Aspergillosis in Four Renal Transplant Recipients

Diagnosis and Effective Treatment with Amphotericin B

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Four patients who had recently received kidney transplants became infected with \textit{Aspergillus fumigatus} while receiving immunosuppressive therapy. Three were shown to have invasive pulmonary mycotic disease, and one of these had documented dissemination. A fourth patient had respiratory symptoms and fever and was found to have mycelial forms consistent with \textit{A. fumigatus} in his sputum, verified by cultures. All four were effectively treated with amphotericin B in low, widely spaced doses. Early diagnosis was apparently the key to successful management of the invasive \textit{Aspergillus fumigatus} infection in these patients.

Discovery of mycelial forms in fresh preparations of sputa or bronchial washings is a valuable clue to active infections. Securing tissue by biopsy is warranted in those patients who develop a pulmonary infiltrate or cavity that is not otherwise causally explained.

\textbf{PULMONARY} and systemic aspergilloses have been common complications in immunosuppressed renal transplant recipients (1-4). The diagnosis has generally been established at autopsy or during the terminal stage, and therapy has been largely ineffective. Rifkind and associates (1) and Finegold, Will, and Murray (5) suggested that early diagnosis would improve therapy. Stinson and colleagues (6) demonstrated the importance of early diagnosis and documented control of aspergillus infections in two of the three cardiac transplant recipients in whom they diagnosed aspergillosis premortally. To emphasize the importance and the method of making an early diagnosis we present four renal transplant patients who became infected with \textit{Aspergillus fumigatus} and describe successful treatment with low, widely spaced doses of amphotericin B.

\textbf{Case Reports}

\textbf{CASE 1}

A 24-year-old man was discharged with a serum creatinine level of 1.6 mg/100 ml on a regimen of prednisone, 30 mg, and azathioprine, 150 mg daily, 10 weeks after renal allograft transplantation, with his sister as the donor. At home he cleaned his neglected pet pigeon’s coop. Within 2 days he developed cough, malaise, and then night sweats and was readmitted within 5 days of discharge. On readmission he was afebrile and had no detectable signs of infection and was believed to have a clear chest X ray. The serum creatinine level had doubled to 3.2 mg/100 ml, and acute allograft rejection was thought to be occurring. The prednisone dose was increased to 200 mg daily.

His dry cough persisted; he developed pleuritic chest pain with a temperature of 38.9 °C [102 °F] rectally. Within a few days he developed whitish sputum that contained only a few leukocytes and a few Gram-positive cocci and from which \textit{Diplococcus pneumoniae} was cultured. His chest X ray now showed a small infiltrate in the right upper lobe (Figure 1A). He was placed on a daily intravenous regimen of 20 million units of penicillin G, for a presumptive diagnosis of bacterial pneumonia. The fever diminished only slightly. His cough worsened and was associated with increasing pleuritic chest pain. Five days after readmission he developed a shaking chill, followed by a temperature spike of 41.1 °C [106 °F] rectally. Eighteen days after readmission a chest X ray (Figure 1B) showed rapid progression of the infiltrate, with cavity formation and the appearance of new disease areas.

Within 7 days he had undergone marked clinical deterioration and was nearly moribund. The only therapeutic hope was to establish a specific diagnosis and...
then initiate specific therapy. An open thoracotomy and lung biopsy were therefore performed in spite of his very critical condition. A fresh-touch preparation of the lung showed branching septate hyphae with conidiophores characteristic of *Aspergillus fumigatus* (Figure 2A). Culture on Sabouraud’s agar confirmed *Aspergillus fumigatus*. Microscopic section of lung showed invasive aspergillosis (Figure 2B).

The previous antibiotic and azathioprine therapy was stopped, and the prednisone dosage was reduced to 15 mg daily. Therapy with amphotericin B, intravenously, was begun; after an initial test dose of 25 mg, the daily dose was 40 mg. This dosage was maintained for 10 days, then arbitrarily changed to 60-mg infusion every other day (1.2 mg/kg body weight).

He remained febrile, and his pulmonary lesions became cavities. Ten days after the establishment of the diagnosis and initiation of treatment, acute endophthalmitis developed in the left eye. Global aspiration showed aspergillosis, and amphotericin B was given intracocularly. The eye worsened, necessitating enucleation within several days; sections and culture showed *A. fumigatus*.

