MAJOR ARTICLE

Invasive Aspergillosis following Hematopoietic Cell Transplantation: Outcomes and Prognostic Factors Associated with Mortality

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Background. Invasive aspergillosis (IA) is a leading cause of infection-related mortality following hematopoietic cell transplantation (HCT). The aim of this study was to determine the probability of survival and prognostic factors associated with outcomes over a long period of time.

Methods. Cases of proven and probable IA diagnosed in HCT recipients at the Fred Hutchinson Cancer Research Center from 1 January 1990 through 31 December 2004 were included. Patient data were collected from a prospectively maintained database and by retrospective clinical chart review. Survival was estimated using Kaplan-Meier curves, and Cox regression models were used for multivariable analyses.

Results. Four hundred five cases were identified. The probability of survival at 90 days after diagnosis was higher for patients identified as having IA between 2002 and 2004 than for patients whose IA was diagnosed in preceding years (45% vs. 22%; $P < .001$). Risk factors independently associated with all-cause mortality include impairment in pulmonary function before HCT, receipt of human leukocyte antigen–mismatched stem cells, neutropenia, elevated bilirubin and creatinine levels, receipt of corticosteroids at $\geqslant 2$ mg/kg per day, disseminated and proven IA, and IA occurring >40 days after HCT. Factors associated with a decreased risk of all-cause mortality included receipt of nonmyeloablative conditioning and peripheral blood stem cells. In a subanalysis of attributable mortality restricted to patients receiving antifungal therapy, receipt of voriconazole was independently associated with protection from IA-related death.

Conclusions. There has been a significant decrease in mortality in patients with a diagnosis of IA following HCT in recent years, coinciding with multiple changes in transplantation practices, including use of nonmyeloablative conditioning regimens, receipt of peripheral blood stem cells, more prompt diagnosis of IA, and use of voriconazole.

Invasive aspergillosis (IA) is a common cause of infection-related mortality in hematopoietic cell transplant (HCT) recipients. Crude mortality 1 year after diagnosis ranges from 70% to 93% [1–4]. Several factors probably contribute to the poor outcomes. These patients have significant immune compromise from cumulative insults, including underlying disease, previous cytotoxic chemotherapies and conditioning regimens, and prophylaxis and therapy for graft-versus-host disease (GVHD). The diagnosis is often made late in the course of infection, when fungal burden is high and antifungal treatment is less likely to be efficacious.

Although the risk factors for the development of IA have been well documented [2, 4–6], factors dictating outcomes are less well described. In published studies, risk factors identified as having an impact on mortality include those reflecting degree of patient immunosuppression and disseminated infection [3, 7]. However, few large studies have focused on host, transplant, and infection variables that could affect survival. This information may be considered particularly important for improving the design of future clinical trials. The purpose of the current study was to describe changes in outcomes over time and to identify prognostic factors associated with outcomes.

PATIENTS AND METHODS

Study patients. HCT recipients given the diagnosis of probable and proven IA from 1 January 1990 through

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31 December 2004 were included. Patients with sinus or cutaneous or soft-tissue aspergillosis and those with mixed mold infections were excluded. Patient data were collected from a prospectively maintained database and by retrospective chart review. This study was approved by the Fred Hutchinson Cancer Research Center (Seattle, WA) institutional review board.

Testing. Conditioning regimens, GVHD prophylaxis, and supportive care of HCT recipients at Fred Hutchinson Cancer Research Center have been described previously [1, 8–11]. Physical examination, chest radiography and/or CT, and blood cultures were performed to identify sources of fever. Additional samples for culture were obtained as clinically indicated. All tissue and bronchoalveolar lavage fluid samples were evaluated for the presence of fungi by use of standard histopathologic and microbiological techniques. In 2003, the Platelia *Aspergillus* galactomannan EIA was introduced and included as part of the diagnostic evaluation of patients presenting with fever and/or radiographic abnormalities. The test was used on serum and bronchoalveolar lavage fluid, using a galactomannan index of $\geqslant$0.5 to define positivity [12, 13]. Use of the assay was dictated by suspicion of disease; routine screening was not performed.

Therapy for documented IA. Before 1997, conventional amphotericin B (0.5–1.0 mg/kg per day) was administered, except for 16 patients from 1992 through 1996 who received a lipid amphotericin B preparation. Subsequently, patients received an equivalent dose (5 kg/kg per day) of lipid amphotericin B. In 1998, voriconazole became available during the conduct of clinical trials and, by 2002, had largely replaced amphotericin B products. After 2002, caspofungin was added to voriconazole for primary treatment of documented IA. Starting in 1996, oral itraconazole was used for maintenance therapy. After 2002, oral voriconazole was used. Therapy typically continued until prednisone treatment was discontinued and radiographic abnormalities resolved.

