MAJOR ARTICLE

Epidemiology and Outcome of Mould Infections in Hematopoietic Stem Cell Transplant Recipients

Kieren A. Marr,[1,2] Rachel A. Carter,[1] Fulvio Crippa,[1,a] Anna Wald,[1,2,3] and Lawrence Corey[1,2,4]

[1]Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, and Departments of [2]Medicine, [3]Epidemiology, and [4]Laboratory Medicine, University of Washington, Seattle

Reports have focused on the emergence of moulds as pathogens in recipients of hematopoietic stem cell transplants. To review the incidence of and risks for mould infections, we examined the records of 5589 patients who underwent hematopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center (Seattle) from 1985 through 1999. After 1992, the incidence of invasive aspergillosis increased in allograft recipients and remained high through the 1990s. Infections with non-*fumigatus Aspergillus* species, *Fusarium* species, and Zygomycetes increased during the late 1990s, especially in patients who received multiple transplants. Although infection caused by *Scedosporium* species was common in patients who had neutropenia, infection caused by Zygomycetes typically occurred later after transplantation, when patients had graft-versus-host disease. The overall 1-year survival rate was equally poor (~20%) for all patients with mould infections. The results of the present study demonstrate the changing epidemiology of mould infections, emphasizing the increasing importance of amphotericin B–resistant organisms and the differences in risks and outcome of infection with different filamentous fungi.

Mortality rates secondary to invasive infection with *Candida albicans* have decreased in hematopoietic stem cell transplant (HSCT) recipients because of the widespread use of fluconazole for prophylaxis [1, 2]. However, several centers have reported that infections caused by moulds have become increasingly common. A study published elsewhere about *Aspergillus* infections that occurred in a cohort of patients who underwent transplantation from 1987 through 1993 at the Fred Hutchinson Cancer Research Center (FHCRC; Seattle) revealed an increased incidence of invasive aspergillosis (IA) in patients who underwent transplantation during the first 6 months of 1993 [3]. Other reports have noted the emergence of infections caused by other moulds, such as *Fusarium* species, Zygomycetes, and dematiaceous fungi [4–7]. These observations are especially worrisome given the overall poor prognosis of invasive fungal infection [3, 8].

The results of in vitro susceptibility studies provide another reason to be concerned about increasing rates of infection with moulds, because *Aspergillus flavus* [9], *Aspergillus terreus* [10], *Scedosporium* species, and *Fusarium* species [11] have been reported to be resistant to amphotericin B, and because *Aspergillus fumigatus* can develop resistance to itraconazole [12]. Although infection with any mould is associated with a high mortality rate, infection with drug-resistant organisms may lead to an especially poor outcome [12].

To gain insights into the changes in and the risks of infection throughout the 1990s, we examined a large

Received 6 August 2001; revised 2 November 2001; electronically published 26 February 2002.

Financial support: Medical School Grant from Merck and the National Institutes of Health (grants K08-A1571 and CA-18029).

[a] Present affiliation San Raffale Hospital and University, Milan, Italy.

Reprints or correspondence: Dr. Kieren A. Marr, Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N. D3-100, Seattle, WA 98109 (kmarr@fhcrc.org).

Clinical Infectious Diseases 2002; 34:909–17

1058-4838/2002/3407-0004$03.00

cohort of HSCT recipients at FHCRC. We specifically sought to determine whether the incidence of invasive infection caused by *Aspergillus* species, Zygomycetes, *Fusarium* species, *Scedosporium* species, and other dematiaceous moulds has changed during the past decade. We attempted to define some of the clinical factors that might account for the increase in invasive infections observed during the late 1990s and to determine the outcome of infection caused by different organisms.

METHODS

Study patients. Patients who received care at the FHCRC from 1 January 1985 through 31 December 1999 were included in this retrospective cohort study. To determine the incidence of infection with non-*Aspergillus* moulds, the records of all patients who either underwent cytotoxic chemotherapy or received an HSCT during the period of 1 January 1985 through 31 December 1999 were examined. Because we have described the cohort of patients at FHCRC who developed IA from 1 January 1987 through 1 June 1993 elsewhere [3], we restricted the IA cohort to those patients who developed IA from 1 January 1993 through 31 December 1999.

Patients who received an HSCT underwent conditioning chemotherapy, with or without total body irradiation, and prophylaxis and treatment of graft-versus-host disease (GVHD), as described elsewhere [13, 14]. The antifungal prophylaxis regimen of fluconazole (400 mg/day) administered for 75 days after the patient received an HSCT was studied in a randomized protocol from 1990 through 1992 [15] and was subsequently adopted as the standard regimen for both allograft and autograft recipients. Patients with aplastic anemia who received HSCTs from unrelated donors received care in rooms with laminar airflow (LAF) before 1992. Subsequently, high-efficiency particulate air (HEPA) filtration (not LAF) was routine. Patients who developed fever while they had neutropenia routinely received ceftazidime monotherapy, with the addition of an aminoglycoside and vancomycin when clinically indicated. Patients who had persistent fever despite administration of antibiotics received amphotericin B (0.5–1.0 mg/kg or an equivalent dose of a lipid preparation) until resolution of fever and neutropenia.

