Epidemiology of *Aspergillus* Infections in a Large Cohort of Patients Undergoing Bone Marrow Transplantation

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To investigate the incidence, risk factors, and outcome of *Aspergillus* infections among marrow transplant recipients, records from 2496 patients were reviewed, and 214 patients had *Aspergillus* organisms identified. Of these, 158 had invasive aspergillosis, 44 were colonized, and 12 had contaminated cultures. The incidence of invasive aspergillosis increased from 5.7% to 11.2% during the study. The onset of infection was bimodal, peaking 16 and 96 days after transplant. For patients within 40 days after transplant, underlying disease, donor type, season, and transplant outside of laminar air flow rooms were associated with significant risk for invasive aspergillosis. For patients >40 days after transplant, age, underlying disease, donor type, graft-versus-host disease, neutropenia, and corticosteroid use were associated with increased risk of aspergillosis. Only 31% of infected patients were neutropenic at the time of diagnosis. The risk factors for aspergillosis depend on the time after marrow transplant and include both host and environmental characteristics.

Invasive *Aspergillus* infection has become the leading infectious cause of death after allogeneic bone marrow transplantation [1–6]. In contrast to many infections in the posttransplant period that arise from normal endogenous flora, *Aspergillus* infections are acquired exogenously, as evidenced by outbreaks of aspergillosis associated with construction [7–11]. Inhalation of spores into the respiratory tract is the presumed mode of acquisition of *Aspergillus* pneumonia. The time from inhalation of the organism to development of disease and factors that predict aspergillosis have not been well defined. Since both clinical and environmental conditions are associated with the development of aspergillosis, changes in the environment as well as measures to improve the immune status of the patient may decrease the frequency of invasive *Aspergillus* infection. In most reports, prognosis of established aspergillosis is dismal; therefore, prevention is of utmost importance.

To gain insight into the natural history of *Aspergillus* infection, we reviewed all cases of *Aspergillus* infection defined by positive culture or histology in 2496 marrow transplant patients at our center over a 6.5-year period. We explored the role of demographic and clinical risk factors for aspergillosis, including the relative importance of host factors, such as neutropenia and type of transplant, versus the role of environmental conditions, such as reverse isolation, construction, and season of transplant. We also attempted to define the prognostic value of a superficial *Aspergillus* isolate for the development of invasive disease and to correlate the pathogenicity of *Aspergillus* organisms with species and site of isolation.

Methods

Study patients and setting. This retrospective cohort study included consecutive patients who received their first marrow transplant at the Fred Hutchinson Cancer Research Center in Seattle between 1 January 1987 and 30 June 1993 (follow-up through 30 September 1993). Patients were conditioned for transplant by use of chemotherapy with or without total body irradiation and received prophylaxis and treatment for graft-versus-host disease (GVHD) as previously described [12, 13]. Patients with aplastic anemia and those receiving unrelated grafts were routinely placed in laminar airflow (LAF) rooms; others were placed at the discretion of the attending physician.

We reviewed microbiology, pathology (autopsies, lung and sinus biopsies, and bronchoalveolar lavage fluid), and discharge diagnosis records to identify all *Aspergillus* isolates occurring no longer than 1 month before transplant. During the period of study, cultures of nose, throat, urine, and rectal and vaginal areas were obtained at the pretransplant evaluation. Patients in LAF rooms were then cultured weekly from mouthwash, rectum, and urine [14]. All patients had daily cultures from blood for fever >38.3°C and from other sites (e.g., throat, rectum) as clinically indicated. Blood was cultured onto BCG or dextrose phosphate agar from biphasic broth bottles (PML Microbiologicals, Tualatin, OR), Peptone Broth II bottles (Becton Dickinson, Cockeysville, MD), or Isolator 10 tubes (DuPont, Wilmington, DE) during the study period. All biopsy and autopsy specimens were evaluated by culture and histology for fungus. Patients routinely received ceftazidime as monotherapy at the onset of neutropenia. If fever developed, an aminoglycoside was added; vancomycin was used if coverage against gram-posi-

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tive organisms was clinically indicated. For patients with persistent neutropenic fever for >96 h, empiric amphotericin, 0.5 mg/kg, was given daily until engraftment. For treatment of *Aspergillus* infections, amphotericin B was administered. Between 1991 and 1993, amphotericin B colloidal dispersion (Sequus Pharmaceuticals, Menlo Park, CA) was under study and was given, in escalating doses, to 28 patients with invasive fungal disease [15].

