

Progress in the Diagnosis and Management of Aspergillosis in Bone Marrow Transplantation: 13 Years' Experience

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Over 13 years, we have seen 16 cases of proven invasive aspergillosis in 446 bone marrow transplant recipients, an incidence of 3.6%. The incidence of infection is low in patients with uncomplicated allogeneic or autologous bone-marrow transplants (<2% and 0, respectively). Of the 16 episodes following transplantation, 10 occurred in patients with late transplant complications who were no longer in protective isolation. In patients who had focal pulmonary lesions (as diagnosed by computed tomographic scanning), culture of bronchoalveolar lavage (BAL) fluid was not an effective diagnostic procedure. In diffuse pulmonary disease due to *Aspergillus*, culture of BAL fluid had a sensitivity of 100%. *Aspergillus* species were isolated from an additional six patients who had no evidence of invasive aspergillosis. Graft rejection was a significant predisposing factor for the development of invasive aspergillosis ($P < .001$, log-rank test), and in our hospital, these patients now receive intravenous amphotericin B as prophylaxis. None of the six patients whose chest roentgenograms showed abnormalities before transplantation and who underwent surgical resection as part of the treatment for invasive aspergillosis developed recurrent infection.

Despite the developments that have been made in the prevention of invasive aspergillosis, it remains a major problem in patients undergoing bone marrow transplantation (BMT), with a fatality rate approaching 100% [1]. Prolonged neutropenia is a major risk factor for invasive aspergillosis. Recovery of neutrophils is essential if the patient is to survive [2], although they may die from massive hemoptysis during this time [2-4]. Fungal infections may recur during subsequent episodes of neutropenia [5], which may be prolonged during BMT, with its coexistent lymphocyte dysfunction.

A mycotic lung sequestrum (MLS) forms when normal lung that is infiltrated by fungal hyphae undergoes ischemic necrosis and separates from the surrounding tissue, often forming a well-defined ball several centimeters in diameter [3]. MLS often presents during neutrophil recovery. Since our observation of deaths due to massive hemoptysis [3], we have considered all subsequent patients in this unit (both transplant and nontransplant) with a presumptive diagnosis of MLS candidates for urgent surgery. Surgical intervention not only reduces the bulk of infected tissue, which is the source of hemorrhage, it also may reduce the risk of recurrence during subsequent transplantation. We review our experience of invasive aspergillosis in a total of 29 patients: 16

undergoing BMT, 6 from whom *Aspergillus* species were isolated without proven infection, and 7 with invasive aspergillosis before transplantation.

Patients and Methods

From January 1978 to December 1991, 332 patients underwent allogeneic BMT (including 12 patients who received marrow from matched unrelated donors) and 114 patients underwent autologous BMT. *Aspergillus* species were isolated from clinical specimens from 22 patients during the course of transplantation. Seven additional patients with a diagnosis of invasive aspergillosis before transplantation underwent BMT. Patients D3 and D4 were described briefly in a paper by Kibbler et al. [3] that reported on MLS. The surgical aspects of the management of patients B4, B5, B6, D9, and D10 were described by Wong et al. [6]. None of the 114 autografts or 12 matched unrelated donor grafts showed any evidence of infection due to *Aspergillus* species. The annual incidence of aspergillosis in recipients of bone-marrow transplants in the Royal Free Hospital (RFH; London) is shown in figure 1.

All BMT patients were cared for in protective isolation throughout their period of neutropenia. Since early 1988, RFH has been progressively introducing high-efficiency particulate air (HEPA) filtration, and from 1990, all transplant recipients have been nursed in rooms with HEPA filtration [7]. Among the patients who developed invasive aspergillosis, 6 were receiving oral amphotericin B, 7 were receiving ketoconazole plus oral amphotericin B, and 3 were receiving fluconazole plus oral amphotericin B as prophylaxis for fun-