As part of pretransplantation evaluation, patients had swabs from the nose, mouth, vagina, and rectum obtained for surveillance cultures. Culture of samples from other sites and invasive and surgical procedures were performed as clinically indicated. All tissue and lavage samples were evaluated for the presence of fungi by use of standard histopathologic and microbiologic techniques. Filamentous fungi were identified according to standard protocols [16].

Most patients in this cohort were treated with amphotericin B deoxycholate or lipid formulations of amphotericin B. A small number of patients (<20) received an investigational triazole antifungal in 1997–1998, either as part of "salvage" therapy or while enrolled in a randomized trial. It was routine to administer colony-stimulating factors (granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor) to all patients who developed infection while they had neutropenia. Also, patients who had localized sinus or pulmonary infection with Zygomycetes underwent debridement or resection of pulmonary lesions.

Identification and definition of cases. We reviewed the pathology records (from autopsies, sinus, skin, and lung biopsies, and bronchoalveolar lavage [BAL] fluid analyses) from all patients to identify reports with findings suggestive of infection (i.e., detection of hyphal fragments). A computerized microbiology database was searched to identify cultures that yielded *Aspergillus* species, Zygomycetes (*Absidia, Apophysomyces, Basidiobolus, Cokeromyces, Conidiobolus, Cunninghamella, Mortierella, Mucor, Rhizomucor, Rhizopus, Saksenaea,* and *Syncephalestrum* species), Hyalohyphomyces (*Fusarium, Penicillium,* and *Paecilomyces* species), and dematiaceous fungi (*Pseudallescheria, Scedosporium, Curvularia, Bipolaris, Exserohilum, Alternaria, Dactylaria, Phialophora, Cladosporium, Xylohypha, Wangiella, Cladophialophora, Fonsecaea, Scolecobasidium, Aureobasidium, Acremonium,* and *Ochroconis* species). Records maintained prospectively by hospital epidemiology personnel were also examined to identify cases.

Reviews of research and clinical charts were performed for all patients with microbiologic or histopathologic findings of fungal infection to classify patients as having "proven," "probable," or "possible" invasive disease or colonization/contamination. A modification of the National Institutes of Health Mycoses Study Group–European Organization for Research and Treatment of Cancer consensus definitions of invasive fungal infections were used to classify disease [17]. Transplant recipients or neutropenic patients with clinical signs and symptoms and a tissue biopsy specimen that revealed growth of an organism or positive results of histopathologic tests were considered to have "proven disease." Patients with clinical signs and symptoms and a BAL specimen that yielded growth on culture or that had positive histopathologic test results were considered to have "probable disease." Patients with $\geqslant$3 clinical signs or symptoms and growth of an organism on culture of a nonsterile fluid sample (i.e., sputum) were considered to have "possible invasive disease" [17]. Patients who had no clinical pulmonary findings and no radiographic abnormalities, but who had invasive sinus disease proven by examination of biopsy specimens and culture, were considered to have "isolated sinus infection." Dissemination to other organs, such as skin, brain, and abdominal organs, was considered to be "proven" only if confirmed by biopsy (or autopsy) and "probable" if clinical or suggestive radiographic findings were apparent. "Colonization" was defined as growth of an organism on culture of a sample

from a nonsterile site in the absence of clinical signs or symptoms. Tissue samples that were obtained at autopsy and that yielded an organism on culture, in the absence of histopathologic confirmation of invasion and in the absence of premortem signs or symptoms of infection, were considered to be contaminated.

Patient risks and statistical analysis. The day of diagnosis of the invasive fungal infection was the day on which the first diagnostic culture or examination was performed. For patients with a diagnosis obtained during postmortem examination, the day of death was considered to be the day of diagnosis. The 1-year cumulative incidence of invasive mould infection was calculated according to year of transplantation. To compare the 1-year cumulative incidence of IA with that determined in our previous cohort (January 1987 through June 1993; reported in [3]), we limited the IA analysis to the cohort of patients who developed infection after they received their first HSCT. This cohort was further restricted to patients who underwent transplantation during the period from 1 January 1993 through 31 December 1998, to allow for adequate follow-up time.

Associations in 2×2 tables were analyzed by use of χ^2 and Fisher's exact tests, when applicable [18]. Patient factors (i.e., age, sex, and underlying disease) and transplantation variables associated with the development of invasive infection due to *Aspergillus* species, *Fusarium* species, and Zygomycetes were analyzed in multivariate Cox regression models. Risk factors for *Scedosporium* infection were not analyzed because of the low numbers of infections. Times to diagnosis of infection were the outcomes in the models. Transplant variables entered into the models include year of transplantation, donor type (autologous, human leukocyte antigen [HLA]–matched related, or HLA-mismatched/unrelated), acute GVHD (grades of $\geq$2), chronic GVHD (clinically extensive), and prolonged engraftment (defined as >20 days until 2 consecutive days with an absolute neutrophil count of >750 cells/mm^3). Variables entered into the model were sequentially eliminated in a stepwise backward fashion, whereby any variable with a category factor that approached significance was retained until all variables with $\geq$1 significant category were identified. A 2-sided P value of <.05 was considered statistically significant. Survival after infection was estimated with Kaplan-Meier curves [19], and the 1-year survival rate after each infection was compared by use of the log-rank test.