Case definitions. Case definitions were constructed prior to chart review to categorize patients with evidence of *Aspergillus* species. Proven invasive aspergillosis was defined as the recovery of any *Aspergillus* species from a normally sterile site (including sinuses), or biopsy or autopsy specimen demonstrating either *Aspergillus* by culture or septate hyphae with acute angle branching consistent with *Aspergillus*, and a compatible clinical presentation. Probable invasive aspergillosis was defined as radiographic evidence compatible with *Aspergillus* species and identification of *Aspergillus* organisms from a contiguous nonsterile site, including sputum, nares, throat, or bronchoalveolar lavage fluid. Radiographic evidence compatible with pulmonary infection was defined as single or multiple pulmonary nodules on chest radiograph or computed tomograph. Sinus disease was defined as opacification, air-fluid levels, or mucosal thickening seen on sinus radiograph or computed tomograph. *Aspergillus* organisms were identified by either culture or histology. Patients with proven or probable invasive aspergillosis are combined in most analyses and referred to as patients with invasive *Aspergillus* infection.

Colonization was defined as a culture positive for *Aspergillus* organisms from a nonsterile site in patients without radiographic or clinical evidence of aspergillosis during follow-up. Contamination was defined as recovery of *Aspergillus* organisms from a normally sterile site in the absence of a compatible clinical or radiographic presentation. Control patients were those who did not meet any of the case definitions.

Statistical analysis. We calculated 1-year cumulative incidence of invasive *Aspergillus* infection by year of transplant. The increasing risk of aspergillosis per year of study was assessed in the Cox regression model described below [16]. The day of diagnosis was defined as the day on which the initial isolate of *Aspergillus* or the initial histopathologic specimen was obtained. For patients whose diagnosis of aspergillosis was confirmed only after death, the date of death was used as the date of diagnosis.

Risk factors for aspergillosis and for death were examined in a Cox regression model. Time to aspergillosis or death was used as the end point, respectively, and censoring was at the time of second transplant or at the end of follow-up to obtain hazard ratios and 95% confidence intervals (CI). Neutropenia, LAF, and GVHD grade 2–4 were considered as time-dependent covariates. Up to one additional period of neutropenia after engraftment and two time intervals in LAF rooms were counted in the analyses. The presence or absence of construction in immediate proximity to the hospital (June 1991 to May 1992 and December 1992 to July 1993) and the season of transplant were analyzed as potential environmental risk factors. Seasons were defined in 3-month blocks, beginning with December-January-February as winter. All variables examined by the univariate model were entered in the multivariate model and subsequently eliminated in stepwise backward fashion. Year of transplant, sex, and age were forced into the model. Follow-up time was censored at day 40 in the analysis of risk factors for developing *Aspergillus* infection before day 40. Statistical significance was defined by two-tailed $P < .05$. However, because of the number of statistical comparisons made, P between .05 and .01 should be viewed as suggestive.

Survival for 1 year after transplant was estimated by use of the method of Kaplan and Meier [17]. Median time to aspergillosis in various groups of patients was compared by use of the rank sum test [18]. Associations in 2×2 tables were examined by use of χ^2 and Fisher's exact tests [19].

Results

Of the 214 case-patients, 158 (74%) had either proven ($n = 139$) or probable ($n = 19$) aspergillosis (table 1). The remaining patients either were colonized or had an isolate designated as a contaminant. Thirty-two proven cases (23%) were diagnosed at autopsy only. Of the patients, 85% with proven and 95% with probable infections had their diagnoses made by positive culture.

Among 158 patients with proven or probable aspergillosis, 97 (61%) were men, and the mean age at transplant was 34.8 years (range, 11 months–62 years) (tables 2, 3). Fourteen patients (8.9%) had chronic myelogenous leukemia, chronic phase (CML-CP), as underlying illness, 14 (8.9%) had malignancy in first remission, 100 (63%) had malignancy in other

Table 1. Initial sites of isolates for patients with proven, probable, and colonizing *Aspergillus* infection.