Over the ensuing days he remained febrile and became increasingly confused, without localizing neurological signs. A brain scan showed several abnormalities consistent with brain abscesses. A lumbar puncture was normal except for cerebrospinal fluid protein level of 150 mg/100 ml.

Slowly, he began to improve, and 10 weeks after initiation of amphotericin B therapy he was well enough for discharge. His serum creatinine level was 1.4 mg/100 ml, and immunosuppression was maintained with prednisone, 15 mg daily, and azathioprine, 125 mg daily. Amphotericin B had been administered every other day for 6 weeks. Now, because of clinical and radiological improvement, the dose interval was widened. For 2 weeks he received infusions twice weekly; then a schedule of weekly doses was begun and maintained for 6 weeks. His serum was fungistatic against his strain of *Aspergillus* at a dilution of 1:4, whether tested 1 or 7 days after the infusion. Encouraged by continued improvement and persuaded by the patient’s insistence, the dose interval was lengthened to every 14 days. His serum remained fungistatic for the organism at a dilution of 1:4 when tested 14 days after an infusion of 60 mg amphotericin B.

With a dose interval of 2 weeks his life style was not compromised. With symptomatic resolution and as his pulmonary (Figure 1C) and brain lesions improved, an arbitrary period of 12 months of therapy was decided on. During this year, his total dose of amphotericin B was only 2.5 g. Throughout this period his allograft

**Figure 1.** Serial chest X rays in Case 1. A. Early chest film, showing minimal infiltrate in right upper lobe. B. Chest film taken 13 days after 1A, showing marked progression of the infiltrate, with cavity formation and the appearance of new infiltrates in the right, middle, and lower lobes. C. Chest film taken 6 months after initiation of amphotericin B therapy, showing marked resolution of the lesions.

**Figure 2A.** Fresh touch preparation of lung tissue in Case 1, showing branching septate hyphae and conidiophore characteristic of *Aspergillus fumigatus*. (Polychrome methylene blue; original magnification, × 125.) B. Microscopic section of lung biopsy in Case 1, showing invasive hyphae with focal necrosis. (Periodic acid-Schiff; original magnification, × 50.)
functioned well, with a consistent creatinine clearance of 80 ml/min.

During the 12-month observation period after amphotericin B therapy was discontinued he has remained well and has had no signs of recurrence of the fungal infection.

Comment: In this patient a specific diagnosis of the infection and appropriate treatment came very nearly too late, even though only 9 days elapsed from the first minimal symptoms to the time of diagnosis by biopsy, when he was nearly moribund. Based on the experience with this patient, we anticipated more fully the fulminant nature of aspergillosis in immunosuppressed patients. Therefore, in the subsequent three patients, diagnosis was established much earlier in their course, treatment was initiated earlier, and their clinical course of the aspergillus infection was much milder.

CASE 2

Three months after receiving a cadaver renal allograft a 45-year-old negro man developed coryza, dry cough, and wheezing while at home, receiving 30 mg of prednisone and 200 mg of azathioprine daily. He was afebrile and had a clear chest on examination, but a chest X ray showed a 2-cm ovoid cavity in the right upper lobe (Figure 3). Tomograms showed multiple cavities. Bronchoscopy was normal. Open thorocotomy and lung biopsy were performed within 48 hours of the onset of symptoms. Surgical specimens showed invasive aspergillosis, and Aspergillus fumigatus was confirmed by culture. He was initially treated with daily 30-mg infusions of amphotericin B. After 4 days the dosage was changed to 40 mg intravenously every other day, which was maintained for 6 weeks. Over this period, he had resolution of his symptoms, and radiological improvement was documented. Because of this and because we were satisfied at finding his serum fungistatic against the organism at a 1:2 dilution 7 days after a dose of amphotericin B, weekly infusions were given.

He continued to have evidence of kidney rejection that was unresponsive to prednisone, azathioprine, and radiotherapy. He subsequently has received a second cadaver allograft and experienced two early rejections, both treated with 200 mg/day dosages of prednisone. Amphotericin B therapy was continued through this period as infusions of 30 mg every 7 days for 3 months. His total dose was 1.4 g. He has shown no evidence of recurring infection and is at home, active and feeling well, taking maintenance doses of prednisone and azathioprine 18 months after discontinuing amphotericin B therapy.

Comment: Early diagnosis in this patient limited his illness to one of minimal morbidity. Low-dose, long-term amphotericin B therapy controlled his infection and presumably prevented any exacerbation subsequent to massive immunosuppressive therapy during acute allograft rejection.