RESULTS

IA. Initial screening of microbiology, pathology, and epidemiology records revealed 438 patients with a potential diagnosis of IA during 1993–1998. A total of 375 cases of IA (218 proven, 124 probable, and 33 possible) were diagnosed in 359 different patients. Chart review confirmed that proven IA involved the lungs in 191 patients, sinuses in 22, and other sites (liver, gut, or skin without other organ involvement) in 5. Probable IA involved the lungs in 106 patients, sinuses in 16, and other sites in 2. Possible IA involved the lungs in 27 patients, sinuses in 5, and liver in 1. Seventy-nine patients were colonized with an *Aspergillus* species but never developed possible, probable, or proven invasive infection. Twenty-seven (34%) of these colonized patients had received $\geq$2 weeks of amphotericin B or itraconazole therapy "preemptively."

The 1-year cumulative incidence of proven or probable IA among autograft and allograft recipients, by year of transplantation, is shown in figure 1. After 1992, the incidence of IA among allograft recipients increased. Compared with results from previous years, there was also an increase in IA among autologous transplant recipients in 1998 (5.3% vs. 1.1%). Two (40%) of 5 of autologous HSCT recipients who developed IA in 1998 had received CD34-selected grafts [20].

Most diagnoses were confirmed by culture of tissue or BAL specimens. Of 297 episodes of proven or probable pulmonary IA, 230 diagnoses (77.4%) were made with confirmatory cultures. Of 38 cases of proven or probable sinus disease, 25 (65.8%) had positive culture results. Five (71.4%) of the 7 "other" diagnoses were confirmed microbiologically. The *Aspergillus* species that caused invasive disease, and those that were found to be colonizing organisms, are listed in table 1. *A. fumigatus* was the most common cause of invasive pulmonary disease. Compared with *A. fumigatus,* other *Aspergillus* species were more frequently colonizing isolates or were associated with sinus disease only. However, the incidence of non-*fumigatus Aspergillus* species as a cause of invasive lung infection increased after 1995. From 1993 through 1995, 23 (18.3%) of 126 positive BAL or biopsy cultures yielded a non-*fumigatus Aspergillus* species, and, from 1996 through 1998, 35 (33.7%)

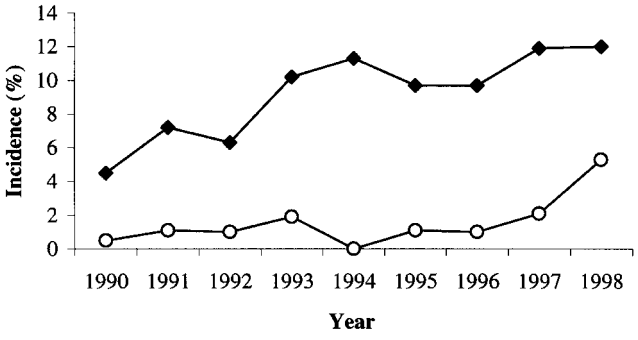

Figure 1. One-year cumulative incidence of proven and probable invasive aspergillosis, by year of transplantation, at Fred Hutchinson Cancer Research Center (Seattle), 1990–1998. The incidences of invasive aspergillosis in allograft recipients (◆) and autograft recipients (○) are shown. Data for years 1990 through 1992 are from a study published elsewhere [3].

Table 1. *Aspergillus* species isolated from patients with different infections who underwent hematopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center (Seattle), 1993–1998.

Aspergillus species isolated	No. (%) of infections, by site			No. (%) of colonizing organisms (n = 98)
	Lung (n = 230)	Sinus (n = 25)	Other[a] (n = 5)	
A. fumigatus	156 (67.8)	15 (60)	2 (40)	40 (40.8)
A. niger	3 (1.3)	4 (16)	2 (40)	34 (34.6)
A. flavus	6 (2.6)	2 (8)	1 (20)	8 (8.2)
A. terreus	5 (2.2)	0 (0)	0 (0)	3 (3.1)
Other[b]	2 (<1)	1 (4)	0 (0)	8 (8.2)
NI[c]	48 (20.9)	3 (12)	0 (0)	4 (4.1)
Multiple[d]	10 (4.3)	0 (0)	0 (0)	1 (1.0)

NOTE. Only cases of proven or probable disease are shown. NI, not identified.

[a] Includes 4 cases of cutaneous infection and 1 case of isolated hepatic infection.

[b] Includes *Aspergillus nidulans* (2 invasive and 2 colonizing organisms), *Aspergillus clavatus* (1 invasive organisms), *Aspergillus versicolor* (6 colonizing organisms), and *Aspergillus glaucus* (2 colonizing organisms).

[c] Not speciated (no sporulation).

[d] Multiple species isolated from 1 culture, including *A. fumigatus* and *A. niger* (in 7 cases), *A. fumigatus* and *A. flavus* (in 2), *A. fumigatus* and *A. terreus* (in 1), and *A. fumigatus, A. niger,* and *A. terreus* (in 1).

of 104 cultures yielded a non-*fumigatus Aspergillus* species ($P = .01$).