Type of infection, site or source	Total	Diagnostic criteria	
		Culture	Histology
Proven	139 (65%)	118 (55%)	21 (10%)
Lung	68	51	17
Sputum	37	37	0
BAL fluid	6	5	1
Skin	12	12	0
Brain	7	5	2
Internal organs*	5	4	1
Sinus	2	2	0
Rectum	1	1	0
Eye	1	1	0
Probable	19 (9%)	18 (15%)	1 (0.5%)
BAL fluid	5	4	1
Sputum	12	12	0
Nose	1	1	0
Rectum	1	1	0
Proven or probable	158 (74%)	136 (64%)	22 (10%)
Colonized	44 (21%)	44 (21%)	0
Sputum	17	17	0
BAL fluid	6	6	0
Rectum	12	12	0
Nose/nasopharynx	7	7	0
Ear	2	2	0
Contaminant	12 (5%)	12 (5%)	0
Total	214	192 (90%)	22 (10%)

NOTE. BAL, bronchoalveolar lavage.
* Internal organs = kidney (3), intestine (1), spleen (1).

Table 2. Univariate and multivariate analysis of risk factors for 62 cases of early aspergillosis (before day 40 after transplant).

Risk factor	Case-patients, no. (%)	Controls, no. (%)	Relative risk	Adjusted relative risk	95% confidence interval
Age, years					
≤18	15 (24.2)	534 (22.5)	1.0	1.0	—
19–40	21 (33.9)	1091 (45.9)	0.70	0.98	(0.50–1.93)
>40	26 (41.9)	753 (31.7)	1.25	1.94	(0.98–3.84)
Diseases group					
Chronic myelogenous leukemia, chronic phase	2 (3.2)	452 (19.0)	1.0	1.0	—
Hematologic malignancy, first remission	2 (3.2)	242 (10.2)	1.88	1.98	(0.27–14.2)
Hematologic malignancy, non-first remission	47 (75.8)	1264 (53.2)	8.61*	8.88*	(2.13–37.1)
Other[†]	11 (17.7)	420 (17.7)	6.12[‡]	5.79[‡]	(1.26–26.6)
Donor type					
HLA-matched, related	24 (38.7)	1064 (44.7)	1.0	1.0	—
Autologous/syngeneic	13 (21.0)	475 (20.0)	1.23	0.86	(0.43–1.70)
HLA-mismatched, related	16 (25.8)	371 (15.6)	1.94[‡]	2.08[‡]	(1.08–4.00)
Unrelated	9 (14.5)	468 (19.7)	0.85	1.5	(0.67–3.36)
Laminar air flow room[§]					
Yes	—	—	1.0	1.0	—
No	—	—	5.9	5.58*	(2.30–13.4)
Season					
Winter	7 (11.3)	593 (24.9)	1.0	1.0	—
Spring	12 (19.4)	644 (27.1)	1.58	1.64	(0.64–4.18)
Summer	28 (45.2)	569 (23.9)	4.04	4.45[‡]	(1.93–10.2)
Fall	15 (24.2)	572 (24.1)	2.21	2.19	(0.89–5.40)
Sex					
Female	19 (30.7)	1048 (44.1)	1.0	1.0	—
Male	43 (69.4)	1330 (55.9)	1.77	1.73	(1.00–2.99)
Year of transplant[‖]	—	—	1.01	1.0	(0.87–1.16)

* $P \leq .01$.
[†] Other diagnoses included aplastic anemia, myelodysplasia, and nonhematologic malignancies.
[‡] $.01 < P \leq .05$.
[§] Included as time-dependent covariate.
[‖] Included as continuous covariate.

than first remission, and 30 (19%) had other underlying disease (including myelodysplasia, aplastic anemia, and multiple myeloma). Fourteen patients (8.9%) had received an autologous marrow transplant. Among the 144 allogeneic marrow recipients, 68 (43%) had received marrow from related and matched donors, 28 (18%) from related and unmatched donors, and 48 (30%) from unrelated donors. Table 2 displays the corresponding demographic information for control patients.