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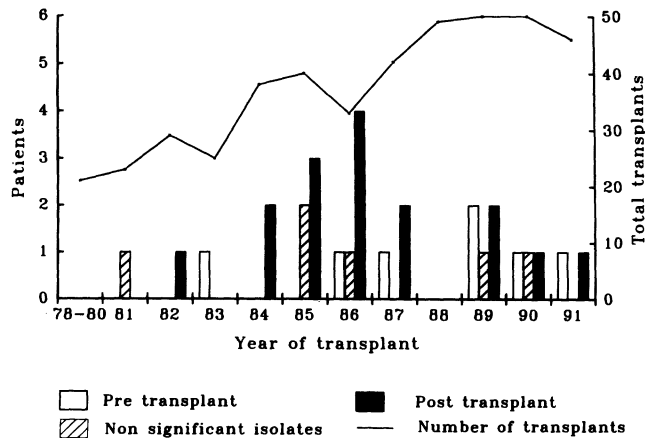


Figure 1. Incidence of isolation of *Aspergillus* species from patients undergoing bone marrow transplantation.

gal infection. At RFH patients remain in the hospital until the transplanted marrow has engrafted. In the absence of complications, patients are seen every 2 weeks for 6 months and then monthly for the first year. Of those patients who died, 17.7% underwent full necropsy, and 8.4% had limited examinations. Postmortem biopsy specimens are routinely cultured for likely pathogens.

During the study period neutropenic patients were treated empirically with intravenous amphotericin B if fever persisted for >72 hours after the initiation of intravenous antibiotic therapy. When diffuse shadowing was seen on the chest roentgenogram of febrile patients, bronchoscopy was performed immediately. Patients with cavitating pulmonary lesions seen on computed tomographic (CT) scans were treated presumptively for invasive aspergillosis. Patients with other radiological findings of pulmonary infection received empirical therapy active against pathogens including fungi. A diagnosis of invasive aspergillosis was made if there was histological evidence of invasive disease or if *Aspergillus* species were isolated from two or more separate specimens from a site of clinical infection. Specimens were incubated at 30°C and 37°C on Sabouraud agar and were identified by standard morphological criteria.

Statistical analysis was performed with the use of the EPI Info v5.01b program, where appropriate (Centers for Disease Control and Prevention, Atlanta, GA). Relative risk (risk ratio [RR]) is expressed with 95% confidence intervals, as are the actuarial incidences.

Results

Isolates of Aspergillus species without clinical evidence of invasive aspergillosis. The 6 patients (median age, 25 years; range, 6–41 years; lymphoma, 1; acute lymphocytic leukemia, 1; acute myeloid leukemia, 1; chronic granulocytic leukemia, 3) with allogeneic transplants who had *Aspergillus*

species isolated that were not considered suggestive of invasive disease are detailed in table 1. The neutrophil count of patient A2 was increasing when the isolation was made. The neutrophil count of patient A4 rose over the week subsequent to the isolation. The neutrophil count of patient A5 rose slowly over the month following isolation.

Clinical diagnosis of invasive aspergillosis (without confirmation by culture or histology). One patient showed a cavitating lesion on a chest roentgenogram 18 days after an allogeneic BMT for chronic granulocytic leukemia at a time his neutrophil count was starting to rise. Investigations included bronchoalveolar lavage (BAL) but not lung biopsy. No pathogens were identified, and the lesion appeared to respond to intravenous amphotericin B. He died with varicella pneumonia 8 months after transplantation. This case is not included in the discussion.

Invasive aspergillosis occurring before BMT. The 7 patients (median age, 19 years; range, 13–36 years; acute lymphocytic leukemia, 3; acute myeloid leukemia, 4) who had a definite diagnosis of pulmonary aspergillosis before BMT are described in table 2. None of these patients showed evidence of invasive aspergillosis after BMT.

All except patient B2 had surgical resection of focal lung lesions (with histological confirmation of diagnosis) a mean of 2 months before transplantation. In patients B4 and B5, the surgery was done to remove an MLS. Because patient B7 had a microbiologically confirmed diagnosis of tuberculosis, the pulmonary lesion, which did not show the characteristic halo sign on a CT scan, was removed only after the patient failed to respond to antituberculous therapy. In patient B1 the resection was for diagnostic purposes, and patients B3 and B6 had elective resections to remove infected foci before transplantation. Patient B6 required in addition to lung resection debridement of an extensive nasal infection, which also was due to *Aspergillus flavus*.