Non*-Aspergillus *moulds. The number of non-*Aspergillus* moulds isolated from any culture during 1985–1999 and whether these isolates were considered to be the cause of invasive disease are shown in table 2. Although *Scedosporium* species, *Fusarium* species, and Zygomycetes were considered to have been the cause of proven or probable disease in the majority of patients from which they were isolated, all other moulds were more often found to be colonizing isolates or contaminants. The most common dematiaceous mould considered to have been involved in disease, other than *Scedosporium* species (including *Psuedallescheria boydii*), was *Alternaria* species, which was implicated in probable pulmonary infections in 2 patients. Two additional patients were considered to have had probable pulmonary disease with *Scopulariopsis* and *Ulocladium* species isolated from BAL samples.

Infections with the most common non-*Aspergillus* moulds (*Fusarium* species, Zygomycetes, and *Scedosporium* species) were examined further. From 1985 through 1999, 31 patients developed infection with *Fusarium* species, 29 with Zygomycetes, and 10 with *Scedosporium* species (figure 2). Two patients developed 2 different infections each, 1 with *Fusarium* species and Zygomycetes and another with both *Fusarium* and *Scedosporium* species. As with *Aspergillus* species, infections caused by *Fusarium* species and Zygomycetes also appeared to increase in frequency at FHCRC during the late 1990s, whereas invasive infection with *Scedosporium* species remained uncommon (figure 2).

Patient characteristics. Characteristics of patients who developed proven and probable infection with all organisms are shown in table 3. The majority of patients who developed proven or probable infection had received an allogeneic HSCT. Aspergillosis was more common in patients who had never received a transplant than was infection with any other mould ($P = .05$). More patients who developed infection with the potentially amphotericin B–resistant organisms (*Fusarium* and *Scedosporium* species) had undergone multiple transplants (5 of 41 patients with *Fusarium* or *Scedosporium* infections vs. 18 of 327 patients with IA received multiple transplants), although the difference was not statistically significant ($P = .16$).

As shown in table 3, patients were more likely to develop infection with *Scedosporium* species in the first 30 days after transplantation, whereas infection with Zygomycetes more frequently occurred late (i.e., >90 days) after transplantation (14 [56%] of 25 patients), corresponding with periods of GVHD and its treatment (figure 3 and table 3). Multivariate risk-factor modeling of patient- and transplant-related variables identified the following risk factors for IA: age >40 years, underlying diagnoses of anything other than chronic myelogenous leukemia in chronic phase (CML-CP) or a hematologic malignancy in first remission, and receipt of an HSCT from an HLA-mismatched or unrelated donor. Underlying myelodysplastic syndrome and severe GVHD were significant risk factors for infection with Zygomycetes, whereas multiple myeloma and receipt of a graft from an HLA-mismatched or unrelated donor were significantly associated with *Fusarium* infection. Prolonged engraftment was not associated with infections in the models performed.

Outcome of infection. Most (9 of 10) of the patients with infection caused by *Scedosporium* species had documented pulmonary disease, and dissemination beyond the sinopulmonary tract was common (6 [60%] of 10 patients). *Fusarium* species caused isolated sinus infection in 6 (19%) of 31 patients. The majority of patients (25 [81%] of 31) had infection involving the lungs, and dissemination was common (23 [74.2%] of 31). In contrast, Zygomycetes more frequently caused isolated sinus disease (12 [41%] of 29). Sixteen (55%) of 29 patients with Zygomycetes infection had disease involving the lungs, and 1 patient (4%) had infection isolated to the abdomen (liver). Compared with infection involving *Scedosporium* and *Fusarium* species, infection with Zygomycetes less frequently disseminated beyond the sinopulmonary tract (8 [27.5%] of 29; $P <$ .001). Also, fewer patients with proven pulmonary IA had documented dissemination beyond the sinopulmonary tract (33 [17.3%] of 191).

The 1-year survival rates for patients after diagnosis of each infection are shown in figure 4. The median duration of survival

Table 2. Non-*Aspergillus* moulds isolated from patients who underwent hematopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center (Seattle), 1985–1999.

Pathogen isolated	No. (%) of moulds		
	No infection[a]	Probable invasive	Proven invasive
Scedosporium species[b] (n = 10)	0 (0)	1 (10)	9 (90)
Fusarium species (n = 39)	8 (20.5)	4 (10.3)	27 (87.1)
Zygomycetes (n = 40)			
Rhizopus species	1 (6.7)	1 (6.7)	13 (86.7)
Mucor species	9 (52.9)	1 (5.9)	7 (41.2)
Other[c]	1 (23.4)	0 (0)	7 (87.5)
Other dematiaceous moulds (n = 41)			
Alternaria species	3 (60)	2 (40)	0 (0)
Exophiala species	2 (66)	1 (33)	0 (0)
Ulocladium species	0 (0)	0 (0)	1 (100)
Other[d]	31 (97)	1 (3)	0 (0)
Paecilomyces species (n = 14)	13 (92.9)	1 (7.1)	0 (0)

[a] Patients with isolate recovered but considered to be a contaminant or a colonizing isolate.

[b] Includes organisms identified as *Pseudallescheria boydii (Scedosporium apiospermum).*

[c] Includes *Absidia* species (in 1 patient with proven disease), *Cunninghamella* species (in 2 patients with proven disease), and unknown Zygomycetes (defined as consistent pathologic findings but no growth on culture; in 5 patients [1 patient was colonized and 4 had biopsy-proven invasion of Zygomycetes-like hyphae]).