Incidence and time of diagnosis of aspergillosis after marrow transplant. During the study period, the incidence of proven or probable aspergillosis increased from 5.7% of patients in 1987 to 11.2% in 1993 ($P = .02$) (figure 1). The proportion of infected patients with probable aspergillosis was 0 in 1987 and ranged from 10% to 18% of patients with aspergillosis in the remaining years.

The distribution of time to invasive *Aspergillus* infection following transplant appeared bimodal (figure 2). The median time to aspergillosis among patients in the first peak was 16 days and in the second peak was 96 days following transplant. Because the underlying host defense undergoes profound changes during these time intervals, we elected to look separately at the risk factors for aspergillosis in 62 patients who developed disease before day 40 after transplant, defined as early invasive aspergillosis, compared with 96 patients who developed disease after day 40, defined as late invasive aspergillosis.

Relationship between neutropenia and time to aspergillosis in autologous and allogeneic transplant recipients. Among patients with invasive aspergillosis, the median time to diagnosis was 16 days (interquartile range, 10–25) for patients with autologous transplants, compared with 64.5 days (interquartile range, 26–92) for patients with allogeneic transplants ($P < .001$). Twelve (86%) of 14 patients with autologous transplants were diagnosed with aspergillosis while neutropenic, compared with 38 (28%) of 144 patients with allogeneic transplants ($P < .001$). Of the 38 patients with allogeneic transplants who developed invasive aspergillosis during neutropenia, 29 developed disease prior to engraftment and within 40 days of transplant and 9 developed disease >40 days after transplant during recurrent neutropenia. All but 2 patients with autologous transplants developed invasive aspergillosis prior to engraftment. Thus, most patients with autologous transplants

Table 3. Univariate and multivariate analysis of risk factors for 96 cases of late aspergillosis (after day 40 after transplant).

Risk factor	Case-patients, no. (%)	Controls, no. (%)	Relative risk	Adjusted relative risk	95% confidence interval
Age, years					
⩽18	9 (9.4)	525 (23.0)	1.0	1.0	—
19–40	45 (46.9)	1046 (45.8)	2.47*	3.03†	(1.46–6.28)
>40	42 (43.8)	711 (31.2)	3.49†	5.03†	(2.41–10.5)
Diseases group					
Chronic myelogenous leukemia, chronic phase	12 (12.5)	440 (19.3)	1.0	1.0	—
Hematologic malignancy, first remission	12 (12.5)	230 (10.1)	1.98	3.60†	(1.57–8.28)
Hematologic malignancy, non-first remission	53 (55.2)	1211 (53.1)	2.09*	3.06†	(1.62–5.78)
Other‡	19 (19.8)	401 (17.6)	2.14*	3.71†	(1.77–7.79)
Donor type					
HLA-matched, related	44 (45.8)	1020 (44.7)	1.0	1.0	—
Autologous/syngeneic	1 (1.0)	474 (20.8)	0.05†	0.09*	(0.01–0.72)
HLA-mismatched, related	12 (12.5)	359 (15.7)	0.94	0.85	(0.44–1.65)
Unrelated	39 (40.6)	429 (18.8)	2.17†	1.67*	(1.04–2.67)
Sex					
Female	42 (43.8)	1006 (44.1)	1.0	1.0	—
Male	54 (56.3)	1296 (55.9)	1.04	0.91	(0.61–1.36)
Acute graft-versus-host disease§ ‖					
Grade 0–1	—	—	1.0	1.0	—
Grade 2–4	—	—	6.42†	2.60†	(1.38–4.87)
Construction					
Absent	54 (56.3)	1680 (73.6)	1.0	1.0	—
Present	42 (43.8)	602 (26.4)	2.14†	1.84†	(1.04–3.25)
Neutropenia‖ ¶					
Absent	—	—	1.0	1.0	—
Present	—	—	4.69†	5.97†	(2.95–12.1)
Corticosteroid use**					
No	12 (12.5)	1109 (48.6)	1.0	1.0	—
Yes	84 (87.5)	1173 (51.4)	6.56†	3.14†	(1.67–5.91)
Year of transplant††	—	—	1.18†	0.98	(0.84–1.17)

* $.01 < P \leqslant .05$.
† $P \leqslant .01$.
‡ Other diagnoses included aplastic anemia, myelodysplasia, and nonhematologic malignancies.
§ Acute graft-versus-host disease was graded by previously published methods [13].
‖ Included as time-dependent covariate.
¶ Neutropenia was defined as absolute neutrophil count $<500/mm^3$ for 3 consecutive days.
** Corticosteroid use was defined as administration of ⩾1 mg/kg prednisone or equivalent for 7 consecutive days.
†† Included as a continuous covariate.

were neutropenic at the time of diagnosis, particularly during the early time period.

