Fungal Infection Following Renal Transplantation

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Twenty-seven deep fungal infections developed in 22 of 171 patients following renal transplantation. These infections included cryptococcosis (ten), nocardiosis (seven), candidiasis (four), aspergillosis (two), phycomycosis (two), chromomycosis (one), and subcutaneous infection with *Phialophora gougeroti* (one). Twelve infections occurred in living-related and ten in cadaveric recipients. Nineteen of the 22 patients were male. Infections occurred from 0 to 61 months after transplantation. Complicating nonfungal infections were present concomitantly in 15 patients. Thirteen patients died, eight probably as a result of fungal infection. Appropriate diagnostic procedures yielded a diagnosis in 20 of 27 infections, and therapy was begun in 18 patients. Serologic, culture, and biopsy procedures useful in making rapid diagnoses are advocated in the hope of increasing survival.

Although renal transplantation has become an accepted means of management of chronic renal failure, the problems of operative complications, rejection, recurrence of original disease, and infection continue to contribute materially to morbidity and mortality. Most infections recognized following transplantation are bacterial, with staphylococci, *Pseudomonas*, and other Gram-negative bacilli being the prime offenders.[1-9] Each reported series, however, includes a substantial number of patients with systemic fungal infection, and in many cases the diagnosis was made late in the course or at postmortem examination.[3,5,6,10] Only one of these groups,[10] however, has described its experience in detail, and antemortem diagnosis and institution of therapy occurred in only two of the 23 patients in that series. We describe all of the patients in our series with systemic fungal infections; the majority of these were diagnosed antemortem, and there were nine survivors after therapy. In addition, we have seen an unusually high incidence of cryptococcosis, and this is described in detail.

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SUBJECTS AND METHODS

A retrospective review was performed of the records of all patients receiving renal transplants from Feb 26, 1965, through May 2, 1973, at Duke Hospital and the Durham Veterans Administration Hospital. All patients were followed up by the nephrology staff in transplant clinics, with long-term outpatient care occasionally managed in cooperation with private physicians. However, all serious problems were handled in our hospitals.

Tests for cryptococcal antibody and antigen were performed by standard techniques.[11] Tissue typing was performed in the transplant laboratory of this medical center as described previously.[12] Patients were divided into three classes: 1 (HL-A identical), 2 (differing at one HL-A allele), and 3 (cadaveric). No living-unrelated donors were used.

All patients received azathioprine or cyclophosphamide, generally 100 mg/day, or as tolerated, and most received corticosteroids in dosages sufficient to prevent acute rejection. A few patients received alternate-day doses of steroids in an attempt to permit normal growth, decrease steroid side-effects, or both. Antilymphocyte globulin was received by some class 2 and 3 patients. Acute rejection episodes were managed with high oral doses of prednisone (200 mg/day), intravenous doses of methylprednisolone (1,000 mg/day), and frequently, local irradiation to the graft.

Criteria for inclusion in this series were as follows: (1) repeated isolation of fungi from patient materials, with clinical disease attributable to the offending agent; (2) single isolation of fungi from patients in whom no other pathogen was isolated; (3) histological demonstration of fungi with or without cultural isolation; and (4) serological identification of cryptococcal antigen in cerebrospinal fluid. Superficial infection, esophagitis, and urinary colonization with *Candida* were not included unless further dissemination was documented. Nocardial infection is included as a "fungal" infection. Cultures and antibiotic sensitivity testing were performed in the microbiology laboratories of our hospitals by means of standard techniques.