[d] Includes *Scopulariopsis, Cladosporium, Bipolaris, Phialophora, Aureobasidium, Curvularia, Exserohilum, Chladophialophora, Wangiella, Fonsecaea, Acremonium,* and *Chaetomium* species.

for patients who received diagnoses before death was longer for patients with documented zygomycosis (66 days) than it was for patients with fusariosis (25 days), *Scedosporium* infection (19 days), or aspergillosis (29 days). However, the overall 1-year survival rate was ~20% in all groups, with the exception of patients who developed infection with *Scedosporium* species, who all died within 1 month of infection (figure 4; $P = .26$).

DISCUSSION

During a decade in which we have made significant advances in the prevention of cytomegalovirus and candidal infections, moulds have emerged as the major cause of mortality related to infection in HSCT recipients. Our study, which summarizes the epidemiology and outcome of invasive mould infections in 1 large transplantation center during the past 15 years, shows that the incidence of IA has at least tripled among both allograft and autograft recipients. Also, the frequency of infections caused by amphotericin B–resistant moulds has increased during the past 5 years, especially among recipients of multiple, high-risk transplants.

In our center, in 1993, we began to routinely administer antifungal prophylaxis with fluconazole (400 mg/day) for 75 days after patients received an HSCT, and this practice has decreased the incidence of candidemia [1], hepatic candidiasis [21], and mortality attributed to *Candida* species [2]. However, the incidence of invasive infection caused by *Aspergillus* species and other moulds increased after 1992. Because we used a very conservative definition of invasive disease and restricted our incidence calculations to those patients who developed disease after receiving a first transplant, the cumulative incidences reported here are probably underestimations.

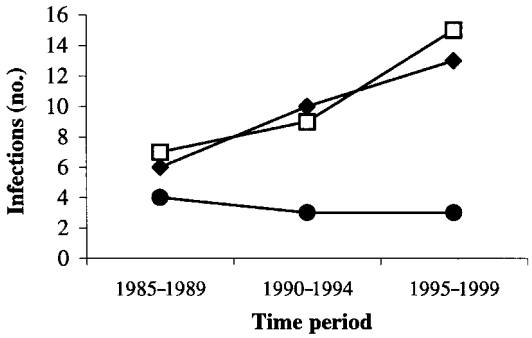

Figure 2. Frequency of non-*Aspergillus* mould infections at Fred Hutchinson Cancer Research Center (Seattle). The number of patients who developed proven or probable infection with *Fusarium* species (□), Zygomycetes (◆), and *Scedosporium* species (●) from 1985 through 1999 are shown.

Table 3. Characteristics of hematopoietic stem cell transplant (HSCT) recipients with proven and probable infections, by pathogen recovered, at Fred Hutchinson Cancer Research Center (Seattle), 1985–1999.

	Patients infected with									
	Aspergillus species (n = 327)			Zygomycetes (n = 29)			*Fusarium* species (n = 31)			*Scedosporium* species (n = 10)
Patient characteristic	n (%)	HR	95% CI	n (%)	HR	95% CI	n (%)	HR	95% CI	n (%)
Male sex	201 (61)	—	NS	21 (72)	—	NS	16 (52)	—	NS	8 (80)
Age at transplantation, years										
<19	39 (13)	1.0	—	7 (24)	1.0	—	6 (20)	1.0	—	3 (33)
19–40	99 (34)	—	NS	14 (48)	—	NS	10 (33)	—	NS	5 (56)
>40	156 (53)	1.8	1.2–2.9[a]	8 (28)	—	NS	14 (47)	—	NS	1 (11)
Underlying disease[b]										
CML-CP	47 (15)	1.0	—	4 (14)	1.0	—	4 (13)	1.0	—	0 (0)
Hematologic malignancy										
First remission	24 (7)	—	NS	3 (10)	—	NS	1 (3)	—	NS	1 (11)
Other	159 (49)	2.2	1.5–3.2[c]	13 (45)	—	NS	17 (55)	—	NS	7 (78)
Myelodysplastic syndrome	44 (14)	1.8	1.1–3.0[a]	5 (17)	4.1	1.1–15.4[a]	1 (3)	—	NS	1 (11)
Multiple myeloma	20 (6)	4.2	2.3–7.6[c]	1 (3)	—	NS	2 (6)	6.9	1.2–40.0[a]	0 (0)
Other	29 (9)	1.9	1.0–3.4[a]	3 (10)	—	NS	6 (19)	—	—	0 (0)
No transplant[d]	33 (10)	NA	NA	0 (0)	NA	NA	1 (3)	NA	NA	1 (10)
Multiple transplants	19 (6)	NA	NA	1 (3)	NA	NA	3 (10)	NA	NA	2 (20)
Transplant type										
Allogeneic, matched related	110 (37)	1.0	—	13 (45)	1.0	—	10 (33)	1.0	—	3 (38)
Mismatched or unrelated	163 (55)	1.5	1.1–2.0[a]	16 (55)	—	NS	17 (57)	2.7	1.1–6.8[a]	5 (62)
Autologous	21 (7)	0.13	0.07–0.25[c]	0 (0)	—	NS	3 (10)	—	NS	0 (0)
Diagnosed before engraftment[e]	22 (8)	NA	NA	2 (9)	NA	NA	2 (8)	NA	NA	2 (33)
Graft-versus-host disease										
Acute[f]	216 (83)	—	NS	21 (78)	3.1	1.0–9.4[a]	20 (80)	—	NS	5 (63)
Chronic[g]	95 (61)	—	NS	13 (76)	—	NS	8 (73)	—	NS	3 (100)