Definitions and statistical analyses. Standard definitions were used for proven and probable IA [14]. Proven disease required histopathologic or microbiological documentation of infection from tissues obtained by biopsy or autopsy or in culture samples from a normally sterile site. Probable disease required microbiological and radiological documentation with compatible signs and symptoms in a patient with a recent history of neutropenia, prolonged corticosteroid use, or treatment with T cell immune suppressants. Microbiological documentation included detection by direct microscopy, culture of *Aspergillus* species, or a positive result of *Aspergillus* galactomannan EIA. Radiological documentation required any of the following new infiltrates on chest CT—halo sign, air-crescent sign, or cavity within an area of consolidation—or any new infiltrate not mentioned above and symptoms of lower respiratory tract infection. The site of infection was recorded as either pulmonary or disseminated (evidence of infection in at least 2 noncontiguous sites or isolated CNS infection). The day of diagnosis of IA was the day on which the first diagnostic microbiological test was performed. For IA diagnosed at autopsy, the date of diagnosis was the date of death.

The primary end points were overall and attributable mortality. Attributable morality was defined as progressive organ failure involving the organ(s) in which IA was diagnosed and the absence of other morbid conditions thought, by the attending physician or pathologist, to have contributed to death, excluding GVHD. Death with IA was defined as either ante- or postmortem evidence of IA, in the presence of other morbid conditions, except GVHD and relapsed underlying disease. Patients with relapsed underlying disease at time of death were considered to have died of relapsed disease. Cause of death was considered "other" for patients not fulfilling criteria for the previous 3 categories.

Host variables included sex, age, pre-HCT pulmonary function, and body mass index (calculated as weight in kilograms divided by the square of height in meters). Underlying disease was classified as good or poor risk: good-risk disease included acute leukemia in first remission, chronic myeloid leukemia in chronic phase, myelodysplasia-refractory anemia, aplastic anemia and nonmalignant hematologic diseases; poor-risk disease included all other diagnoses. Transplant-related variables included donor type, stem cell source, conditioning regimen (ablative vs. nonablative), and receipt of second myeloablative allogeneic transplant. IA-related variables included timing of IA following HCT (early, $\leqslant$40 days; late, >40 days) [1], disseminated or pulmonary, proven or probable diagnosis, and *Aspergillus* species. Antifungal therapy (primary) was defined as therapy with a systemic antifungal agent with anti-*Aspergillus* activity given for $\geqslant$5 consecutive days, prescribed either empirically or therapeutically. Impact of time-dependent variables was also measured, considering those values apparent around the time of diagnosis. Neutropenia (defined as 2 days with count of <750 neutrophils/μL), monocytopenia (defined as 2 days with count of <100 monocytes/μL), and lymphopenia (defined as 2 days with a count of <300 lymphocytes/μL) at the time of diagnosis of IA were included as time-dependent variables. Other time-dependent variables included acute GVHD (grade 3 or 4), chronic GVHD (clinically extensive), cytomegalovirus disease [15], hyperbilirubinemia (total serum bilirubin level of >6.5 mg/dL), and elevated creatinine (serum creatinine level of $\geqslant$2.5 mg/dL) in the 7 days before diagnosis of IA. Cumulative corticosteroid doses were calculated for the week following diagnosis of IA and were recorded in average daily prednisone equivalents (milligrams per kilogram). When corticosteroid doses were not available for each date of interest, estimated doses were calculated using standard tapering schedules.

Overall survival was estimated with Kaplan-Meier curves and

compared between groups using log-rank P values. Mortality attributable to IA was estimated with cumulative incidence curves, in which deaths due to causes other than IA were considered to be competing risk events. Mortality in the setting of GVHD was considered to be caused by IA, not GVHD. The hazard of attributable mortality was compared between groups using log-rank P values. Multivariable analyses were performed to determine factors associated with overall and attributable mortality. Variables with P values of <.10 in univariate models were entered into Cox regression models, and 2-sided tests were considered significant at the .05 level.

