold Infections Diagnosis

	IMI[a]	IA[b]	Mucormycosis[c]	IMI due to Other Molds[d]
	n = 51, No. (%)	n = 34, No. (%)	n = 9, No. (%)	n = 8, No. (%)
Certainty of diagnosis				
Proven	10 (19.6)	4 (11.8)	5 (55.6)	1 (12.5)
Probable	41 (80.4)	30 (88.2)	4 (44.4)	7 (87.5)
Time post-HCT, median (IQR), d	177 (3–1299)	177 (3–1365)	177 (1–421)	245 (13–727)
Site of infection				
1 site	40 (78.4)	28 (82.4)	6 (66.7)	6 (75)
>1 site[e]	11 (21.6)	6 (17.6)	3 (33.3)	2 (25)
Lung	44 (86.3)	31 (92.2)	9 (100.0)	6 (75.0)
Sinus	7 (13.7)	4 (11.8)	0 (0.0)	3 (37.5)
Brain	2 (3.9)	1 (2.9)	1 (11.1)	0 (0.0)
Skin	5 (9.8)	2 (5.9)	2 (22.2)	1 (12.5)
Other[f]	5 (9.8)	4 (11.8)	1 (11.1)	0 (0.0)
Histopathology, performed[g]	10 (19.6)	4 (11.8)	5 (55.6)	1 (12.5)
Positive	10 (100.0)	4 (100.0)	5 (100.0)	1 (100.0)
Culture[g]				
Sputum, performed	11 (21.6)	10 (29.4)	1 (11.1)	0 (0.0)
Positive	7 (63.6)	7 (70.0)	0 (0.0)	0 (0.0)
BAL, performed	30 (58.8)	19 (55.9)	6 (66.7)	5 (62.5)
Positive	15 (50.0)	7 (36.8)	5 (83.3)	3 (60.0)
Sinus, performed	7 (13.7)	5 (14.7)	0 (0.0)	2 (25.0)
Positive	4 (57.1)	2 (40.0)	0 (0.0)	2 (100.0)
Brain, performed	2 (3.9)	2 (5.9)	0 (0.0)	0 (0.0)
Positive	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)
Blood, performed	28 (54.9)	21 (61.8)	5 (55.6)	2 (25.0)
Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other,[h] performed	11 (21.6)	9 (26.5)	0 (0.0)	2 (25.0)
Positive	5 (45.5)	3 (33.3)	0 (0.0)	2 (100.0)
GMA-EIA, blood[g]				
Performed	42 (82.4)	31 (92.2)	7 (77.8)	4 (50.0)
Positive (≥0.5)	25 (59.5)	23 (74.2)	0 (0.0)	2 (50.0)
GM-EIA ODI, median (IQR)[i]	0.97 (0.57–3.51)	0.97 (0.57–3.51)		0.88 (0.83–0.92)
GMA-EIA, BAL[g]				
Performed	32 (62.7)	23 (67.6)	6 (66.7)	3 (37.5)
Positive (≥1.0)	9 (28.1)	8 (34.8)	0 (0.0)	0 (0.0)
GM-EIA ODI, median (IQR)[i]	1.66 (0.53–6.7)	2.16 (0.84–6.7)		0.53
PCR, BAL[g]				
Performed	24 (47.1)	15 (44.1)	6 (66.7)	3 (37.5)
Positive	9 (37.5)	7 (46.7)	2 (33.3)	0 (0.0)
PCR, median (IQR), copies/mL	1780 (1000–10 100)	1780 (1000–10 100)		
b-D-glucan (≥90 pg/mL)[g]				
Performed	8 (15.7)	8 (23.5)	0 (0.0)	0 (0.0)
Positive	6 (75.0)	6 (75.0)	0 (0.0)	0 (0.0)
Median (IQR)	155.5 (97–500)	155.5 (97–500)		

Abbreviations: BAL, bronchoalveolar lavage; GM-EIA, galactomannan enzyme immunoassay; HCT, hematopoietic cell transplant; IA, invasive aspergillosis; IMI, invasive mold infection; IQR, interquartile range; ODI, Optical Density Index; PCR, polymerase chain reaction.

[a]There were 45 patients with 1 IMI and 3 patients with >1 IMI: 1 patient had 2 separate episodes of IMI with mucormycosis followed by IA, and 2 patients had an IMI with 2 different pathogens concomitantly identified: 1 patient had a pulmonary IMI due to *Rhizomuccor pucillus* and *Scopulariopsis* spp., and another had a lung infection due to *Aspergillus* spp. and sinusitis due to *Fusarium* spp.

