Intranasal Amphotericin B Reduces the Frequency of Invasive Aspergillosis in Neutropenic Patients

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PURPOSE: **To retrospectively study the prophylaxis of invasive aspergillosis in neutropenic patients and to relate the frequency of this fungal disease to any causal or modifying factors that could be identified.**

PATIENTS AND METHODS: **Between 1977 and 1988, 130 patients underwent 158 intensive treatment episodes to control acute leukemia, lymphoma, and aplastic anemia, and the frequency of complicating aspergillus infection was determined.**

RESULTS: **Proven invasive aspergillus infections occurred in 22 cases, 12 of which were fatal. Invasive aspergillosis was suspected in a further 16 cases and all these patients recovered with amphotericin B treatment. Colonization by *Aspergillus* in the absence of clinically significant infection was seen in 31 treatment episodes. Invasive aspergillosis involved mainly the upper and lower respiratory tract and skin. Control of the infection was closely related to the control of the underlying disease, with subsequent return of normal marrow function and resolution of neutropenia. The incidence of aspergillus infection has decreased dramatically since 1985, most probably due to the introduction of intranasal amphotericin B. This occurred despite the persistence of aspergillus spores in the hematology ward air during the 1986 to 1988 period.**

CONCLUSION: **Intranasal aerosolized amphotericin B may protect against invasive aspergillosis, even when neutropenic patients are cared for in conventional wards without HEPA filtration.**

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Invasive aspergillus infections remain a major problem in immunocompromised patients, including those undergoing intensive anticancer treatment or receiving organ transplants [1–3]. Patients experiencing a prolonged period of bone marrow suppression, as occurs during remission induction in acute leukemia or with bone marrow transplantation, are particularly prone to opportunistic infection with this group of organisms. Aspergillus infection rates of between 5% and 60% have been reported in these patient groups [4–9]. While neutropenia and altered immunity remain the most important predisposing factors [10,11], infections have been linked to environmental factors such as ventilation systems [12,13], activity on adjacent construction sites [14–17], and building materials [18].

Infections with *Aspergillus* species have been a problem in patients undergoing intensive treatment in our hematology unit from 1977 through 1985. The rate of infection declined to low levels from 1986 to 1988. A retrospective study was therefore carried out in all patients who received intensive treatment, including bone marrow transplantation, for leukemia, lymphoma, and aplastic anemia between 1977 and 1988 to define the frequency of aspergillus infection and to relate this to any causal or modifying factors that could be identified. Aspergillus spore counts were monitored periodically during 1987 and 1988 when a large amount of construction activity commenced close to the hematology unit.

PATIENTS AND METHODS

Patients

The patient records for all patients receiving intensive treatment between January 1977 and December 1988 were reviewed. The patients with acute myeloid leukemia were treated with standard daunorubicin- and cytosine arabinoside-containing protocols with or without thioguanine [19–21]. Patients with acute lymphoblastic leukemia were treated with the OPAL (vincristine, prednisone, Adriamycin, L-asparaginase) protocol [22], although some Burkitt-like tumors were treated with