NOTE. Transplant characteristics are shown only for patients who developed infection after receipt of an HSCT at Fred Hutchinson Cancer Research Center (*Aspergillus,* n = 294; Zygomycetes, n = 29; *Fusarium,* n = 30; and *Scedosporium,* n = 9). Multivariable risk factor analysis for IA included 2077 evaluable control patients who received an HSCT during 1993–1998, and the multivariable risk factor analyses for Zygomycetes and *Fusarium* infection included 5146 evaluable control patients who received an HCST during 1985–1999. Hazard ratios (HRs) and 95% CIs are shown for all significant variables. CML-CP, chronic myelogenous leukemia in chronic phase; HR, hazard ratio; NA, variable not analyzed in the multivariate model; NS, not significant in the multivariate model.

[a] $P < .05$.

[b] Other diagnoses included aplastic anemia, breast cancer, chronic lymphocytic leukemia, metachromatic leukodystrophy, chronic granulomatous disease, paroxysmal nocturnal hemaglobinuria, adenocarcinoma, nephroblastoma, eosinophilic syndrome, and aplasia (not specified). Data regarding underlying disease were not available for 4 patients with invasive aspergillosis and 1 patient from whom a *Scedosporium* species was isolated; human leukocyte antigen match data were not available for an additional patient with *Scedosporium* infection.

[c] $P < .001$.

[d] Includes patients who developed infection before transplantation.

[e] "Engraftment" was defined as an absolute neutrophil count of ⩾750 cells/mm^3 for 2 consecutive days.

[f] Grade of ⩾2 for evaluable patients (*Aspergillus* species were isolated from 260 patients; Zygomycetes, from 27, *Fusarium* species, from 25; and *Scedosporium* species, from 8).

[g] Includes evaluable patients (*Aspergillus* species were isolated from 155 patients; Zygomycetes, from 17; *Fusarium* species, from 11; and *Scedosporium* species, from 3).

The reason that the incidence of invasive mould infection has increased is not entirely clear. Potential explanations include the possibility that more patients develop disease later after transplantation because of an increase in the early posttransplantation survival rate, or that fluconazole prophylaxis decreases the use of empiric amphotericin B by affecting the incidence of fever while patients have neutropenia [22]. Other transplant-related factors that could alter the risk for invasive mould infections, including conditioning chemotherapy regimens, prophylaxis strategies for cytomegalovirus infection, and the introduction of different sources of stem cells (e.g., peripheral blood, cord blood), have also changed during this period.

Our data suggest that the incidence of IA peaked in our institution during 1998, with an additional small increase in the incidence of IA occurring among autograft recipients. One potential explanation for the increase in infection among autologous transplant recipients may be related to the introduction of CD34 selection, which might increase risks for infection

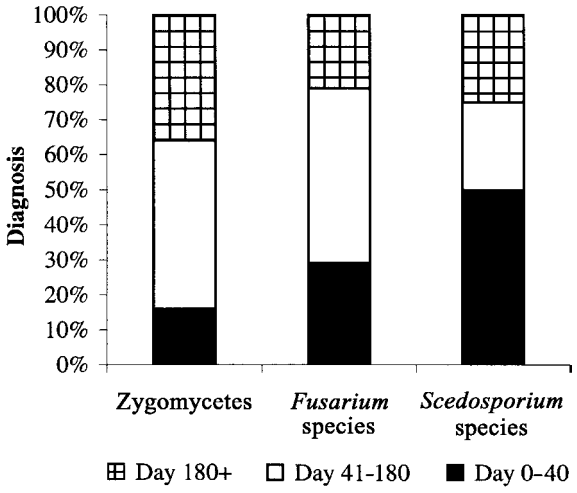

Figure 3. Day of diagnosis of non-*Aspergillus* mould infections. Shown is the proportion of infections that occurred during each period, with day 0 corresponding to the day of receipt of the hematopoietic stem cell transplant. Only patients who developed infection after the receipt of their first hematopoietic stem cell transplant are included (*Scedosporium* infection in 8 patients; Zygomycetes infection in 25, and *Fusarium* infection in 28).

by delaying reconstitution of lymphocytes and monocytes [20, 23].

A. fumigatus remains the most formidable *Aspergillus* pathogen, given that it was recovered in the majority of pulmonary and disseminated infections. Because clinicians did not routinely screen for sinus disease in the presence of proven pulmonary disease, our data are likely an underestimation of the frequency of combined sinus-lung disease. However, consistent with reports published elsewhere [3, 24], more cases of isolated sinus infection were caused by the non-*fumigatus Aspergillus* species, especially *Aspergillus niger* and *A. flavus*. It is possible that certain non-*fumigatus Aspergillus* species thrive in the sinus environment for undefined reasons, such as conidial size or adherence properties. A particularly low virulence potential of *A. niger,* compared with that of other *Aspergillus* species, may be reflected by the higher proportion of these organisms obtained as colonizing isolates.