RESULTS

Patient Population

One hundred seventy-one patients received 179 renal transplants from 95 living-related and 84 cadaveric do-

Table 1.—Immunosuppressive Agents

Agents	Interval After Transplantation, Days						
	0-9	10-19	20-39	40-89	90-179	180-364	365-730
Azathioprine, mg/day							
Class 1	143 ± 3 (28)	85 ± 7 (27)	84 ± 7 (27)	83 ± 9 (25)	88 ± 8 (25)	90 ± 8 (23)	93 ± 9 (18)
Class 2	92 ± 5 (57)	82 ± 5 (55)	82 ± 5 (50)	88 ± 5 (40)	88 ± 5 (40)	85 ± 5 (33)	82 ± 6 (28)
Class 3	98 ± 4 (72)	69 ± 5 (67)	62 ± 5 (51)	81 ± 6 (32)	83 ± 5 (31)	84 ± 6 (23)	90 ± 8 (13)
Prednisone, mg/day							
Class 1	45 ± 18 (28)*	57 ± 16 (27)*	51 ± 14 (27)	30 ± 6 (26)	19 ± 4 (25)*	13 ± 3 (23)*	10 ± 2 (18)*
Class 2	177 ± 28 (56)	180 ± 25 (55)*	76 ± 10 (50)	45 ± 3 (44)	35 ± 3 (40)*	27 ± 2 (33)	20 ± 2 (28)*
Class 3	123 ± 17 (71)	141 ± 23 (67)*	93 ± 10 (51)	65 ± 5 (41)*	46 ± 2 (31)*	34 ± 2 (23)	29 ± 3 (12)*
Antilymphocyte globulin, ml/patient/interval							
Class 1	0 ± 0 (28)	0 ± 0 (27)	0 ± 0 (27)	0 ± 0 (24)	0 ± 0 (25)	0 ± 0 (23)	0 ± 0 (18)
Class 2	2 ± 1 (57)	2 ± 1 (55)	4 ± 2 (50)	3 ± 3 (44)	6 ± 4 (40)	9 ± 6 (33)	0 ± 0 (28)
Class 3	60 ± 14 (73)*	34 ± 8 (68)*	32 ± 3 (51)*	45 ± 6 (42)*	24 ± 6 (31)*	17 ± 9 (23)	23 ± 16 (13)

*$P < .01$ when compared to next nearest number. Data are expressed as mean ± SE. Highest mean is compared to second highest mean; this in turn is compared to lowest mean. This was done by standard Fisher t test whenever valid (ie, variance within two classes estimated as equal) and using the Welch approximation to t when two classes were found to have unequal variance. Sample sizes for each interval are given in parentheses. These data were compiled on initial 158 of 179 transplants studied in this series.

Table 2.—Renal Function

Variables	Interval After Transplantation, Days						
	0-9	10-19	20-39	40-89	90-179	180-364	365-730
Blood urea nitrogen, mg/100 ml							
Class 1	34 ± 4 (28)	24 ± 2 (27)*	23 ± 2 (27)	20 ± 2 (26)	16 ± 1 (25)*	15 ± 1 (23)*	16 ± 1 (18)
Class 2	44 ± 3 (56)	42 ± 4 (54)*	33 ± 3 (49)	27 ± 2 (44)	22 ± 1 (40)*	22 ± 2 (33)	23 ± 3 (28)
Class 3	82 ± 3 (72)*	79 ± 5 (67)*	47 ± 5 (51)	39 ± 4 (42)*	20 ± 2 (31)*	24 ± 2 (23)	26 ± 3 (13)
Plasma creatinine, mg/100 ml							
Class 1	3.1 ± 0.4 (28)	1.1 ± 0.1 (27)*	1.2 ± 0.1 (27)	1.1 ± 0.1 (26)	1.1 ± 0.1 (25)	1.2 ± 0.1 (23)	1.2 ± 0.1 (18)
Class 2	3.1 ± 0.3 (57)	1.8 ± 0.2 (55)*	1.3 ± 0.1 (50)	1.2 ± 0.1 (44)	1.2 ± 0.1 (40)	1.3 ± 0.1 (33)	1.5 ± 0.1 (28)
Class 3	8.1 ± 0.6 (70)*	4.7 ± 0.4 (67)*	2.3 ± 0.3 (51)*	1.8 ± 0.1 (42)*	1.5 ± 0.1 (31)	1.3 ± 0.1 (23)	1.4 ± 0.2 (13)
Creatinine clearance, ml/min							
Class 1	65 ± 5 (28)*	76 ± 4 (26)*	75 ± 3 (25)	83 ± 4 (24)*	85 ± 4 (24)*	84 ± 5 (22)*	87 ± 5 (17)
Class 2	48 ± 3 (55)*	54 ± 3 (53)*	63 ± 4 (46)	67 ± 3 (41)	64 ± 3 (39)	65 ± 4 (33)	63 ± 4 (28)
Class 3	19 ± 2 (70)*	32 ± 3 (66)*	52 ± 3 (51)	56 ± 3 (42)	60 ± 4 (31)	66 ± 5 (23)	70 ± 6 (13)