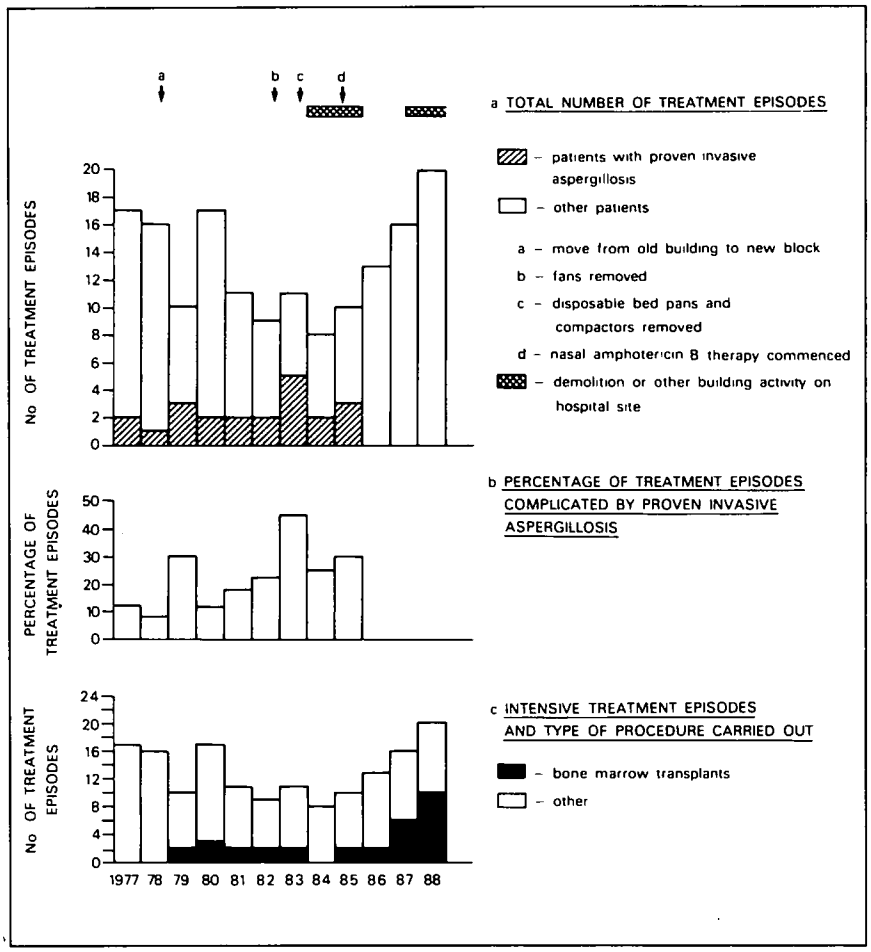

Figure 1. A, total number of treatment episodes. **B,** percentage of treatment episodes complicated by proven invasive aspergillosis. **C,** intensive treatment episodes and type of procedure carried out.

the protocol reported by McGrath *et al* [23]. The 21 allogeneic bone marrow transplants were conditioned with cyclophosphamide/total body irradiation [24] or busulfan/cyclophosphamide [25]. The 14 autologous bone marrow transplants were performed following a number of standard autograft conditioning protocols.

Patients receiving intensive treatment underwent routine cultures as follows: swabs from both nostrils, axillae, groin, and throat were performed on admission and repeated weekly. Specimens of urine, feces, sputum (if possible), and blood via an indwelling central venous catheter and swabs from the venous catheter exit site were cultured on admission and weekly thereafter. More frequent additional cultures were taken when clinically indicated.

Each patient was assigned to one of four groups: (1) no aspergillus colonization or infection, (2) aspergillus colonization only, (3) suspected aspergillus infection, (4) proven invasive aspergillosis. *Colonization only* was defined as positive cultures for *Aspergillus* species from two consecutive specimens from the same site. Single positive cultures from separate sites were not considered to represent colonization. No "colonization only" patients were treated with amphotericin B. *Proven invasive aspergillosis* was established by histologic evidence of hyphal invasion or when ulcerating lesions grew *Aspergillus* species on culture. *Suspected aspergillus infection* included patients with neutropenia, persistent fever, and/or pulmonary infiltrates unresponsive to antibacterial agents in whom histologic confirmation of invasive aspergillosis was not obtainable. All "suspected" cases were treated with amphotericin B.

The Hematology Ward

During 1977 and 1978, patients were nursed in a general hospital ward built in the early part of the 20th century. From 1979, all patients were nursed in a newly built ward in single rooms. Neither ward had any air conditioning. In the old ward, the airflow was largely dictated by open windows and the prevailing wind. The new ward had a ducted air system with a fan producing positive airflow to the center of the building and to two of the single rooms used for the hematology patients. There were also

two types of negative pressure, one for the utility rooms, toilets, and bathrooms and one for the multi-bed rooms. The positive airflow was only filtered by a rough dust filter. Adjacent to the new ward was a building site; periods of demolition and building are shown in **Figure** 1. Disposable bedpans were used from 1979 through 1983.