CASE 3

A 40-year-old white man received maintenance dosages of 20 mg prednisone daily and 150 mg azathioprine daily after receiving a cadaver renal allograft. While at home, 8 weeks after transplantation, he developed a dry, nonproductive cough and pleuritic chest pain. He was afebrile and his chest was normal on examination but on X ray showed a left upper lobe cavity (Figure 4). Sputum Gram-stain showed branching septate hyphae. Bronchoscopy was negative. Open thorocotomy with wedge resection of the cavity was performed within 48 hours after the onset of symptoms. The surgical specimen showed invasive aspergillosis, and culture showed Aspergillus fumigatus.

He was immediately treated with amphotericin B, 40 mg every other day. His course was complicated, 2 weeks after thorocotomy, by the development of a recurring pseudocyst of the pancreas that required external drainage. Over the next 5 weeks he developed a colonic-cutaneous fistula with enterococcal, then pseudomonas, septicemia. Terminally, he had a massive gastrointestinal hemorrhage.

Autopsy showed that the aspergillosis was limited to a single lung cavity, with no evidence of systemic spread. The kidney showed mild rejection changes but no evi-
dence of amphotericin B toxicity. Over the 30 days of treatment he had received 560 mg of amphotericin B. His chest lesion seemed to be improving radiologically, and his allograft functioned well, with a stable creatinine clearance of 60 ml/min. His death was considered to be secondary to septicemia (pseudomonas and enterocolitis) and gastrointestinal hemorrhage.

Comment: Although this patient's course was fatal, the aspergillosis was effectively controlled in that systemic spread did not occur and radiological resolution of his lung cavity was occurring when he died.

CASE 4
This 36-year-old negro man developed cough, chest pain, and wheezing 30 days after a second cadaver renal allograft, while receiving 30 mg prednisone and 150 mg azathioprine daily.

He was afebrile and had a normal chest X ray and tomograms. Fresh sputum preparations showed many branching septate hyphae (Figure 5), and culture confirmed A. fumigatus. Because of our experience with the three preceding patients, we considered the pulmonary symptoms and the hyphae in the sputum evidence enough in the immunosuppressed patient to begin amphotericin B therapy. He was treated with 695 mg of amphotericin B over the next 14 weeks. His pulmonary symptoms resolved, but he experienced chronic allograft rejection that was unresponsive to high-dose prednisone. The allograft was subsequently removed and showed only chronic rejection, with no evidence of amphotericin B toxicity. He returned to dialysis, and the amphotericin B was withdrawn when immunosuppression was stopped. He had no further pulmonary symptoms. He died 5 weeks later of pancreatitis. Autopsy showed no evidence of residual aspergillosis infection.

Comment: In this immunosuppressed host, branching septate hyphal forms in the sputum were believed to be indicative of fungal germination and active infection.

Diagnosis of Aspergillosis

Fungal infections can be rapidly progressive in the immunosuppressed patient, as witnessed by Case 1 and documented by others (6, 7). Host defences are compromised, and immunosuppressive therapy predisposes to fungal infection (8, 9). Further, use of antibiotics may predispose to mycotic infection (7, 10), although this relationship is less clear (11, 12). The renal transplant recipient is therefore a prime candidate for infection with ubiquitous fungi, such as Aspergillus.

The symptoms and signs of aspergillosis are nonspecific and overlap with other infectious and noninfectious processes. Manifestations include cough, scant sputum, pleuritic chest pain, bronchospasm, and fever, although the temperature may be normal early in the course. Prednisone may camouflage the signs and minimize the symptoms. The radiological picture is not diagnostic and consists of infiltrating lesions with and without cavitation.

Skin tests for A. fumigatus are nonexistent, and serological tests are unreliable and time-consuming and are often falsely negative in patients with invasive aspergillosis (13). An agar gel precipitation test for aspergillosis was done on two of our patients and was negative in both (Case 1 and 2). Blood cultures are not helpful in asperillus infections (7) and were negative in our patients.

With pulmonary symptoms the sputum is the logical place to look to establish the diagnosis. However, finding Aspergillus species on culture of sputa has been thought by some (14, 15) not to be diagnostic of active infection because the organism is so ubiquitous that contamination by airborne spores occurs commonly. Others (7, 11), however, have warned that the presence of Aspergillus on culture should alert one to the possibility of active infection.