Because cultures of her facial lesion were persistently positive, patient B6 received prophylaxis with intravenous liposomal amphotericin B (2–4 mg/kg per day; total dose, 17 g). Patient B1 received only oral ketoconazole and amphotericin B suspension. Patient B3 and patient B4, respectively, received alternate-day prophylaxis with 0.25 and 0.5 mg of intravenous amphotericin B/kg. Because of a recurrence of invasive aspergillosis in the previous cycle of chemotherapy, patient B2 received in excess of 1.2 g of amphotericin B during the period of neutropenia. Patient B5 received just over 1 g of amphotericin B after developing pleuritic pain at her thoracotomy site and a small pleural effusion. Patient B7, who was neutropenic for 9 weeks after transplantation, received itraconazole (100 mg twice a day iv for 2 weeks then 300 mg of suspension twice a day orally) as prophylaxis during transplantation.

Invasive aspergillosis after BMT. The 16 patients (median age, 25 years; range, 17–49 years; acute lymphocytic

Table 1. Isolates of *Aspergillus* not associated with invasive disease.

Patient	<i>Aspergillus</i> isolate (time after transplantation)	Site of isolation × no. of isolates	Outcome (duration of follow-up from time of isolation)	Other diagnosis	Immunosuppression
A1	<i>A. fumigatus</i> (3 mo)	Sputum × 1 (BAL negative × 2)	Died (5 w)	PCP, slow engraftment	Steroids
A2	<i>A. fumigatus</i> (6 mo)	Sputum × 1 (BAL negative)	Died* (10 w)	PCP and CMV IPN (PM), asthma and transient cytopenia	Steroids, cyclosporin A, neutropenia
A3	<i>A. fumigatus</i> (3 mo)	Chest drain × 2 (BAL negative)	Died (1 w)	Febrile IPN, GVH	Steroids, cyclosporin A
A4	<i>Aspergillus</i> species (1 w)	Sputum × 1 (BAL negative)	Died* (7 w)	Pneumococcal [†] sepsis, ARDS, GVH	Steroids, cyclosporin A, neutropenia
A5	<i>A. fumigatus</i> (6 mo)	Ear × 1	—† (8 w)	<i>Mycobacterium chelonae</i> bacteremia, relapsed leukemia	Chemotherapy, neutropenia
A6	<i>Aspergillus</i> species (6 w)	BAL × 1	Died (4 w)	PCP, respiratory failure, GVHD	Steroids, methotrexate

NOTE. BAL = bronchoalveolar lavage; PCP = *Pneumocystis carinii* pneumonia; CMV = cytomegalovirus; IPN = interstitial pneumonitis; GVHD = graft-vs.-host disease; PM = postmortem findings; ARDS = adult respiratory distress syndrome.

* Autopsy performed (results in next column).
 † Lost to follow-up (afebrile at last report).
 ‡ Patient also had other bacterial infections.

leukemia, 8; acute myeloid leukemia, 7; chronic granulocytic leukemia, 1) who developed invasive aspergillosis after transplantation are described in tables 3 and 4. The overall incidence of invasive aspergillosis was 3.6%, but all cases were seen in patients who received allogeneic transplants from related donors, giving an incidence of 5% (actuarial incidence, 8% ± 1.5%) in this subpopulation.

T-cell depletion of donor marrow was used as prophylaxis for graft-vs.-host disease (GVHD) in the six patients with aspergillosis before transplantation who received allogeneic transplants. Of the patients who developed aspergillosis after transplantation, 14 (87.5%) of 16 had received T cell-depleted allografts, a similar proportion to that in the entire population (82.5%).