Relationship between placement in LAF rooms and the time to development of aspergillosis. The median time to invasive aspergillosis was 78 days (interquartile range, 47–104) for 69 patients placed in LAF rooms compared with 40 days (interquartile range, 13–77) for 89 patients not placed in LAF rooms ($P < .001$). Six patients were diagnosed with invasive aspergillosis while in LAF rooms, with a median time to invasive disease of 23 days (interquartile range, 20–33). Among patients who developed invasive aspergillosis after leaving LAF rooms, the median time between discontinuation of LAF and diagnosis of disease was 55 days (interquartile range, 28–81).

Risk factors for development of early invasive aspergillosis. The univariate and adjusted risk factors for early aspergillosis are presented in table 2. All variables shown are included in the multivariate model. In the multivariate analysis, patients with hematologic malignancy in other than first remission, or with other underlying conditions, were more likely to develop invasive aspergillosis than were patients with CML-CP (relative risk [RR], 8.9; 95% CI, 2.1–37.1; and RR, 5.8; 95% CI, 1.3–26.6, respectively). Patients whose transplants did not take place under LAF conditions and those who received their transplants during the summer compared to winter were also at increased risk (RR, 5.6; 95% CI, 2.3–13.4; and RR, 4.5; 95% CI, 1.9–10, respectively). Age, sex, and year of transplant were not significant risk factors. Acute GVHD, pretransplant cytomegalovirus serology, conditioning regimen, construction, neutropenia, and use of corticosteroids were also not significantly associated with early aspergillosis.

Risk factors for development of late invasive aspergillosis. The univariate and adjusted risk factors for late aspergillosis

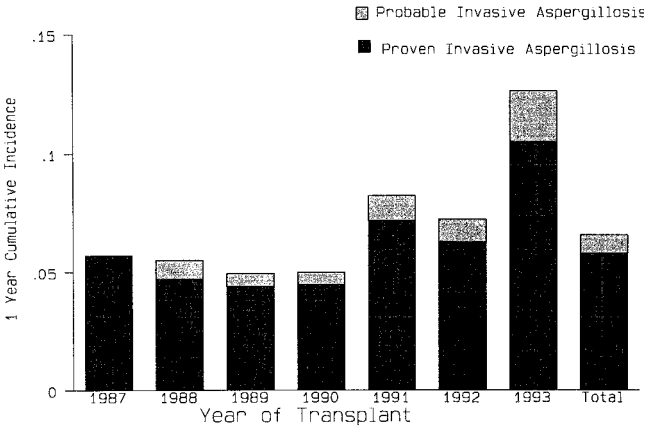

Figure 1. One-year cumulative incidence of aspergillosis by year of transplant; for 1993 only 9-month incidence is shown.

are summarized in table 3. In the multivariate model, patients with diagnoses other than CML-CP had increased risk of aspergillosis. The relative risks of aspergillosis were 3.6 (95% CI, 1.6–8.3) for patients with hematologic malignancy in first remission, 3.1 (95% CI, 1.6–5.8) for patients with hematologic malignancy in other than first remission, and 3.7 (95% CI, 1.8–7.8) for patients with other diagnoses. Patients who received marrow from unrelated donors were 1.7 times (95% CI, 1.0–2.7) more likely to develop aspergillosis than were patients with matched, related donors. Receipt of autologous or syngeneic marrow was protective (RR, 0.09; 95% CI, 0.01–0.7). Patients who developed acute GVHD and those who received treatment with corticosteroids were also at increased risk (RR, 2.6; 95% CI, 1.4–4.9; and RR, 3.1; 95% CI, 1.7–5.9, respectively). Risk of aspergillosis was 3-fold (95% CI, 1.5–6.3) higher for patients aged 19–40 and 5-fold (95% CI, 2.4–10.5) higher for patients aged >40 relative to patients ≤18 years. Patients who received transplants at the time of construction were at increased risk compared with patients whose transplants were at other times (RR, 1.8; 95% CI, 1.0–3.3). Neutropenia also predisposed patients to *Aspergillus* infection (RR, 6.0; 95% CI, 3.0–12). However, among neutropenic patients, the duration of neutropenia did not differ significantly among patients who developed aspergillosis and those who did not. Transplantation during a later year increased the risk of aspergillosis in the univariate but not in the adjusted analysis. Conditioning regimen, season, cytomegalovirus serology, and LAF did not significantly affect risk for late invasive aspergillosis.