*$P < .01$ when compared to next nearest number. Sample sizes are given in parenthses. Table 1 footnote explains statistical analysis.

nors. There were 30 HL-A identical transplants in the living-related group. Dosage of immunosuppressive agents are summarized in Table 1. Statistically significant differences ($P < .01$) were observed, primarily in steroid dosages, between all three groups. Only the class 3 recipients received substantial amounts of antilymphocyte globulin. Renal function data (levels of blood urea nitrogen, plasma creatinine, and creatinine clearance) are presented in Table 2. Better renal function was observed in the class 1 patients. After the initial three months, the class 2 and class 3 patients were similar. The mortality in the entire group was 47%.

Infected Patients

In 22 patients, deep fungal infection developed, ten in the class 3 and 12 in the class 2 groups, for an incidence of 12.7% and 19.7%, respectively. None occurred in the class 1 recipients. The number and kinds of infection, organs involved, and methods of diagnosis are summarized in Tables 3 and 4. Infections included cryptococcosis (ten), nocardiosis (seven), candidiasis (four), pulmonary aspergillosis (two), phycomycosis (two), chromomycosis (one), and subcutaneous infection with *Phialophora gougeroti* (one). Five patients had infection with two organisms: *Cryptococcus* and *Nocardia*, *Cryptococcus* and Chromomycetes, *Candida* and Phycomycetes, *Nocardia* and Phycomycetes, and *Nocardia* and *Candida*, for a total of 27 infections in 22 patients.

Clinical Course

The onset of infection following transplantation was from 0 to 61 months with a mean of 14 months (Table 4). Infection was present in one patient at the time of transplantation and occurred in another one month after transplant nephrectomy. Leukopenia (less than 4,000 cells/cu mm) was present at the onset of infection in only three patients. Levels of renal function and immunosup-

pressive medication of patients with and without fungal infection were analyzed, and no statistically significant differences were observed.

Complicating infections were present in 15 of the 22 patients, in five of nine who survived, and ten of 13 who died (Table 4). Fourteen of these patients had simultaneous bacterial infection, with *Staphylococcus aureus* being the most common agent (six patients). Other organisms included *Escherichia coli* (four), *Klebsiella pneumoniae* (two), *Enterobacter aerogenes* (one), *Salmonella typhimurium* (one), *Bacteroides* species (two), *Streptococcus pneumoniae* (one), *Pseudomonas aeruginosa* (two), and *Mycobacterium kansasii* (one). *Pneumocystis carinii* was demonstrated at autopsy in two patients and suspected clinically in another on whom an autopsy was not performed. In eight patients, bacterial infection obscured and delayed the diagnosis of fungal disease, especially in patients infected with *Nocardia* (patients 11, 13, 14, and 16), *Candida* (patients 14, 17, and 19), and *Phialophora* (patient 20).

Material from the site of infection yielded the responsible organism by culture in 20 of 27 infections occurring in 18 patients; however, the importance of positive cultures on two occasions was not appreciated until nephrectomy (patient 19, *Candida* pyelonephritis with papillary necrosis) or autopsy (patient 22, invasive pulmonary aspergillosis). Antemortem diagnoses were made in two additional patients by Gram stain of sputum (patient 11, pulmonary nocardiosis) and cryptococcal antigen test of cerebrospinal fluid (patient 2, probable cryptococcal meningitis). In all, an antemortem diagnosis of fungal infection was made in 19 patients, and appropriate therapy was begun.