Graft failure/rejection occurred in 39 of the 332 transplant recipients. Seven (18%) of these patients developed invasive aspergillosis ($P = .0009$, two-tailed Fisher's test; RR = 5.84, 2.31–14.80), giving an actuarial risk of 39% ± 22%, as compared with a risk of 5.5% ± 1% for the rest of the group ($P < .001$, log-rank test; figure 2). Aspergillosis developed in 6 (one also rejected the graft) of the 69 patients who had GVHD that was more severe than grade 1 ($P = .11$, two-tailed Fisher's test; RR = 2.29, 0.86–6.07). The majority of patients with GVHD worse than grade 1 were enrolled early in our series, when prophylaxis with T-cell depletion for GVHD was still being developed.

Of the six patients who developed invasive aspergillosis before discharge from the hospital after BMT (median time

from transplantation to clinical infection: 3 weeks), only one developed it after HEPA filtration was introduced in 1988. The other 10 patients appeared to have acquired the infection in the community, having left the hospital after transplantation (median time from presentation to clinical infection: 19 weeks).

Diagnosis. Fourteen patients with a definite diagnosis (including four with isolates before transplantation) underwent BAL. BAL fluid was negative for all seven patients with a focal lesion, whereas *Aspergillus* species were isolated from BAL fluid of all seven patients with diffuse pulmonary lesions. Of the patients with *Aspergillus* isolated before transplantation, in only one was diagnosis accelerated by cytological examination of specimens. In the BMT patients in this series, cytological and direct microscopic examination of BAL fluid did not detect any cases. Of the six patients with nonsignificant isolates, only one had a positive result on BAL, which was coincident with the diagnosis of *Pneumocystis carinii* pneumonia. The overall sensitivity of BAL was 50%, and its specificity was 80%; however, if patients who had only focal lesions are excluded, the sensitivity is 100%. *Aspergillus* species were isolated successfully after transplantation for 14 of 16 patients and before transplantation for 6 of 7 patients. The three patients whose cultures were negative had an appropriate history and had fungal elements compatible with *Aspergillus* species seen on histological examination. In 3 of 16 cases the definitive diagnosis was not made until after death. One case was unexpected (patient C1), and

Table 2. Patients with aspergillosis diagnosed prior to bone marrow transplantation.

Patient	Disease	Follow-up post-transplantation (mo)	Outcome	Diagnostic specimen	Species of <i>Aspergillus</i> isolated
B1	ALL	7	Died (no autopsy) (relapsed leukemia)	Resected lung	Histology only
B2	AML*	4	Died (no autopsy) (HSV encephalitis)	BAL and biopsy	<i>A. fumigatus</i> [†]
B3	AML	60	Alive	Percutaneous biopsy [‡] (negative BAL)	<i>A. fumigatus</i>
B4	AML	38	Alive	Resected lung (negative BAL)	<i>A. fumigatus</i>
B5	ALL	23	Alive	Sputum × 2 [‡] , resected lung	<i>A. fumigatus</i> [§]
B6	ALL	13	Died (no autopsy) (relapsed leukemia)	Nasal swab [#] , resected lung	<i>A. flavus</i>
B7	AML	11	Alive	Resected lung (negative BAL)	<i>A. fumigatus</i>

NOTE. ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; HSV = herpes simplex virus; BAL = bronchoalveolar lavage.

* Developed graft-vs.-host disease requiring therapy with cyclosporin A, thalidomide, and steroids.

[†] Histologic studies also suggested zygomycetes infection.

[‡] Confirmed in surgical specimen.

[§] Sputum also contained *A. niger*; *A. fumigatus* was only isolate from biopsy specimen.

^{||} Patient had received an autologous bone marrow transplant.

[#] Facial lesion (also present in resected lung).

a strong clinical impression of aspergillosis in patients D3 and D4 was confirmed postmortem.

Therapy for and outcome of aspergillosis after BMT. The day of diagnosis of aspergillosis was defined as the day on which fever plus abnormal chest roentgenographic findings or pleuritic pain occurred or at the start of a fever lasting >72

hours that was unresponsive to empirical therapy in the absence of the above criteria. In 12 patients for whom full details are available, a median delay of 1 day (range, 0–5 days) from diagnosis to initiation of therapy was noted in a retrospective examination.