RESULTS

Four hundred five patients with probable or proven IA were included in the study. Characteristics of the study cohort are summarized in table 1. Characteristics of IA, therapies, and outcomes are detailed in table 2. Over the 15-year period, the number of IA cases ranged from 55 to 95 per 3-year interval. In recent years, fewer cases were proven, and the rate of disseminated IA appeared to decrease. Most patients (90.9%) died after diagnosis of IA; approximately one-third (31.6%) of deaths were considered to be attributable to IA.

Outcomes: changes over time. Figure 1 demonstrates the Kaplan-Meier probability of survival in the overall cohort, divided into five 3-year time periods. The probability of survival at 30 days, 90 days, and 1 year for patients given diagnoses during 2002–2004 was significantly higher than in the preceding years (1990–2001): 30 days, 69% versus 33% ($P < .0001$); 90 days, 44% versus 22% ($P < .0001$); and 1 year, 28% versus 13% ($P < .0001$). Figure 2 demonstrates the probability of attributable mortality. Attributable mortality at 30 days and 90 days was significantly higher in patients whose IA was diagnosed during 1990–2001 than in patients whose IA was diagnosed in subsequent years (29% vs. 17% [$P = .008$] and 32% vs. 23% [$P < .01$]).

Risk factors for death. Risks for death are shown in table 3. In multivariable analysis, the only host variable increasing risk for death was the presence of severe pulmonary function

Table 1. Characteristics of the cohort of 405 patients with proven or probable invasive aspergillosis (IA) following hematopoietic cell transplantation (HCT), 1990–2004.

Characteristic	Value
Age, median years (range)	42.2 (0.6–68.7)
Male sex	240 (59.3)
Body mass index,[a] median (range)	25.1 (15.0–52.4)
Severe PFT abnormality before HCT[b]	21 (5.2)
Good-risk underlying disease[c]	125 (30.9)
Allogeneic HCT	381 (94.1)
Stem cell source	
Bone marrow	274 (67.7)
Cord blood	3 (0.7)
Peripheral blood	128 (31.6)
HLA match	
Matched	287 (70.9)
Mismatched	94 (23.2)
Myeloablative HCT	367 (90.6)
Cytomegalovirus disease between HCT and diagnosis of IA	75 (18.5)
Cytomegalovirus disease within 7 days of diagnosis of IA	47 (11.6)
Elevated bilirubin level[d]	87 (21.5)
Elevated creatinine level[e]	48 (11.9)
Acute GVHD, grade ⩾3	185 (45.7)
Chronic GVHD, clinically extensive	141 (34.8)

NOTE. Data are no. (%) of patients, unless otherwise indicated. GVHD, graft-versus-host disease.

[a] Calculated as weight in kilograms divided by the square of height in meters.

[b] Pulmonary function testing abnormality: forced vital capacity in 1 min and adjusted diffusing capacity <60% of predicted value for patient age and sex.

[c] Acute leukemia in first remission, chronic myeloid leukemia in chronic phase, myelodysplasia-refractory anemia, aplastic anemia, and nonmalignant hematologic diseases; poor-risk disease included all other diagnoses.

[d] Total serum bilirubin level of >6.5 mg/dL.

[e] Serum creatinine level of >2.5 mg/dL.

Table 2. Characteristics of invasive aspergillosis (IA) in hematopoietic cell transplant (HCT) recipients and patient outcomes.

Characteristic	Value
Type of IA	
Proven	235 (58.0)
Pulmonary only	295 (72.8)
Late[a]	302 (74.6)
Days from HCT to IA, median (range)	77.0 (2.0–2842.0)
Year of diagnosis of IA	
1990–1992	68 (16.8)
1993–1995	92 (22.7)
1996–1998	95 (23.5)
1999–2001	95 (23.5)
2002–2004	55 (13.6)
Diagnosis made by autopsy[b]	
1990–1992	18 (27.7)
1993–1995	12 (13.0)
1996–1998	12 (12.6)
1999–2001	18 (18.9)
2002–2004	2 (3.6)
Disseminated IA[b]	
1990–1992	31 (46)
1993–1995	28 (23)
1996–1998	22 (23)
1999–2001	20 (21)
2002–2004	7 (13)
Proven IA[b]	
1990–1992	56 (82)
1993–1995	60 (66)
1996–1998	52 (54)
1999–2001	51 (54)
2002–2004	16 (29)
Primary antifungal therapy[c]	
Systemic anti-mold therapy for ⩾5 days	286 (70.6)
Conventional amphotericin B	131 (32.3)
Lipid amphotericin B	80 (19.8)
Vori-containing regimen	58 (14.3)
Vori plus caspofungin	33 (8.1)
Vori monotherapy	25 (6.2)
Death over course of study	368 (90.9)
Death due to IA	128 (31.6)
Death with IA	126 (31.1)
Death due to relapsed underlying disease	65 (16.0)
Death due to other	49 (12.1)
Time from IA diagnosis to death or last follow-up, median days (range)	13.0 (0.0–3361.0)

NOTE. Vori, voriconazole.