Microbiologic Techniques and Air Sampling

In response to an apparent increase in aspergillus infection in leukemic patients, a detailed microbiologic environmental survey of the hematology ward was conducted in 1983 to determine possible reservoirs of *Aspergillus* species. Areas were swabbed with sterile cotton swabs and cultured onto blood and Sabouraud's dextrose agar (SDA) (Oxoid) for bacteria and fungi. Settle plates (blood agar) were left exposed for 2 or 3 hours. Air samples were cultured by the aid of a slit air sampler (approximately 0.25 m^3 air) onto blood agar. Solid materials and fluid were cultured directly onto SDA and/or blood agar or filtered and cultured on blood agar. Plates were incubated at 28°C for 1 to 2 weeks for fungal culture. Fungi were identified as *Aspergillus* species, *Mucor* species, or other fungi.

At the beginning of 1987, regular surveillance of aspergillus spore counts was commenced. This sampling continued throughout most of 1987 and January and February 1988. Air sampling was performed using a Pool Bionalyse Italiana surface air system sampler supplied by Medic DDS, New Zealand, as suggested by the Department of Health and Human Services, National Institutes of Health, Bethesda, Maryland (personal communication). This machine was a compromise between efficiency and portability, but the same machine was used for each sample. Dichloran Rose-Bengal chloramphenicol agar (Oxoid) was used to collect the samples; the chloramphenicol supplement inhibits bacteria and the dichloran inhibits Zygomycetes. The duration of each sample was 100 seconds (300 liters of air sampled). Plates were incubated in air at 28°C for 3 days. *Aspergillus* species were identified by their colonial morphology, both by means of the naked eye and by stereomicroscopy. When there was doubt as to whether the fungus was an *Aspergillus* species, microscopy was performed. Initially some isolates were also subcultured onto Czapek-Dox solution agar [26] and colonies were further examined microscopically for typical conidiophore and foot-cell morphology. Twenty-three constant sites, within and outside the old and new hospital blocks,,were sampled on each occasion. Six sites were sampled in the new hematology unit and two in the ward that previously housed the hematology unit. Two other wards were sampled in the new hospital block (eight sites) as well as the main air intake to the new block and three sites outside the buildings.

Other variables were recorded for the day of sampling, including sample order, time of day, amount of movement in sampling area, temperature, windows and doors open or closed, direction in which the sampler was facing, rainfall, humidity, pressure, and wind speed and direction.

Statistical Methods

The data from the aspergillus spore counts were analyzed using single- and multi-factor analysis of variance and, when the environmental effects were incorporated, analysis of covariance using the mean aspergillus counts/m^3.

Measures to Prevent and Treat Infection

Measures to prevent infection were essentially unchanged during the 1979 to 1988 period. All patients were nursed in single rooms except for some patients prior to 1978. Gown and mask isolation with rigorous hand washing was practiced, although the use of masks was abandoned in 1984. Low-bacterial food was given and each patient had his or her own medical equipment (stethoscope, etc.); no flowers were allowed. No attempt was made to decontaminate the skin or vagina. Mouth care consisted of Neosporin mouthwash, amphotericin B lozenges 10 mg five times daily, and nystatin suspension 200,000 units every 6 hours. Prophylactic poorly absorbed antibiotics were given when the neutrophil count fell below 0.5×10^9/L and consisted of framycetin and colistin. Nystatin tablets 500,000 units every 6 hours were also given.

The management of fever was also essentially unchanged during the 12-year period. In the neutropenic patient (neutrophil count less than 0.5×10^9/L), antibiotics were started if a temperature of 38°C or more persisted for more than 2 hours in the absence of any obvious explanation. Gentamicin/cefazolin, gentamicin/ticarcillin, and gentamicin/piperacillin were the antibiotics in use during most of the period. Persistent fever led to the empiric use of amphotericin B intravenously within 3 to 4 days. The policy to institute the early use of amphotericin B was introduced during 1980/1981. Hickman central venous catheters were used from 1979 and nearly all patients had these inserted from that time.