Our standard therapy was amphotericin B with a target

Table 3. Patients with aspergillosis involving more than one site diagnosed after bone marrow transplantation.

Patient	Disease	Complication	Immuno-suppressive treatment at diagnosis	Weeks from transplant to infection [†] (days survival)	Duration of neutropenia [‡]	Diagnostic specimen	Organs involved	Species of <i>Aspergillus</i> isolated
C1	ALL	Rejection	Steroids	15 (20)	3	PM	Brain, lung	Histology
C2	CGL	Rejection	Steroids and cyclosporin A	4 (12)	4	Facial	Sinus, lung	<i>A. flavus</i>
C3	AML	GVHD	Steroids	13 (21)	...	BAL and skin	Skin, lung	<i>A. flavus</i>
C4	AML	GVHD*	Steroids, cyclosporin A, and thalidomide	18 (27)	9	BAL and PM	Heart, kidney, lung	<i>A. flavus</i>
C5	AML	Autograft for rejection*	...	93 (55)	83	Percutaneous lung biopsy (BAL negative)	Sinus, lung	<i>A. flavus</i>
C6	ALL	GVHD	Steroids	3 (16)	Rising	BAL and PM	Lung, thyroid	<i>A. fumigatus</i>

NOTE. ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CGL = chronic granulocytic leukemia; GVHD = graft-vs.-host disease; BAL = bronchoalveolar lavage; PM = postmortem biopsy.

* Cytomegalovirus isolated on BAL.

[†] Start of terminal fever is considered the first day of infection.

[‡] Neutropenia is defined as a neutrophil count of <1 × 10⁹/L.

[§] Neutrophil count >1 × 10⁹/L for 8 weeks.

^{||} *Pneumocystis carinii* pneumonia 19 days before.

Table 4. Patients with invasive aspergillosis at one site diagnosed after bone marrow transplantation.

Patient	Disease	Complication	Immunosuppressive treatment	Weeks from transplant to infection (days survival)*	Weeks of neutrophil count $>1 \times 10^9/L$ prior to diagnosis† (weeks of neutropenia)	Diagnostic specimen	Species of <i>Aspergillus</i> isolated	Outcome‡
D1	ALL	GVHD	Steroids	6 (3)	2	Tracheal aspirate and biopsies	<i>A. fumigatus</i>	Died with tracheal stenosis
D2	ALL	GVHD	Steroids and azathioprine	108 (11)	98	BAL, sputum§	<i>Aspergillus</i> species	Died with diffuse lung disease
D3	AML	2 (21)	Rising	PM, BAL negative	Histology only	Died with hemoptysis (MLS)
D4	AML	Failed graft	. . .	2 (35)	Neutropenic (2)	PM, BAL negative	<i>Aspergillus</i> species	Died with hemoptysis (MLS)
D5	ALL	Relapse	Chemotherapy	60 (17)	Neutropenic (7)	Ear swab	<i>A. fumigatus</i>	Died with invasive aural infection
D6	AML	Rejection (multiple)	Steroids and ALG	18 (3)	Neutropenic (4)	Sputum, BAL, PM	<i>A. fumigatus</i>	Died with MLS and diffuse lung disease¶
D7	ALL	Rejection and GVHD	Steroids and thalidomide	20 (12)	15 (fell)	Sputum, BAL, PM*	<i>A. fumigatus</i>	Died with diffuse and focal lung disease
D8	AML	Relapse	Chemotherapy	37 (9)	Neutropenic (1)	Sputum**	<i>A. fumigatus</i>	Died with pneumonitis
D9	ALL	3 (21)	Rising	BAL negative, resected MLS	<i>A. fumigatus</i>	Died 7 days after surgery with CMV pneumonitis (PM)
D10	ALL	Rejection	Autograft	6 (22 mo)††	Rising	Resected MLS	<i>A. fumigatus</i>	Alive after surgery

NOTE. ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; GVHD = graft-vs.-host disease greater than grade 1; ALG = antilymphocyte globulin; PM = postmortem findings supportive of diagnosis; BAL = bronchoalveolar lavage; MLS = mycotic lung sequestrum; CMV = cytomegalovirus.