[a] Infection diagnosed >40 days after HCT.

[b] The denominator is the number of IA cases diagnosed during the relevant 3-year period.

[c] Systemic antifungal therapy with anti-*Aspergillus* activity given for at least 5 consecutive days.

abnormality before HCT. Type of transplant had an impact on risk of death, with receipt of nonmyeloablative conditioning and peripheral blood stem cells decreasing the risk and HLA mismatch increasing the risk. Complications associated with increased risk included the presence of neutropenia at time of IA diagnosis, elevated creatinine and bilirubin levels, and treatment with corticosteroids at ⩾2 mg/kg per day. Factors related to an increased risk of death included disseminated infection, a diagnosis of "proven" IA, and IA occurring late (>40 days after HCT). Year of IA diagnosis was univariately associated with risk of death and of attributable mortality but was not included in multivariable models because of its collinearity with voriconazole receipt.

Risk factors for attributable mortality. Risk factors for attributable mortality are shown in table 4. In this model, receipt of nonmyeloablative conditioning was protective from IA-related death (hazard ratio [HR], 0.2; 95% CI, 0.1–0.6), whereas HLA mismatch, elevated creatinine and bilirubin levels, treatment with corticosteroids at ⩾2 mg/kg per day, and disseminated and late IA were independently associated with higher risk of attributable mortality. Although receipt of specific antifungal agents was not independently associated with outcomes, in the univariate model, receipt of voriconazole was associated with a lower risk of IA-related death than was receipt of other antifungals (table 4 and figure 3). No differences in outcomes were seen in patients who received the voriconazole and caspofungin combination, compared with those who received voriconazole monotherapy, in either univariate or multivariable analysis (table 4), although few patients were treated with the combination ($n = 33$).

To further analyze the impact of antifungal therapy, we evaluated risks for IA-related death in 192 patients who received a systemic mold-active agent for at least 5 days, and excluded patients who had severe organ dysfunction (increased bilirubin or creatinine levels), which were strong predictors of death. In this multivariable subanalysis, neutropenia at time of IA diagnosis (HR, 3.5; 95% CI, 1.5–8.0; $P = .003$), receipt of corticosteroids at ⩾2 mg/kg per day (HR, 3.8; 95% CI, 1.6–8.8; $P = .002$), and disseminated IA (HR, 5.9; 95% CI, 3.0–11.5; $P < .0001$) were risks for IA-related death, whereas receipt of voriconazole monotherapy was associated with a trend to protection from IA-related death (HR, 0.4; 95% CI, 0.1–1.0; $P = .06$). In this limited cohort, receipt of combined voriconazole and caspofungin still had no effect on attributable mortality in univariate analysis ($P = .73$).

DISCUSSION

This study describes the largest single-center experience of IA outcomes following HCT. Our data clearly demonstrate an increase in survival after infection since the late 1990s, associated with concurrent advances in clinical practice. Changes that have

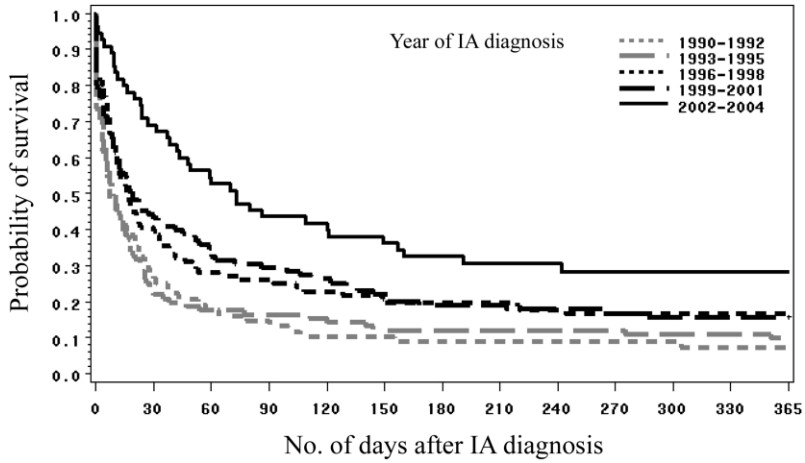

Figure 1. Kaplan-Meier probability of overall survival after diagnosis of invasive aspergillosis (IA) following hematopoietic cell transplantation, according to year of IA diagnosis. Year of diagnosis is divided into five 3-year time periods: 1990–1992, 1993–1995, 1996–1998, 1999–2001, and 2002–2004. Survival for diagnosis years 2002–2004 is compared with that for 1990–2001 ($P < .0001$).