More cases of proven pulmonary disease caused by potentially amphotericin B–resistant non-*fumigatus Aspergillus* species and *Fusarium* species occurred in our cohort during the late 1990s. Our data suggest that patients who undergo multiple transplantations might be at increased risk for infections caused by these and amphotericin B–resistant moulds (including *Scedosporium* species). Because FHCRC has performed more second and "tandem" (autologous followed by allogeneic) transplantations since 1995 (data not shown), this factor might account for this small increase. It is unknown whether the risk is related to a host factor, such as a longer duration until engraftment, or to a microbial factor. It is tempting to speculate that the receipt of increased doses or longer durations of amphotericin B therapy in highly immunosuppressed patients who have received multiple transplants might select for infections with amphotericin B–resistant organisms.

One interesting finding of the present study is the difference in the timing of the onset of various fungal infections. Elsewhere, we have described a "bimodal" timing of IA, with peaks that correlate with neutropenia (before engraftment) and GVHD [3]. The results of the present study emphasize the importance of severe neutropenia in the pathogenesis of disease caused by *Scedosporium* species, whereas GVHD and its therapy (corticosteroids) have a stronger association with Zygomycetes infection [25].

Consistent with prior studies, analysis of risk factors identified older age, receipt of an HSCT from an HLA-mismatched or unrelated donor, and underlying disease as important predictors of IA [3]. That GVHD was not significant in this model is likely explained by the inclusion of transplant-type variables in the overall cohort, causing the effect of GVHD to be overwhelmed by the HLA-mismatched or unrelated donor transplant-type variable. Indeed, rerunning the analysis in the absence of a variable for transplant type yielded a significant impact of GVHD (data not shown). The impact of specific transplant variables, such as GVHD, its therapy (corticosteroids), and time until engraftment, should be assessed in future analyses of cohorts restricted to allograft recipients.

The risk factor analyses indicate that underlying diseases are important variables that influence risks for specific fungal infections after receipt of an HSCT. Studies published elsewhere have shown that patients with a diagnosis of CML-CP have a lower risk of developing IA than do patients who have other hematologic malignancies not in first remission [3]. Our data

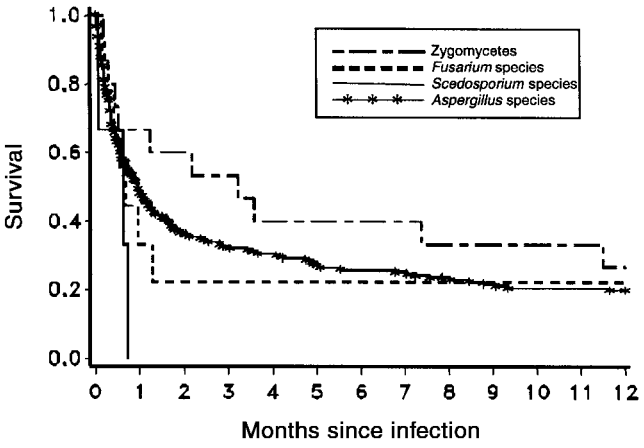

Figure 4. The 1-year survival rate after proven and probable infection with *Aspergillus* species, Zygomycetes, *Fusarium* species, and *Scedosporium* species.

indicate added risks for IA and Zygomycetes in people who receive an HSCT for myelodysplastic syndrome and risks for IA and fusariosis in people who receive an HSCT for multiple myeloma. These findings are consistent with the severity of GVHD and transplant-related mortality after HSCT for myelodysplastic syndrome [26] and with the high transplant-related mortality after receipt of an allogeneic HSCT for multiple myeloma [27]. An increased occurrence of IA in nonallografted patients with multiple myeloma has been described elsewhere, with risks thought to be attributable to intensified chemotherapeutic regimens [28]. Identification of specific risks in the HSCT setting awaits future studies.

Patients who develop infection with *Fusarium* and *Scedosporium* species had frequent dissemination of infection to other tissues. This finding is consistent with reports published elsewhere and may be related to in vivo sporulation and production of "adventitial forms," which are thought to increase the overall fungal burden of these infections [4, 11]. Although a minority of patients with proven IA in this study had documented disseminated disease, it is important to note that the lack of standardized screening by clinicians, and our definition (requirement of biopsy confirmation) may lead to an underestimation of actual dissemination. Finally, the particularly poor outcome of disseminated infection with *Scedosporium* species is consistent with the virulence and antifungal drug resistance of this organism as well as the occurrence of infection during neutropenia. The longer median duration of survival after infection with Zygomycetes is consistent with the later onset of disease and a decreased ability of this organism to disseminate hematogenously [25]. Unfortunately, the 1-year survival rate after infection with Zygomycetes is still as poor as that for infection with *Aspergillus* or *Fusarium* species, which may be explained by the severity of GVHD and development of other complications after transplantation.

That infections caused by other moulds occur infrequently supports theories regarding the low virulence of these organisms relative to *Aspergillus* species. The observation that *Alternaria* and *Exophiala* species were the cause of invasive disease in several patients is consistent with the findings of studies published elsewhere [5, 29].