Survival of patients with invasive aspergillosis. The 1-year survival estimate for patients with invasive aspergillosis was 7% (7.4% for proven and 5.3% for probable disease), compared with 54% for control patients ($P < .001$) (figure 3). Only 8 patients who developed invasive or probable aspergillosis survived 1 year after transplant, and only 8 were alive 6 months after the diagnosis of invasive disease. In contrast to patients with invasive disease, 46% of patients colonized with *Aspergillus* species were alive 1 year after transplant.

Aspergillus infection was the strongest risk factor for death among patients with both early and late aspergillosis (RR, 11.2; 95% CI, 7.33–17.0; and RR, 5.5; 95% CI, 4.3–7.2, respectively). Other variables predictive of death were GVHD, older age, and underlying disease other than CML-CP. Patients with transplants from matched, related donors had better survival than those with autologous transplants (RR, 0.74; 95% CI, 0.62–0.88). Neutropenia, cytomegalovirus seropositivity, and corticosteroid administration were additional risks for death. The risk of death decreased significantly during the study period.

Predictive value of a superficial isolate of Aspergillus. We examined the association of an initial isolate of *Aspergillus* obtained from a mucosal site and the presence or development

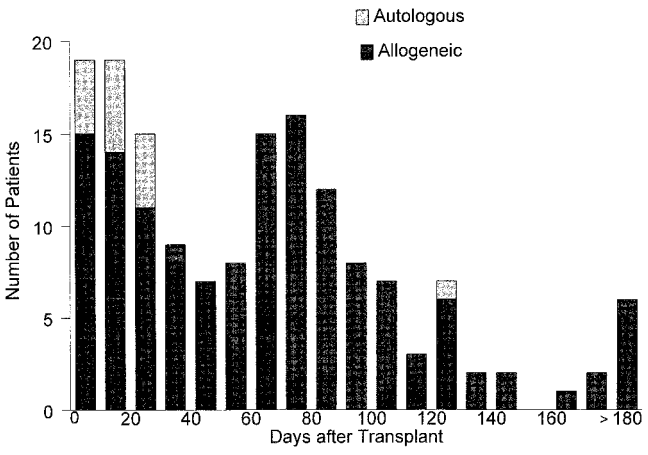

Figure 2. Time from transplant to diagnosis of aspergillosis, stratified by allogeneic and autologous type of transplant, in days.

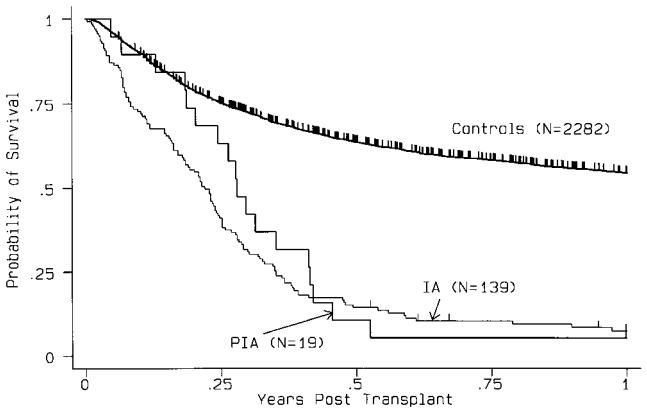

Figure 3. Kaplan-Meier estimates of survival of patients with documented and probable invasive aspergillosis, patients colonized with *Aspergillus* species, and control patients. IA, documented invasive aspergillosis; PIA, probable invasive aspergillosis. Tic marks indicate patients lost to follow-up.