contributed to improved outcomes include the use of nonmyeloablative conditioning regimens, the use of peripheral blood stem cells, more prompt diagnosis of IA, and widespread use of voriconazole.

In this study, nonmyeloablative conditioning regimens (part of investigative protocols since 1997) were independently associated with a lower risk for death. Although recipients of nonmyeloablative conditioning typically are older, have more comorbidities, and have received more previous cytotoxic chemotherapies than recipients of myeloablative conditioning, they typically experience fewer hematologic and nonhematologic toxicities, translating into a lower incidence of nonrelapse mortality [16]. There is some preservation of immunity following nonmyeloablative conditioning; neutropenia either does not develop or is of shortened duration, and mixed T cell chimerism allows for persistence of host T cells [17]. It is notable that nonmyeloablative conditioning was no longer associated with protection from death when the analysis was restricted to the 192 patients without severe organ dysfunction and who had received antifungal therapy for at least 5 days, consistent with a recent study of 51 cases of IA after HCT [7] that did not identify conditioning regimen to have a meaningful impact on attributable mortality. It is possible that the nonmyeloablative conditioning regimens are of particular benefit for people who have some degree of organ (liver and kidney) impairment at baseline.

Receipt of peripheral blood stem cells, another variable associated with reduced duration of cytopenia [8], was also in-

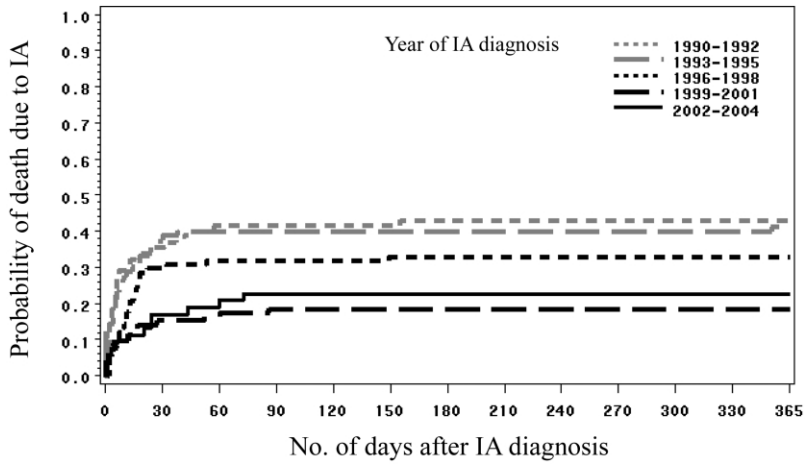

Figure 2. Probability of attributable death after diagnosis of invasive aspergillosis (IA) following hematopoietic cell transplantation, according to year of diagnosis of IA. Year of diagnosis is divided into five 3-year time periods: 1990–1992, 1993–1995, 1996–1998, 1999–2001, and 2002–2004. Probability of death for diagnosis years 2002–2004 is compared with that for 1990–2001 ($P < .01$).

Table 3. Risk factors for death due to all causes in 405 hematopoietic cell transplant (HCT) recipients with proven or probable invasive aspergillosis (IA).