[b]*Aspergillus* species included 13 *A. fumigatus*, 3 *A. terreus*, 3 *A. ustus*, 2 *A. niger;* in 18 cases, the *Aspergillus* spp. were not identified.

[c]*Mucorales* species included 6 *Rhizomucor pucillus,* 2 *Rhizopus* spp., and 1 *Absidia*.

[d]Other molds included 3 *Fusarium* spp. and 1 each of: *Alternaria* spp., *Hormographiella aspergillata*, *Scedosporium* spp., *Schizophyllum commune, Scopulariopsis* spp.

[e]Eleven patients had an IMI that affected >1 site. Nine patients had an IMI on 2 sites: 3 patients had IA diagnosed in the lung and sinus, 1 patient had IA diagnosed in the lung and brain, 1 patient had IA diagnosed in the skin and humeral head, 1 patient had *Rhizomucor pucillus* recovered from the lung and pleural fluid, and 1 patient had a lung infection due to *Rhizomucor pucillus* and skin infection due to *Alternaria* spp. One patient had *Rhizomucor pucillus* identified in 3 sites including the lung, brain, and skin. One patient had *Aspegillus* spp. recovered on 6 different sites: lung, skin, abdomen, pancreas, heart, and the iliac fossa.

[f]Other sites of infection included the abdomen (2), bone (2), and heart (1).

[g]Percentages represent the proportion of positive tests over the number of tests performed.

[h]Other included 11 culture specimens that were tested: 3, 2, 2, 1, 1, 1, and 1 from pleural fluid, sinus, skin, osteoarticular fluid, tunneled central line, cerebrospinal fluid, and lung transbronchial biopsy, respectively. Five specimens were positive by culture for the following pathogens: *Hormographiela aspergillata* from pleural fluid, *Aspergillus* spp. from pleural fluid, tunneled central line and skin and *Alternaria* spp. from skin.

[i]The median GM-EIA ODI presented refers to the first positive result per patient.

More than two-thirds of allogeneic HCT recipients diagnosed with an IMI had died 1 year after their infection was diagnosed, an observation consistent with prior data from the early 2000s [1–5, 26]. Moreover, IMI was identified as an important independent predictor of mortality at 1 year post-HCT. It is unclear why patients with IMI continue to have such dismal long-term outcomes. It is less likely for IMI to have a direct effect on 1-year all-cause mortality. Historically, 6-week mortality has been considered more representative of IMI-attributable mortality [35]. An effect of IMI treatments and their associated toxicities on clinical outcomes cannot be ruled out, although this needs to be further evaluated. Patients with moderate to severe acute GvHD and a prior allogeneic HCT were at higher risk to develop an IMI in this cohort. The above suggest that sicker patients may be more likely to develop an IMI and hence succumb as a result of their cumulative underlying immunosuppression. Whether IMI represents and/or may be used as a surrogate marker of severe immunosuppression and higher mortality remains to be studied. However, the above underscore the need for better understanding of those patients at higher risk and the need to target them for closer monitoring and clinical care optimization.

Despite the relatively low cumulative incidence of IMI, consistent with prior series, our data provide new insights on the timing of IMI after an allogeneic HCT [1, 14, 26]. Almost one-third of patients developed an IMI before engraftment. This may be, in part, associated with fluconazole being used as routine primary antifungal prophylaxis at our center. However, a prospective clinical trial comparing fluconazole with voriconazole as antifungal prophylaxis in allogeneic HCT recipients between day 0 and day 100 post-HCT failed to show a significant IMI reduction associated with voriconazole administration [36]. Stratification of patients at higher risk for IMI to receive anti-mold-active prophylaxis may allow for observation of a more significant effect of such prophylactic strategies. Considering the higher incidence of IMI in patients with a prior allogeneic HCT observed in our study, we have thus changed our primary antifungal prophylaxis from fluconazole to posaconazole for those patients. The vast majority of IMIs were diagnosed late post-HCT. This coincides with the time when most allogeneic HCT recipients return home, a large number of them potentially diagnosed with chronic GvHD. Although in the vast majority those patients receive mold-active antifungal prophylaxis, they may still develop a bIMI. It has been suggested that suboptimal posaconazole blood concentrations may be associated with higher rates of bIMI [37, 38]. However, recent data from the Swiss Transplant Cohort Study suggest that allogeneic HCT recipients may still develop bIMI despite therapeutic posaconazole blood levels [39]. It is likely that their persistent high-degree immunosuppression, along with a more intensive exposure to mold spores as outpatients, may, in part, contribute to their developing an IMI late post-HCT despite effective antifungal prophylaxis. With more allogeneic HCT recipients surviving for longer and exposed to variable environmental hazards, the dynamic relationship between host and environmental risk factors and antifungal prophylaxis is an evolving field requiring more data. Of note, only 10 infections were diagnosed between 30 and 180 days post-HCT, suggesting that close monitoring and administration of posaconazole as primary antifungal prophylaxis in patients with early acute GvHD remain effective strategies [9]. Despite the relatively fewer cases early post-HCT, mortality in this patient group was significantly