of invasive disease in 104 patients. Fifty-seven patients (55%) were diagnosed with invasive disease within 10 days of the initial isolate. Among the remaining 49 patients who had no evidence of disease for at least 10 days, 44 (90%) did not progress to invasive disease, while 5 (10%) developed invasive disease after an interval ranging from 11 to 44 days. Overall, 62 of 104 patients with a mucosal *Aspergillus* isolate developed invasive disease; thus, the positive predictive value of such an isolate for invasive aspergillosis was 60%. Among neutropenic patients, the predictive value of a mucosal isolate for invasive disease was 94%. The mean number of days with a sputum culture positive for *Aspergillus* organisms was 2.3 in patients with invasive disease and 1.8 in colonized patients; the median was 1 for both groups.

The univariate comparison of risk factors for the development of invasive disease in colonized patients showed that patients who had invasive disease were more likely to have had an allogeneic transplant with a mismatched or unrelated donor than were patients who remained colonized ($P = .03$). Sixty-nine percent of patients with invasive aspergillosis received corticosteroids, compared with 45% of colonized patients ($P = .004$). Sixty percent of patients with invasive aspergillosis had acute GVHD at the time of infection, compared with 43% of colonized patients ($P = .02$). Other characteristics did not differ significantly between patients with invasive disease and colonization.

Relationship between species of Aspergillus, site of initial isolation, and invasive infection. Microbiologic identification of *Aspergillus* species was available for 183 patients, including 133 patients with invasive aspergillosis, 41 colonized patients, and 9 patients with contaminants. Of 133 initial isolates of *Aspergillus* from patients with invasive disease, 101 (75%) were isolated from the respiratory tract (sputum, 48; bronchoalveolar lavage fluid, 9; and lung biopsy, 44). Other frequent sites were internal organs obtained at autopsy (15) and skin (13). *Aspergillus fumigatus* accounted for most invasive isolates (107), followed by *Aspergillus flavus* (16), *Aspergillus terreus* (9), and *Aspergillus niger* (1).

Among 41 patients colonized with a known species of *Aspergillus*, 22 had isolates recovered from the respiratory tract, 12 from the rectum, 5 from the nasopharynx, and 2 from the ear. Most common isolates were *A. fumigatus* (22), followed by *A. niger* (11) and *A. flavus* and *A. terreus* (2 each).

We examined associations between the site of infection and the species of *Aspergillus*. Isolates from the respiratory tract and those of *A. fumigatus* were associated with invasive disease. In contrast, isolates from the rectum were associated with colonization, and *A. niger* was the predominant isolate in these patients. For example, only 1 (8.3%) of 12 *A. niger* isolates was associated with invasive disease, compared with 107 (80%) of 133 *A. fumigatus* isolates (odds ratio, 0.02; 95% CI, 0.003–0.18). Among patients with *A. fumigatus,* 2 (29%) of 7 rectal isolates were associated with invasive disease versus 88 (83%) of 106 pulmonary isolates. Among patients with *A. niger,* none of 7 rectal isolates was associated with invasive disease compared with 1 of 3 pulmonary isolates (combined odds ratio, 0.07; 95% CI, 0.013–0.37). Among patients with invasive disease, 6 (38%) of 16 isolates of *A. flavus* were from skin, compared with 5 (4.7%) of 107 isolates of *A. fumigatus* (odds ratio, 12.2; 95% CI, 3.3–45.3).

Contaminants. Ten of twelve patients with *Aspergillus* contaminants had a single *Aspergillus* isolate recovered from blood. None of these patients had evidence of fungal disease at the time of the positive isolate and none developed invasive aspergillosis during the follow-up.