	Unadjusted		Adjusted	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*
Recipient-related factors				
Year of IA diagnosis (2002–2004 vs. previous)	0.6 (0.5–0.9)	.008	…	…
Good-risk underlying disease[a]	0.7 (0.5–0.9)	.001	NS	NS
Severe impairment on PFT[b]	1.6 (1.0–2.8)	.07	2.0 (1.1–3.8)	.03
Transplant-related factors				
HLA mismatch	1.3 (1.0–1.6)	.04	1.4 (1.1–1.8)	.02
Unrelated donor	1.3 (1.1–1.6)	.008	NS	NS
Receipt of PBSCs	0.7 (0.6–0.9)	.009	0.8 (0.6–1.0)	.08
Nonmyeloablative conditioning	0.6 (0.4–0.9)	.006	0.6 (0.4–1.0)	.05
Transplant complications				
Neutropenia[c]	2.3 (1.7–3.1)	<.0001	1.6 (1.1–2.3)	.02
Lymphopenia[d]	1.9 (1.5–2.4)	<.0001	…	…
Monocytopenia[e]	1.3 (1.0–1.6)	.05	…	…
Acute GVHD[f]	1.8 (1.5–2.2)	<.0001	NS	NS
Hyperbilirubinemia[g]	43.8 (33.5–57.1)	<.0001	2.9 (2.0–4.1)	<.0001
Elevated creatinine level[h]	10.4 (7.5–14.2)	<.0001	2.0 (1.3–3.0)	.001
Cytomegalovirus disease[i]	2.1 (1.6–2.8)	<.0001	NS	NS
Corticosteroid dosage of ⩾2 mg/kg per day	3.1 (2.2–4.4)	<.0001	1.6 (1.1–2.4)	.008
IA-related factors				
Disseminated IA	4.1 (3.3–5.2)	<.0001	1.9 (1.4–2.6)	<.0001
Late IA	3.7 (2.6–5.3)	<.0001	2.5 (1.7–3.7)	<.0001
Proven IA	2.7 (2.0–3.7)	<.0001	1.3 (1.0–1.6)	.08
Voriconazole monotherapy	0.6 (0.4–1.0)	.06	NS	NS

NOTE. GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; PBSCs, peripheral blood stem cells; …, variable was not entered in the multivariable model.

[a] Acute leukemia in first remission, chronic myeloid leukemia in chronic phase, myelodyplasia-refractory anemia, aplastic anemia, and nonmalignant hematologic diseases; poor-risk disease included all other diagnoses.

[b] Severe pulmonary function test abnormality: forced vital capacity in 1 min and adjusted diffusing capacity <60% of predicted value for patient age and sex.

[c] Neutrophil count of <750 on 2 consecutive days concurrent with IA, compared with IA in absence of neutropenia.

[d] Lymphocyte count of <300 on 2 consecutive days concurrent with IA, compared with IA in absence of neutropenia.

[e] Monocyte count of <100 on 2 consecutive days concurrent with IA, compared with IA in absence of neutropenia.

[f] Grade, ⩾3.

[g] Total serum bilirubin level of >6.5 mg/dL.

[h] Serum creatinine level of ⩾2.5 mg/dL.

[i] Diagnosed between HCT and diagnosis of IA.

dependently associated with reduced risk of death due to all causes. We investigated the impact of neutropenia, monocytopenia, and lymphopenia at the time of diagnosis of IA on outcomes. In univariate analysis, all 3 were associated with increased risk of overall and attributable mortality, although only neutropenia remained significant in multivariable models. In contrast, a recent analysis of 51 cases of IA following HCT identified monocytopenia at the time of diagnosis of IA, but not neutropenia, to be an independent risk factor for attributable mortality [7]. There are ex vivo and animal data to support the importance of both neutrophils and monocytic cells in controlling IA [3, 7, 18–20].

Another change in clinical practice coinciding with improved outcomes was the introduction of voriconazole. The superiority of voriconazole, compared with conventional amphotericin B, for treatment of IA has been demonstrated in a randomized trial [21]. In our study, receipt of voriconazole as primary antifungal therapy was associated with reduced risk of death in the univariate analyses (figure 3) but not in the multivariable models of all-cause and IA-related death. However, receipt of voriconazole was independently associated with reduced risk of IA-related death in the model restricted to patients who did not have severe underlying organ impairment. It is likely that elevated creatinine and bilirubin levels function as variables that signify severely ill patients in whom the presence of multiple comorbidities limit the potential for antifungal therapy to alter observed outcomes.

We did not see a survival advantage among patients who

Table 4. Risk factors for attributable death in 391 hematopoietic cell transplant (HCT) recipients with proven or probable invasive aspergillosis (IA).