Measures Taken to Reduce Invasive Aspergillus Infection

In 1983, *Aspergillus* species were isolated from the fans used to cool febrile patients and the use of these fans was discontinued (Figure 1A). Dispos-

able bedpans made of papier-mâché were heavily contaminated with *Aspergillus* species. Their use and the use of the bedpan disposal unit were introduced in 1979 and this continued until 1983. It was not known whether the disposable bedpans had always been heavily contaminated with *Aspergillus* species or whether it was a recent occurrence.

The report from Meunier-Carpentier *et al* [27] prompted the use of amphotericin via aerosol in early 1985. Seven milligrams of amphotericin B in 7 mL was placed in a De Vilbiss atomizer (model 251 atomizer - De Vilbiss, Somerset, Pennsylvania) and the aerosolized amphotericin solution was instilled intranasally to each nostril four times daily. On average, 5 mg of amphotericin was administered every 24 hours. No adverse effects have been noted. This treatment was started on admission to the ward and continued until discharge or death. This means that intranasal amphotericin was always started prior to chemotherapy.

RESULTS

Patients

A total of 158 intensive treatment episodes involving 130 patients were reviewed. There were 68 females and 62 males with a median age of 38 years. A 5-year-old girl, who had a bone marrow transplant in the hematology unit, is included but all other patients were aged between 13 and 74 years. Eighty-three patients had acute myeloid leukemia, 36 had acute lymphoblastic leukemia, four had chronic myeloid leukemia, and four had non-Hodgkin's lymphoma. The patients with chronic myeloid leukemia and non-Hodgkin's lymphoma were all undergoing bone marrow transplantation. Three patients with aplastic anemia who were undergoing bone marrow transplantation are included.

Each treatment episode was assessed and assigned to one of the four groups described in Patients and Methods. Eighty-nine (56.3%) treatment episodes were uncomplicated by invasive aspergillus infection or colonization. Aspergillus colonization of nasal mucosa and/or sputum was documented in 31 (19.6%) treatment episodes, but these patients did not develop clinical evidence of fungal infection and no patient received systemic antifungal therapy. Twenty-eight had *Aspergillus* species isolated from routine nasal swabs and the remaining three had positive sputum cultures for *Aspergillus* species. Only one patient in the "suspected" or "proven" groups was colonized prior to the onset of clinical infection. The criteria we adopted for colonization meant that this was often documented only after fairly prolonged neutropenia. By this time, many of the patients in the "proven" or "suspected" groups had developed clinical disease. Sixteen patients (10.1%) had suspected invasive aspergillosis and recovered with amphotericin B treatment and with resolution of neutropenia. Eight of 16 patients with suspected invasive aspergillus infection had positive cultures for *Aspergillus* species. Of these eight patients, an *Aspergillus* species was isolated from sputum alone in three, nasal swabs only in three, bronchial washings in one, and nasal mucosa plus sputum in one. Before the introduction of the amphotericin B nasal spray, colonization with *Aspergillus* species occurred in 41 of 107 treatment episodes; after the introduction, the colonization rate was 14 of 52 treatment episodes, a finding that was not significantly different.

Twenty-two patients (13.9%) had proven invasive aspergillosis and the infection was fatal in 12 of these patients. **Table I** gives details of these 22 patients. Pulmonary involvement was the most common, although ulcerating lesions of the skin and nasal septum were also observed. Six patients had disseminated infection. Only one patient with proven pulmonary aspergillosis recovered with antifungal treatment. Underlying disease activity in the 22 patients with proven aspergillosis was analyzed. Of the 12 fatal cases, only one patient achieved a complete remission at any stage; at the time of death, nine patients had persistent leukemic infiltration of bone marrow and the remaining three patients had hypoplastic marrow. Of the 10 successfully treated patients with invasive infection, eight achieved complete remission, one achieved partial remission, and one transplant recipient had a satisfactory engraftment. Of the 22 patients with confirmed invasive infection, 21 patients had positive cultures from at least one site. An *Aspergillus* species was isolated from sputum in 11 patients, nasal mucosa in 12 patients, skin in six patients, feces in three patients, and throat and mouth in one patient each. Eleven patients had positive isolates from two separate sites, although clinically only six of these patients had disseminated infection. Colonization prior to the onset of clinical infection was documented in only one patient.