In summary, infections caused by filamentous fungi, especially *A. fumigatus,* increased in our center during the 1990s. Increased numbers of infections caused by amphotericin B–resistant moulds and poor outcomes emphasize the need to develop more-effective prevention and treatment strategies. Such strategies may rely on new antifungal agents that have increased activity against amphotericin B–resistant moulds (i.e., investigational triazoles); however, continued efforts are needed to develop diagnostic strategies that enable both rapid and microbe-specific diagnoses.

References

1. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis **2000**; 181:309–16.
2. Marr K, Seidel K, Slavin M, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. Blood **2000**; 96:2055–61.
3. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis **1997**; 175:1459–66.
4. Boutati E, Anaissie E. *Fusarium,* a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. Blood **1997**; 90:999–1008.
5. Morrison V, Haake R, Weisdorf D. Non-*Candida* fungal infections after bone marrow transplantation: risk factors and outcome. Am J Med **1994**; 96:497–503.
6. Rossmann S, Cernoch P, Davis J. Dematiaceous fungi are an increasing cause of human disease. Clin Infect Dis **1996**; 22:73–80.
7. Kontoyiannis D, Wessel V, Bodey G, Rolston K. Zygomycosis in the 1990s in a tertiary care center. Clin Infect Dis **2000**; 30:851–6.
8. Ribaud P, Chastang C, Latge J, et al. Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. Clin Infect Dis **1999**; 28:322–30.
9. Seo K, Akiyoshi H, Ohnishi Y. Alteration of cell wall composition leads to amphotericin B resistance in *Aspergillus flavus.* Microbiol Immunol **1999**; 43:1017–25.
10. Sutton DA, Sanche SE, Revankar SG, Fothergill AW, Rinaldi MG. In vitro amphotericin B resistance in clinical isolates of *Aspergillus terreus,* with a head-to-head comparison to voriconazole. J Clin Microbiol **1999**; 37:2343–5.
11. Perfect J, Schell W. The new fungal opportunists are coming. Clin Infect Dis **1996**; 22:S112–8.
12. Denning DW, Radford SA, Oakley KL, Hall L, Johnson EM, Warnock DW. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection. J Antimicrob Chemother **1997**; 40:401–14.
13. Buckner C, Clift R, Appelbaum F, et al. Effects of treatment regimens in patients allografted for acute and chronic myelogenous leukemia. Bone Marrow Transplant **1991**; 7:6–8.
14. Clift R, Buckner C, Thomas E, et al. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclosphosphamide and total body irradiation with busulfan and cyclosphosphamide. Blood **1994**; 84:2036–43.
15. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation—a prospective, randomized, double-blind study. J Infect Dis **1995**; 171: 1545–52.
16. Larone D. Medically important fungi: a guide to identification. Washington, DC: ASM Press, **1995**.
17. Ascioglu A, Rex J, DePauw B, et al. Defining opportunistic invasive fungal infectionsin immunocomprimised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.
18. Fisher L, vanBelle G. Biostatistics: a methodology for the health sciences. New York: John Wiley & Sons, **1993**.
19. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc **1958**:457–81.
20. Holmberg L, Boeckh M, Hooper H, et al. Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. Blood **1999**; 94:4029–35.
21. van Burik JH, Leisenring W, Myerson D, et al. The effect of prophylactic fluconazole on the clinical spectrum of fungal diseases in bone marrow transplant recipients with special attention to hepatic candidiasis. Medicine (Baltimore) **1998**; 77:246–54.
22. Alangaden G, Chandrasekar P, Bailey E, et al. Antifungal prophylaxis

with low-dose fluconazole during bone marrow transplantation. Bone Marrow Transplant **1994**; 14:919–24.
23. Crippa F, Holmberg L, Carter RA, et al. Infectious complications after autologous CD34-selected peripheral blood stem cell transplantation. Biol Blood Marr Transplant **2002** (in press).
24. Drakos P, Nagler A, Or R, et al. Invasive fungal sinusitis in patients undergoing bone marrow transplantation. Bone Marrow Transplant **1993**; 12:203–8.
25. Ribes J, Vanover-Sams C, Baker D. Zygomycetes in human disease. Clin Microbiol Rev **2000**; 13:236–301.
26. Jurado M, Storer B, Appelbaum F, et al. Hematopoietic stem cell transplantation (HSCT) for advanced myelodysplastic syndrome (MDS) after conditioning with busulfan (BU) and fractionated total body irradiation (TBI) [abstract 1671]. In: Programs and abstracts of the 42nd Annual Meeting of the American Society of Hematology (San Francisco). Washington, DC: American Society of Hematology, **2000**: 387.
27. Bensinger W, Buckner C, Anasetti C, et al. Allogeneic marrow transplantation for multiple myeloma: an analysis of risk factors on outcome. Blood **1996**; 88:2787–93.
28. Denning DW, Marinus A, Cohen J, et al. An EORTC multicenter prospective survey of invasive aspergillosis in haematological patients: diagnosis and therapeutic outcome. EORTC Invasive Fungal Infections Cooperative Group. J Infect **1998**; 37:173–80.
29. Singh N, Chang F, Gayowski T, Marino I. Infections due to dematiaceous fungi in organ transplant recipients: case report and review. Clin Infect Dis **1997**; 24:369–74.