Variable	Unadjusted		Adjusted	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Recipient-related factors				
Year of IA diagnosis (2002–2004 vs. previous)	0.5 (0.3–1.0)	.03	…	…
Good-risk underlying disease[a]	0.6 (0.4–0.9)	.001	NS	NS
Transplant-related factors				
Unrelated donor	0.8 (1.0–2.0)	.07	NS	NS
Receipt of PBSCs	0.5 (0.4–0.8)	.004	NS	NS
HLA mismatch	2.0 (1.4–2.9)	.0003	2.6 (1.7–4.0)	<.0001
Nonmyeloablative conditioning	0.2 (0.1–0.6)	.003	0.3 (0.1–1.0)	.05
Transplant complications				
Neutropenia[b]	2.5 (1.5–4.1)	<.001	NS	NS
Lymphopenia[c]	2.6 (1.8–3.9)	<.0001	NS	NS
Acute GVHD[d]	2.0 (1.4–2.8)	<.001	NS	NS
Chronic GVHD[e]	1.9 (1.1–3.2)	.02	NS	NS
Hyperbilirubinemia[f]	47.3 (31.4–71.3)	<.0001	1.9 (1.0–3.3)	.03
Elevated creatinine level[g]	14.8 (8.9–24.7)	<.0001	2.2 (1.1–4.5)	.03
Cytomegalovirus disease[h]	2.3 (1.2–4.3)	.01	NS	NS
Corticosteroid dosage of ⩾2 mg/kg per day	4.1 (2.4–6.9)	<.0001	1.9 (1.0–3.6)	.04
IA-related factors				
Disseminated IA	7.9 (5.5–11.3)	<.0001	3.6 (2.2–5.7)	<.0001
Late IA	4.7 (2.5–9.0)	<.0001	2.4 (1.2–4.7)	.02
Proven IA	2.2 (1.5–3.2)	<.0001	NS	NS
Antifungal agent(s)[i]				
Vori vs. non-Vori agent	0.4 (0.2–0.8)	.01	NS	NS
Lipid vs. conventional amphotericin	0.5 (0.3–1.0)	.04	…	…
Vori plus caspofungin vs. Vori alone	2.3 (0.6–9.4)	.23	…	…

NOTE. Cause of death was unknown for 14 patients who were excluded from the analysis. GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; PBSCs, peripheral blood stem cells; Vori, voriconazole; …, variable was not entered in the multivariable model.

[a] Acute leukemia in first remission, chronic myeloid leukemia in chronic phase, myelodyplasia-refractory anemia, aplastic anemia, and nonmalignant hematologic diseases; poor-risk disease included all other diagnoses.
[b] Neutrophil count of <750 on 2 consecutive days concurrent with IA, compared with IA in absence of neutropenia.
[c] Lymphocyte count of <300 on 2 consecutive days concurrent with IA, compared with IA in absence of neutropenia.
[d] Grade, ⩾3.
[e] Clinically extensive.
[f] Total serum bilirubin level of >6.5 mg/dL.
[g] Serum creatinine level of ⩾2.5 mg/dL.
[h] Diagnosed between HCT and diagnosis of IA.
[i] Analysis restricted to 286 patients receiving antimold therapy for ⩾5 days.

received first-line voriconazole and caspofungin combination therapy, in contrast to a study comparing receipt of combination therapy with voriconazole and caspofungin with receipt of voriconazole alone (historical controls) for salvage therapy of IA, in which combination therapy was independently associated with a better outcome at day 90 [22]. One study comparing any anti-*Aspergillus* agents in combination with monotherapy for IA in patients with hematologic malignancies did not detect any difference in outcome [23], although another study found differences only in the select group of patients with compromised renal function [24]. These conflicting results illustrate the difficulty in generating definitive conclusions based on retrospective analyses. There are multiple potential explanations: other changes in transplant or host variables could account for differences in observational studies, and the multiple comorbidities apparent in these patients "dilute" our ability to measure potentially small therapeutic effects of the combination regimen in multivariable modeling. The utility of combination antifungal therapies as primary therapy for IA clearly needs to be analyzed in a randomized trial.

Receipt of high-dose corticosteroids is a risk factor for the development of IA [1, 4, 6, 25], and our data indicate that a dosage of ⩾2 mg/kg per day at the time of IA diagnosis was independently associated with an increased risk for death in all