* Start of terminal fever is considered the first day of infection, and days survival is from start of terminal fever to death.

† Rising = steady increase to $>1 \times 10^9/L$ at time of diagnosis; fell = fall in neutrophil count to $<1 \times 10^9/L$ after diagnosis.

‡ Survival is calculated from retrospective clinical date of emergence of infection.

§ *Pseudomonas* also in sputum.

¶ Generalized varicella 4 weeks earlier.

* Positive throat swab 3 months beforehand.

** Multiple isolates.

†† Time from first transplant.

daily dosage of 1 mg/kg. Details of dosage were not available for two patients (who had no clinically significant toxic reactions), and two died within 3 days of diagnosis. Six patients developed renal impairment (serum creatinine level greater than twice the initial value), one developed high fevers with rigors (unresponsive to concomitant meperidine therapy),

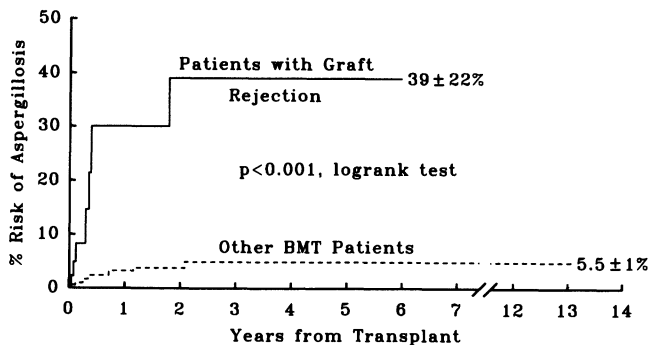


Figure 2. Actuarial incidence of invasive aspergillosis in patients undergoing bone marrow transplantation (BMT) with and without graft rejection. Rejection was significantly associated with an increased risk of invasive aspergillosis.

and one had marked systemic discomfort. In five patients treatment had to be modified because of toxicity (renal toxicity in patients C4, C5, D4, and D9; discomfort in patient D3).

Of the five patients with MLS, two died of massive hemoptysis and one died with disseminated infection. Of the two who underwent surgical resection, one subsequently died of cytomegalovirus (CMV) pneumonitis and the other remains well. Of the other 11 deaths, 9 were due to disseminated aspergillosis, 1 was due to cerebral lesions, and 1 patient died with widespread obstruction of the airways (despite intubation). Thus, the mortality due to aspergillosis was 14 (87.5%) of 16, with an additional death due to CMV pneumonitis after the patient underwent successful resection of an MLS (patient D9). Of the seven patients who had invasive aspergillosis before BMT, four remain alive (median follow-up, 31 months).

Discussion

Fewer than 10 survivors of invasive aspergillosis after BMT have been reported worldwide [8]. The incidence of invasive aspergillosis during BMT performed in units with

filtered air is at least 4% [9] but may be much higher in units without air filtration. The associated mortality is 94% [1]. Pulmonary aspergillosis carries an attributable mortality of 85% [10]. During 1990 ~4,000 BMTs were performed in Europe alone [11], a figure that illustrates the potential impact of this problem. Although the major risk factor for invasive aspergillosis is granulocytopenia [12], cell-mediated immunodeficiency (as in BMT) also is associated with invasive disease [9, 13]. Improvements in antibacterial prophylaxis [14] and prophylaxis with azoles active against yeasts [15] will increase the relative importance of aspergillosis in immunocompromised patients. Indeed, patients with bacteriologically sterile airways appear to be at increased risk of invasive aspergillosis [16]. The spores are ubiquitous, and while HEPA filtration substantially reduces spore counts, sporadic infections will continue to occur [7]. The place for pharmacological prophylaxis with amphotericin B administered intravenously [9], by nebulized inhalation [17], or by nasal spray [18] or with itraconazole [19] has not been established. Furthermore, infection with *Aspergillus* species may be clinically silent for several weeks [20] only to present after extended periods of protective isolation. This clinically silent period also is reflected in the risk of recurrence of aspergillosis in later episodes of neutropenia [5].