Figure 1 shows an annual analysis of the study period. In Figure 1A, the total number of treatment episodes is shown and correlated with the number of proven aspergillus infections. Other relevant information such as ward relocation, elimination of equipment contaminated with *Aspergillus* species, and the initiation of intranasal amphotericin is also shown. In Figure 1B, the annual percentage of treatment episodes complicated by proven aspergillosis is illustrated. In Figure 1C, the gradual increase in the number of bone marrow transplant

TABLE I

Details of Patients with Proven Invasive Aspergillus Infection

Patient Number	Age/Sex	Hematologic Diagnosis	Days Neutropenic Prior to Diagnosis of Infection	Colonization Prior to Clinical Onset of Infection	Site(s) of Infection	Method of Diagnosis	Amphotericin B Treatment Details (days/dose mg)	Outcome
1	58/M	AML	27	No	Lung	Postmortem	43/1,560	Relapse, died
2	54/F	AUL	31	No	Lung	Postmortem	12/460	Relapse, died
3	58/F	AML	24	No	Pericardium/lung	Postmortem	8/255	Relapse, died
4	26/M	AML	11	No	Lung	Postmortem	33/1,125	Relapse, died
5	68/M	AML	29	No	Lung	Postmortem	Not given	Relapse, died
6	68/M	AML	28	No	Lung	Postmortem	9/350	Hypoplasia, died
7	64/M	AML	16	No	Lung/back ulcer	Postmortem	24/915	Relapse, died
8	69/F	AML	17	No	Lung	Postmortem	13/420	Hypoplasia, died
9	56/M	AML	38	No	Lung/brain	Postmortem	10/240	Hypoplasia, died
10	46/F	AML		No	Lung	Postmortem	Not given	Relapse, died
11	49/F	AML	20	No	Pericardium/lung	Postmortem	22/877	Relapse, died
12	26/F	ALL	35	No	Lung	Postmortem	12/380	Relapse, died
13	19/M	ALL	28	No	Skin, muscle, eye	Culture from abscess	38/912	CR with control of fungal infection
14	27/F	ALL/BMT	18	Nostrils	Chest wall	Culture from lesion	7/195	Chest wall lesion resolved, CR
15	66/M	AML	28	No	Ulcers, gastrointestinal tract	Biopsy	Not given	CR but subsequent relapse
16	46/M	ALL	11	No	Chest & back	Culture from lesion	58/410	Infection resolved, CR
17	32/M	AML	26	No	Nasal septum	Biopsy	40/1,695	Infection resolved, CR
18	50/F	AML	18	No	Atrial catheter	Culture from lesion	18/540	PR, infection resolved
19	28/F	ALL	27	No	Nasal septum	Biopsy	Topical/26 days	CR, infection resolved
20	14/F	ALL	12	No	Nasal septum	Culture from lesion	Topical 9 days/ 1,350	CR, infection resolved
21	55/F	AML	23	No	Nasal septum	Biopsy	2/40	CR, infection resolved
22	56/M	AML	29	No	Lung, chest wall	Chest wall biopsy	66/2,620	CR, infection resolved

CR = complete remission; PR = partial remission; AML = acute myeloid leukemia; AUL = acute undifferentiated leukemia; ALL = acute lymphoblastic leukemia; BMT = bone marrow transplant.

procedures is shown. Figures 1A and D document the virtual elimination of proven invasive aspergillosis during the 1986 to 1988 period despite unchanged patient management practices, apart from the introduction of intranasal amphotericin B, and the continuing presence of aspergillus spores in the air. It seems reasonable to conclude that the introduction of intranasal amphotericin led to this decrease in invasive aspergillosis, although the high rate of infection continued through 1985.