English and Henderson (15) suggested that the demonstration of mycelium in the sputum by direct microscopy was indicative of intrapulmonary fungal germination and recommended this method to evaluate the significance of a positive culture. Because of this and the experience with our first three patients, we considered the finding of hyphae, on direct preparation of the sputum, to be diagnostic of active pulmonary A. fumigatus infection in our fourth patient. We had cultured A. fumigatus organisms from each of the first three patients but were not at that time satisfied that this alone was diagnostic, without biopsy confirmation.

The evaluation of fresh sputa is easy (Figure 5A), especially with phase contrast microscopy (Figure 5B). We used no special techniques or expectorants to obtain sputa. A specimen from a deep productive cough was used, and, if cough was nonproductive, bronchoscopy with saline washings was performed. Some (7) have found percutaneous tracheal aspiration a useful method of specimen collection.

The finding of hyphae in the sputum of a patient provides the clue to early diagnosis. Unfortunately the sputum is not always positive, despite active infection (1, 7). Therefore, collection of tissue by percutaneous needle biopsy (6) or by open thoracotomy should be done in the immunosuppressed patient who develops an unexplained pulmonary infiltrate, provided there is no definite contraindication to biopsy.

Amphotericin B Therapy

Amphotericin B is the most effective antibiotic available for invasive aspergillosis (16). The dosage
and frequency of administration have been adjusted to infuse amounts just below systemic toxicity. Using a new approach, Drutz and associates (17) and Goodman and Koenig (18) suggested a dose sufficient to provide peak serum levels at least twice those necessary for inhibition of the infecting organism. This dose, which was usually lower than previously recommended, was then administered arbitrarily over 10 weeks. They found a lower than usual total dose very effective and well tolerated.

In our patients a similar therapeutic approach was attempted. The serum amphotericin B concentrations by the method of Taylor and colleagues (19) did not, however, reach those found by Drutz and associates (17) but were consistent with those reported by Fields, Bates, and Abernathy (20). The concentration of amphotericin B in the serum in our patients was between 0.15 and 0.3 μg/ml regardless of the dose, which was as high as 1.5 mg/kg body weight per day. The serum bioassays were performed 24 to 48 hours after the infusion of amphotericin B in our patients.

Guided by the experience of Drutz and associates (17) and Goodman and Koenig (18), we administered amphotericin B in low doses. Further, we found that widening the dose interval to 1 to 2 weeks, as in Cases 1 and 2, provided effective fungistatic levels and, more importantly, provided continued clinical and radiological improvement. The duration of therapy was arbitrary. Once clinical and radiological control of the aspergillosis had been accomplished, continued therapy depended largely on the necessity of using high doses of prednisone in control of rejection. While the patients were in the hospital we gave 0.5 to 1.5 mg/kg body weight every day for several doses. The interval was then widened to every second or third day. As soon as clinical and radiological improvements occurred, the dose interval was widened to 7 days. With continued improvement, 14-day intervals were used, with a dosage of 1 mg/kg body weight, as described in Case 1. These patients' serums were fungistic against their strain of Aspergillus at dilutions of 1:2 or 1:4 whether the drug was administered every 2 or every 14 days. The drug was well tolerated. It was given on an outpatient basis for many months, and no renal toxicity or other serious side effects (21-23) were observed. In all four patients the aspergillus infections were controlled or cured even though immunosuppressive therapy was continued.

Clearly, more investigation is necessary to define the amount and duration of amphotericin B therapy in aspergillosis and other fungal infection. The metabolic pathways and body distribution of amphotericin B need also to be more clearly defined, since the rapid uptake of colloidal amphotericin would tend to make it less likely that a serum bioassay would be a useful measure of therapeutic effectiveness.

Careful attention should be given to the prevention of fungal infection in immunosuppressed patients. Our first patient presumably acquired aspergillosis from his pet pigeons. Such exposures should not be permitted. Aspergillus fumigatus was isolated from the air ducts in our isolation room, and pigeon excretions were found at the external air inlets. This may, therefore, have been a source of infection. Gage and co-workers (24) found a similar source for aspergillosis in patients after cardiac surgery. Since then our air intake system has been revised so that pigeons cannot reach it. In the year since the re-
vision we have had no aspergillosis in 20 subsequent renal transplant patients.

ACKNOWLEDGMENTS: Presented in part to April 1971, 52nd Annual Session of the American College of Physicians, Denver, Colo.

Received 27 March 1972; revision accepted 7 June 1972.

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