The initial clinical manifestation in 14 of 16 patients was fever unresponsive to empirical antibiotic therapy. Patient B5 developed a productive cough without fever, and *Aspergillus fumigatus* and *Aspergillus niger* were isolated from a sputum sample, prompting further investigation. Patient C1 presented with neurological disturbance, and the diagnosis was only made postmortem. Otherwise, apart from the frequent occurrence of pleuritic chest pain, no specific features were noted until radiological abnormalities developed. Frequent chest roentgenography in patients with persistent fever is an essential adjunct to the diagnosis of invasive aspergillosis [21], and CT scanning may detect very small lesions [22]. Endobronchial aspergillosis is an emerging problem in neutropenic patients [23, 24] and in lung transplant [25] and bone marrow transplant [26] recipients. As with other forms of pulmonary aspergillosis, chest roentgenograms may fail to detect early lesions, but in our experience, CT scanning (in conjunction with BAL) detected such lesions in some of our non-BMT patients.

Diagnosis of aspergillosis may be difficult. Albelda et al. [27] demonstrated that the isolation of aspergilli in BAL fluid was a good positive predictor of invasive disease, but we did not find it predictive in patients with focal lesions suggestive of MLS, a result that may explain the poor predictive value of BAL in some studies [28]. CT scans of early focal lesions of invasive aspergillosis usually show segmental infarcts before the lesions progress to the well-described halo sign [29]. While BAL is helpful in the diagnosis of diffuse pulmonary infiltrates [30], its use in patients with focal lesions may delay diagnosis unnecessarily. The microbiologi-

cal diagnosis is best confirmed by open lung biopsy/resection, which has proved to be safe [6]. Unlike Kahn et al. [31], we did not find that cytological examination of BAL fluid improved the diagnostic sensitivity of BAL for fungal infections.

Where tissue is available, histological examination provides conclusive evidence of invasive fungal disease, but the histological appearance of many filamentous fungi is sufficiently similar to make culture a necessary aid in identifying them by species (and may influence management). Of cases in which specimens were cultured in addition to being examined histologically, only one case identified as positive histologically was found negative by culture. Antigen tests are available for use in immunocompromised patients and show promise [32], and the use of the polymerase chain reaction is under investigation; however, for such a ubiquitous organism, the interpretation of results might be difficult.

Although isolation of the organism from normally sterile sites is strong evidence for invasive aspergillosis, Yu et al. [33] have shown that the isolation of *Aspergillus* species from sputum is suggestive of invasive disease only in neutropenic patients. Isolation of *A. flavus* or *A. fumigatus* is most likely to be associated with invasive aspergillosis [34]. Treger et al. [35] showed that isolation of *A. fumigatus* or *A. flavus* in more than one sputum specimen was strongly associated with invasive disease. In a study of an outbreak of invasive *A. flavus* infection, positive nasal cultures (often in association with the presence of superficial ulcers—perhaps “preinvasive” lesions) were highly predictive of disease [16]. Overall, it appears that ~40% of neutropenic patients from whom *Aspergillus* species are isolated do not have invasive aspergillosis [17, 18, 36]. In immunocompromised patients with sinusitis, nasal cultures positive for *Aspergillus* are associated with invasive disease in 58% of cases, while cultures for only 10% of patients are negative [37]. *A. flavus* has a propensity to cause sinus disease [38], but in our experience such disease usually is associated with coexisting pulmonary lesions.

All but one of the patients with a single “superficial” isolate (table 1) had a definite alternative diagnosis, in contrast to the definite cases of invasive aspergillosis (tables 3 and 4). The clinical course for this first group suggests that these isolates represent colonization or environmental contamination rather than invasive disease. When another diagnosis is suspected and a single isolation of *Aspergillus* is made, therapy should not be focused exclusively on invasive aspergillosis. Some of our patients who had a single isolate of an *Aspergillus* species might have developed invasive aspergillosis if empirical intravenous amphotericin B had not been used.