Microbiologic Monitoring

The microbiologic and environmental survey undertaken in 1983 found reservoirs of *Aspergillus* species. Doors, walls, ceiling vents, windowsills, trolleys, disposable bedpans, and table fans were found to be contaminated with fungi in patients' rooms and adjacent service areas. Air samples revealed *Aspergillus* species and *Mucor* species in most areas, although samples from two single rooms were free of fungi. The building materials used in the construction of the rooms and ceiling were not contaminated with *Aspergillus* species. The results from the air sampling in 1987 and 1988 for aspergillus spores are shown in **Figure 2.** This illustration demonstrates that counts were very high at the beginning of 1987 and have tended to decrease since that time. However, aspergillus spores have been found in all months sampled. There was no significant increase in spore counts after intensive construction started in 1987.

COMMENTS

Invasive aspergillosis is a significant problem in patients receiving intensive chemotherapy/radiotherapy. A number of factors have been suggested as predisposing these patients to invasive infection. The underlying disease process may suppress the immune response, cytotoxic drugs disrupt normal mucosal barriers and render the patient neutropenic for significant periods of time, steroids diminish inflammatory responses, and antibiotic therapy disrupts normal microbial flora. All these factors increase host susceptibility to infection [4,11,28–30].

In addition, extrinsic factors augment the possibility of opportunistic infections in these patients [31]. Both increases and decreases in the frequency

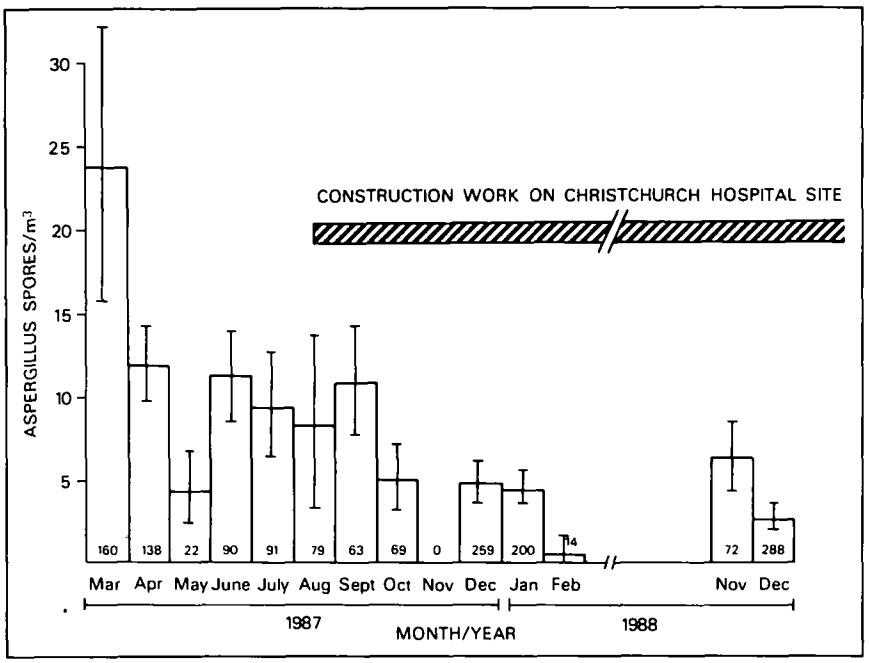

Figure 2. Aspergillus spore counts inside and outside Christchurch Hospital. The numbers of samples are shown at the base of the bars.

of invasive aspergillus infections have been attributed to relocation of patients into new hospital buildings [12,18,32,33]. Outbreaks of invasive aspergillus infections in immunocompromised patients have been linked to contaminated or malfunctioning air filtering systems [13,15,16,33]. Conversely, success in reducing the rate of aspergillus colonization and infection has been attributed to effective mechanical air filtering systems [12,31,33]. Nearby construction activity has been implicated in several outbreaks of invasive aspergillus infection [14,16], and in one report, cellulose-based fireproofing material within the hospital was incriminated [18].