Discussion

This study describes the largest cohort of patients with invasive aspergillosis following marrow transplantation published to date. Several new observations have emerged from this study. First, the onset of invasive aspergillosis after marrow transplant appeared bimodal, and most risk factors for early versus late aspergillosis differed. Second, the LAF environment protected against early but not late aspergillosis, suggesting that most patients who receive their transplants in LAF rooms probably acquired the infection exogenously after discontinuation of LAF. Third, most allogeneic marrow transplant patients were not neutropenic at diagnosis of aspergillosis, in contrast to patients with autologous transplant. Finally, we found that the prognosis of a patient with an *Aspergillus* isolate depended on both the site of isolation and the species recovered.

The bimodal distribution of onset of *Aspergillus* infections after transplant has not been noted previously, possibly because of smaller numbers of patients in most series. The current literature reports a wide range of median day of onset, ranging from 4 to 115 days [4, 2, 6, 20]. Among patients undergoing autologous transplants, aspergillosis occurred during neutropenia and was rare after engraftment. In contrast, aspergillosis in patients receiving allogeneic transplants was more likely to occur after engraftment [6].

In patients with cancer, neutropenia has been reported as the major risk factor for aspergillosis [21], and the recovery of granulocytes appears essential for survival [21, 22]. Among patients with autologous grafts whose risk was highest prior to engraftment, use of granulocyte colony-stimulating factor and possibly granulocyte transfusions may be important adjunctive therapy. However, the majority of our patients were not neutropenic when aspergillosis was diagnosed. The development of GVHD in patients with allogeneic transplants complicates the prevention and successful treatment of *Aspergillus* infection, as it necessitates prolonged administration of immunosuppressive medications, including corticosteroids. Unfortunately, we did not have sufficient data on corticosteroid use to measure the associated risk more precisely, as the risk of infection may occur after exceeding a certain dose [23].

The protection against *Aspergillus* infections afforded by LAF has been reported previously [24] and has become of

increasing interest, as this form of protection remains expensive and requires inpatient hospitalization. In outbreaks of aspergillosis associated with hospital construction, patients housed in LAF rooms have been protected from infection [7, 25–27] or had a late onset of infection. For example, in a study of 18 patients, all of whom remained in LAF rooms for 30 days after transplant, aspergillosis occurred at a mean of 115 days after transplant [2]. These studies, as well as ours, suggest that the risk for *Aspergillus* infection increases when immunosuppression is combined with the lack of a protective environment. In the present study, the median time interval between transplant and diagnosis of aspergillosis in patients who received their transplants outside of LAF rooms was comparable to the interval between discontinuation of LAF and aspergillosis in patients who received their transplants in LAF rooms (40 and 55 days, respectively). Thus, the incubation period in a susceptible host appears relatively short. The increased risk of *Aspergillus* infection during the summer has not been noted previously, although significantly increased numbers of airborne spores have been noted during the summer [28]. As multiple environmental factors appear to play a role in the acquisition of invasive aspergillosis, manipulation of the environment may reduce the risk of disease, and promotion of alternative strategies to reduce airway exposure becomes critical.

The association of *A. flavus* and sinusitis in immunocompromised patients has been noted previously [29–31]; *A. flavus* also predominates in the literature about cutaneous *Aspergillus* infections [32, 33]. In our study, *A. niger* appeared to be less virulent than *A. fumigatus,* and its recovery from rectal swabs suggested that the acquisition of the organism may occur via the gastrointestinal rather than the respiratory route. Whether patients colonized in the gastrointestinal tract with *A. niger* do not require antifungal therapy remains to be determined and may depend on the presence of other risk factors, such as acute GVHD.

Despite prompt initiation of antifungal therapy, recovery of an *Aspergillus* isolate from a superficial site signaled the presence of invasive disease in most patients. Unlike the case in other patient populations [34, 35], the number of sputum isolates did not distinguish patients who were colonized from those with invasive disease. Even most patients with invasive aspergillosis had only a single isolate from sputum. Clearly, current diagnostic methods lack sensitivity, as most marrow transplant patients who present with *Aspergillus* in their sputum have already progressed to invasive disease.

This study has identified a bimodal distribution of *Aspergillus* infection after marrow transplantation and risk factors associated with each peak. Preventive strategies should focus on reducing both environmental and host risk factors, including decreasing exposure of the airways by less costly and more effective methods than LAF and reducing the risk associated with neutropenia. Future challenges also include the development of more sensitive methods for detecting early infection and more effective treatment of established disease.

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