As is apparent from our series, once a clinical diagnosis of invasive aspergillosis is made the outlook is poor. Despite the toxicity of amphotericin B, empirical therapy with this agent is now standard practice and has probably done much to reduce the incidence of diagnosed fungal infection [39]. Despite prompt initiation of such treatment, which has been

shown to be effective in patients receiving chemotherapy for leukemia [40], the mortality rate in our patients was as high as that in published series [1], and 5 of 12 patients were unable to tolerate the target dosage. Use of liposomal amphotericin B [41, 42] may allow patients to tolerate larger doses. Therapy with itraconazole [8] also is a potential advance.

MLS usually develops as bone marrow function recovers and often is associated with fatal hemoptysis. Surgery reduces both the risk of death due to hemoptysis [3] and the infective load. In addition to its diagnostic and therapeutic roles, surgery also may reduce the risk of recurrence during subsequent BMT. The outcome for patients in our series who underwent BMT after an episode of invasive aspergillosis contrasts with that previously described for BMT patients, who had a high rate of recurrence of infection [5]. No patient in our series had a recurrence of fungal disease. Karp et al. [43] have suggested that during the less severe immunosuppression associated with chemotherapy, high-dose amphotericin B may be adequate, with signs of recurrent infection in only 2 of 9 patients. Patient B7 had a prolonged period of neutropenia, and the success of resection plus itraconazole therapy in this patient is encouraging. We now have extensive experience with open-excision biopsy in patients undergoing therapy for leukemia [6]. By the end of 1991, only two patients had died after 19 procedures. Neither death was attributable to the surgery. One patient (D9) died 7 days postoperatively of CMV pneumonitis. The other died 17 days postoperatively with progressive pulmonary infiltrates (necropsy was not performed). The other 17 patients (including one who required re-operation for hemorrhage) recovered. The operative morbidity compares well with that for series of patients not undergoing therapy for hematologic malignancy. Jewkes et al. [44] reported a 15% incidence of major complications and a 7% incidence of operative mortality. The operative mortality in the series of Daly et al. [45] was between 5% (for simple aspergillomas) and 34% (for complex aspergillomas). Lupinetti et al. [46] and Young et al. [47] reported on a recent surgical series in which bone-marrow transplant recipients/neutropenic patients have been free of surgical complications.

Some studies suggest an appreciable risk of aspergillosis during autologous BMT [41]. No patient receiving an autograft in our series developed invasive aspergillosis, unlike the experience of Wingard et al. [9], in which the incidence was similar in autograft and allograft recipients. The high risk of infection in patients (figure 2) who have rejected their graft is likely to represent the summation of three major risk factors: the patient will have been removed from protective isolation (10 of our episodes were diagnosed during admissions subsequent to transplantation); will be neutropenic; and will have received immunosuppressive therapy at a time when cell-mediated immunity is already impaired. Only two of the 16 patients had an otherwise uncomplicated transplant. As

Conneally et al. [17] found in three of four cases of aspergillosis in BMT patients, there is a major risk of infection if hematological complications (such as graft rejection) occur after neutrophil recovery.

We have shown that patients with previous invasive aspergillosis may safely undergo T cell-depleted BMT. If the thoracic CT scan or chest roentgenogram remains abnormal, we resect any focal pulmonary lesions before BMT. Despite the use of antifungal therapy, the infection remains life-threatening, and prevention is essential. With our current prophylactic regimen, those patients whose transplants are complicated by GVHD or rejection appear to be those principally at risk of aspergillosis. We now use prophylactic intravenous amphotericin B (1 mg/kg every third day) in patients with graft rejection. Minimizing the incidence of graft rejection and GVHD are as important in reducing mortality as is the use of prophylactic antifungal regimens, filtered air, early diagnostic maneuvers (e.g., serological tests), and the aggressive management of suspected cases.

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