No special treatment was given to the air supply in either the old (1977 to 1978) or new (1979 to 1988) hematology wards. The air supply was served by a rough filter that would not retain aspergillus spores, and *Aspergillus* was isolated from some of the vents with positive air pressure. Air also entered the ward by corridors, stairwells, and windows. There was intermittent building activity on the Christchurch Hospital site during the study period. From April to June 1984, two old wards were demolished on this site and further demolition of adjacent buildings took place in August and September of 1984. In late 1984, construction of a new ward block began and this continued through 1988. Figure 1A compares the timing of the building activity with the incidence of aspergillus infections over the last few years and clearly shows that the number of cases of invasive aspergillus infection has decreased in the face of construction activity and aspergillus spores in the air.

Following the microbiologic survey in 1983, some air vents were sealed in the center of the building. Bedside fans were no longer used to cool febrile neutropenic patients, and reusable bedpans were reintroduced. These changes are displayed in Figure 1A. From early 1985, prophylactic nasal instillation of amphotericin B spray has been given routinely to Christchurch patients undergoing intensive antileukemic treatment. This was prompted by a report by Meunier-Carpentier *et al* [27] who noted a reduction in the incidence of invasive aspergillosis in neutropenic patients receiving such prophylaxis. This group subsequently confirmed these findings in a randomized trial [34]. Colonization and suspected and proven invasive aspergillosis persisted through 1985, but no proven cases have been seen since that time.

Aisner *et al* [35] reported that nasal colonization by *Aspergillus* was an important predictor of subsequent invasive infection. In their study of 11 patients with nasal colonization, 10 later developed invasive aspergillosis. In our series, only one patient had nasal colonization prior to the onset of proven aspergillus infection. However, our criteria for colonization were more stringent than those of Aisner *et al*, who required only one culture to be positive. In our 13 patients with pulmonary aspergillosis, seven had *Aspergillus* species isolated from the nasal mucosa. These results contrast with Aisner's findings. Of these 13 patients with pulmonary infection, nine (69%) had *Aspergillus* species isolated from the sputum. This contrasts with previous reports that have emphasized that such cultures are unreliable in this situation [29,36–39].

Patients in this series with suspected or proven aspergillosis were treated with intravenous amphotericin B in a dose of 0.5 mg/kg/day; none were treated with higher dosage regimens [39,40]. In the early part of the study (1977 to 1980), the use of amphotericin B was delayed in some patients, as seen in Table I. After 1980, amphotericin B was given in febrile neutropenic patients after 3 to 4 days if no response to broad-spectrum antibiotics was observed. We currently use higher doses of amphotericin B as recommended in recent reports [39,40], but none of these patients are included in this report. Granulocyte transfusions were used from 1977 but have not been used since October 1981. Other agents such as flucytosine and rifampicin were not used. Ten patients with proven invasive aspergillosis recovered with amphotericin B treatment. Early suspicion of fungal infection, initial empiric antifungal treatment, and recovery of normal bone marrow function were features of these cases. Only one of these 10 patients had pulmonary disease, but several "suspected" cases of pulmonary infection that improved with amphotericin treatment may have been added to the "proven" group had histologic confirmation been available.

The significant morbidity and mortality associated with aspergillus infection in these patients indicate that prevention of aspergillosis should be the aim. The conventional way of achieving this is to care for the patient in an environment of HEPA (high-efficiency particulate air)-filtered air [12,30]. This is expensive and not easy to introduce as an emergency measure. The practice of instilling intranasal amphotericin B reported by Meunier [34] was adopted by us as a positive measure to try and reduce the incidence of invasive aspergillosis and appears to have been successful.

We have been unable to identify any other significant change in the management of these patients that could have been responsible for the dramatic decrease in invasive aspergillosis. This is even more surprising considering that analysis of the air in the hematology unit and adjacent areas during the 1987 to 1988 period revealed *Aspergillus* species. Despite this, no clinically significant problems due to invasive aspergillosis occurred.

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