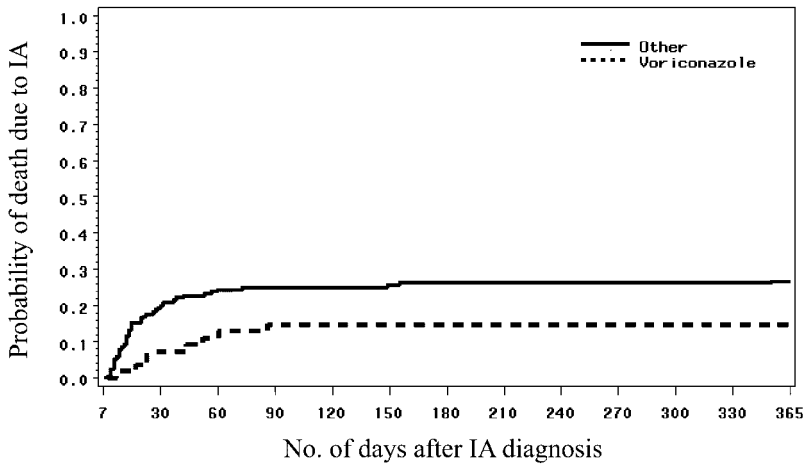

Figure 3. Probability of attributable death after diagnosis of invasive aspergillosis (IA) following hematopoietic cell transplantation among patients surviving past day 7 and receiving ($n = 54$) or not receiving ($n = 176$) voriconazole as primary therapy. The probability of mortality was significantly lower in voriconazole recipients ($P = .03$).

models. The adverse impact of high-dose corticosteroid therapy has been consistently identified in other studies of invasive mold infections [3, 7, 25]. Corticosteroids affect the host immune response to *Aspergillus* by preventing killing of phagocytosed *Aspergillus fumigatus* conidia by alveolar macrophages [26] and by blunting alveolar macrophage production of proinflammatory cytokines (IL-1α and TNF-α) and chemokines (macrophage inhibitory factor–1α) important for recruiting neutrophils and monocytes [27]. Corticosteroids may also affect the type of T helper cell response to IA. PBMCs from healthy human subjects produce Th1-type cytokines to *A. fumigatus* in vitro [28, 29]. Corticosteroids are associated with Th2 responses and poor outcomes [18, 29, 30].

Infection-related factors independently associated with increased risks for death in this study included disseminated infection and infection diagnosed "late" after HCT. Dissemination is typically seen with delayed diagnoses and/or severely immunocompromised hosts, and the fungal burden tends to be higher than in localized pulmonary infection. We were not able to determine the precise impact of timing of diagnosis of IA on outcome; however, it is likely that dissemination in this setting serves as a surrogate for delayed diagnosis, which is associated with poor outcome [31]. In the later years of the study period, fewer cases of IA were diagnosed at autopsy than in earlier years, and the incidence of disseminated and proven disease decreased (table 2). We speculate that improved diagnostic tests, such as CT and the galactomannan EIA, have led to more prompt diagnosis of IA in recent years. In addition, the recent availability of less toxic antifungal agents may have lowered the threshold for clinicians to start treatment before a definitive diagnosis was made.

Late IA (diagnosed >40 days after HCT) was identified as an independent risk factor for death. IA following HCT is not a homogenous disease. Both animal and human studies suggest that the pathogenesis of IA differs depending on the etiology of the immune deficit [18, 19, 32]. The predominant immune defect associated with early (preengraftment) IA is neutropenia, whereas secondary neutropenia, GVHD, high-dose corticosteroid treatment, and cytomegalovirus disease are factors associated with the development of late (post-engraftment) IA [1]. A possible explanation of our finding is that many of the risk factors associated with the development of late IA (GVHD, cytomegalovirus disease, and high-dose corticosteroid treatment) are slow to resolve or respond to therapy, whereas neutropenia typically resolves within 2–4 weeks after stem cell infusion.

Our finding that HLA mismatch was independently associated with increased risk for death is consistent with the well-documented adverse impact of HLA mismatch on outcomes [33]. Among the complications of HCT included in our analysis, the presence of hepatic and/or renal impairment was independently associated with increased risk for death. There are multiple etiologies for organ dysfunction in HCT recipients, including veno-occlusive disease, drug toxicities, and sepsis—all of which are associated with morbidity and mortality. Our finding that severe abnormalities on pulmonary function testing before HCT is prognostic for overall mortality is consistent with a study demonstrating greater risks for early respiratory failure and mortality in patients with compromised pulmonary function before HCT [34].

The results of this study indicate that the outcomes of IA following HCT have improved greatly. Prognostic variables identified largely reflect the degree of patient immunocompromise and disseminated infection, emphasizing the importance of prompt diagnosis and therapy and reducing immunosuppression once a diagnosis is established. Although we have

shown that receipt of voriconazole was protective in a subgroup of patients, we were not able to demonstrate an advantage of combination therapy with voriconazole and caspofungin.

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