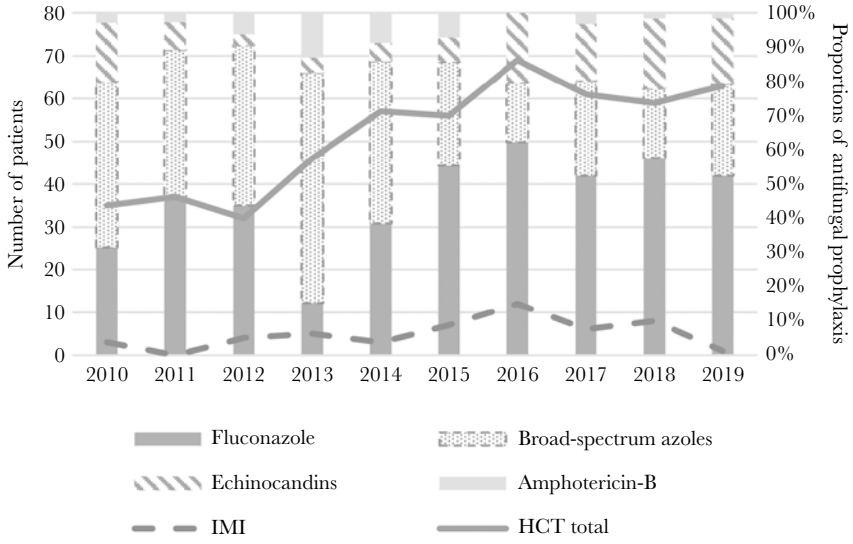

Figure 2. Absolute numbers of allogeneic hematopoietic cell transplant (HCT) performed during the study period and invasive mold infections (IMIs), with proportions of antifungal prophylaxis administered at the time of HCT, presented by calendar year. Only primary antifungal prophylaxis prescribed at the time of HCT was considered in this figure, without taking into consideration subsequent changes on antifungal prophylaxis performed during the patient's post-HCT course.

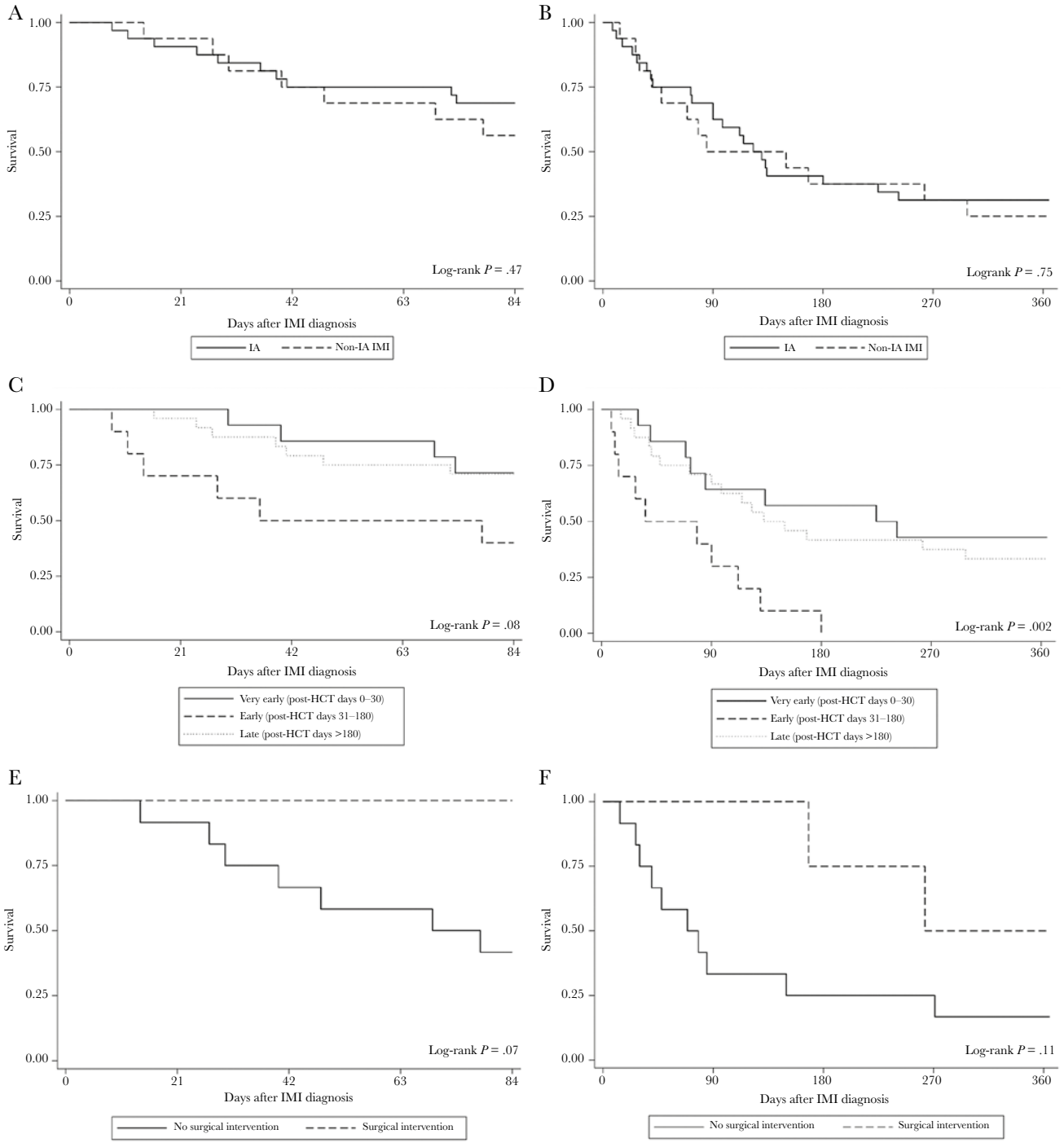

Figure 3. All-cause mortality presented as Kaplan-Meier survival curves for allogeneic hematopoietic cell transplant (HCT) recipients based on the (i) type of IMI: proven or probable invasive aspergillosis (IA) vs other-than-*Aspergillus* invasive mold infection (IMI) by (A) 84 days and (B) 1 year after IMI diagnosis; (ii) timing of IMI diagnosis: very early (0–30 days), early (31–180 days), and late (>180 days) post-HCT by (C) 84 days and (D) 1 year after IMI diagnosis; (iii) surgical intervention or not for patients with a proven or probable non-IA IMI by (E) 84 days and (F) 1 year after IMI diagnosis.

higher. In fact, by 6 months post–IMI diagnosis, all 10 patients with early post-HCT IMI had died. This may reflect the underlying immune status of those patients, most of them heavily treated for acute severe GvHD, succumbing to HCT-associated complications, rather than an IMI itself. Notably, no azole-resistant *Aspergillus fumigatus* isolates were identified in this study, consistent with environmental data in Switzerland [40]. Our findings further point to the need for close follow-up of patients with severe early GvHD during the first 6 months post-HCT to optimize patient care and improve clinical outcomes.

We report improved mortality rates in patients with non-IA IMI compared with historical data, essentially mirroring that

of IA [2, 7, 27, 41–43]. Traditionally, non-IA IMIs, particularly mucormycosis, have been correlated with higher mortality [2, 7, 27, 41]. However, our findings are in line with recently published data on the outcomes of IMIs due to *Mucorales*, suggesting a potential trend for improving outcomes in patients with mucormycosis [42]. It is likely that higher rates of prompt diagnosis, based on high clinical suspicion and our institutional protocols and aggressive diagnostic approaches, might have allowed for prompt treatment initiation and better outcomes. Although not always feasible, attempts were made to surgically remove lesions attributed to *Mucorales* and other molds, which are associated with improved clinical outcomes as shown by our survival analyses. However, due to the small number of non-IA IMIs, further conclusions cannot be drawn.

This study has important limitations, including its retrospective single-center design and the small number of IMIs included. Furthermore, mortality was not feasible to attribute, considering the retrospective nature of this study and relevant potential biases. However, our data show a shift in late IMI diagnosis in allogeneic HCT recipients and confirm the improved, albeit stagnant over the last 2 decades, survival in patients with IA. In addition, we show clear survival improvement in patients with non-IA IMI. A high 1-year all-cause mortality in patients diagnosed with an IMI remains a major problem. More studies and actions are urgently required to better understand and overcome the survival plateau reached in allogeneic HCT recipients with IMI.

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