Thirteen patients died, six directly as a result of fungal infection, two of a combination of infectious causes, and five of other complicating illnesses. Postmortem examinations were performed on ten of these 13 patients. A previously undiagnosed fungal infection was identified at five autopsies. Three of these involved the agent causing death (patients 17, 21, and 22) and two were incidental findings (patients 9 and 15). In only two of five patients with two infections were both fungi identified during life.

The incidence of infection in male patients (19 of 119 or 15.6%) was considerably greater than that in female patients (three of 52 or 5.8%), but the difference was not statistically significant.

TYPES OF INFECTION

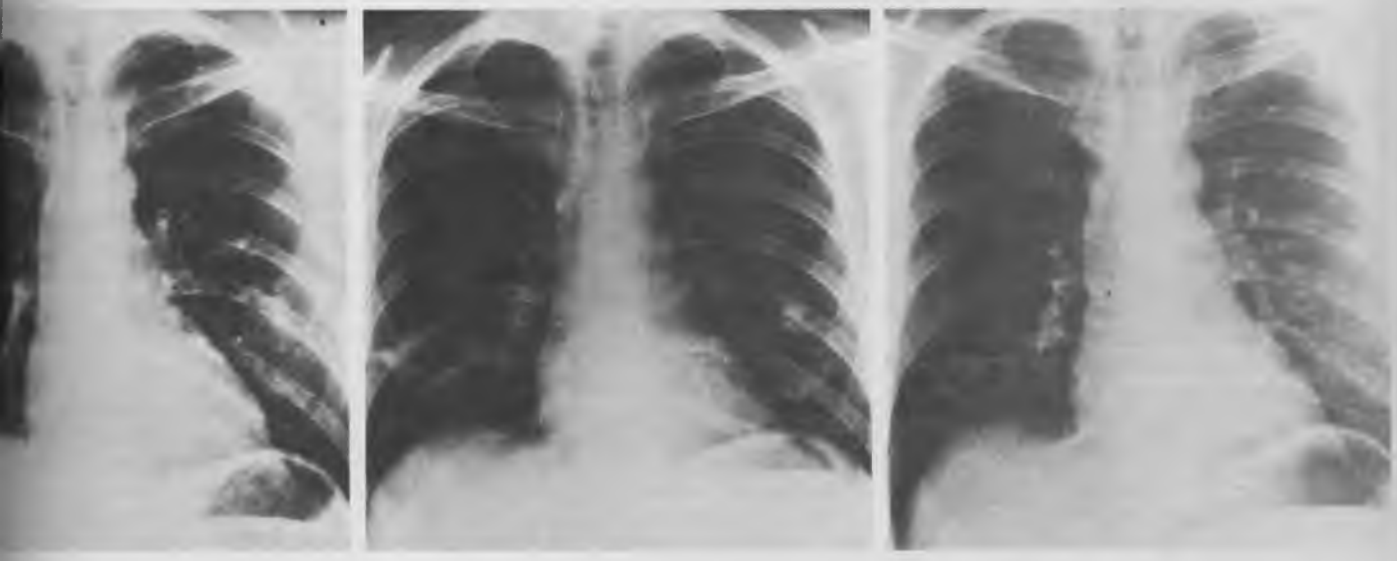
Cryptococcosis

Cryptococcal meningitis was the most common disease entity, occurring in ten of 171 patients for an incidence of 5.8%. The usual course was that of subacute meningitis; however, two patients had a fulminant course and died within three days of hospitalization. The following findings pre-

Table 3.—Types of Infections

Type of Infection	No. of Patients	Deaths	Sites of Infection			
			Central Nervous System	Lung	Skin	Genito-urinary Tract
Cryptococcus	10	6	10	5	1	0
Nocardia	7	3	3	6	1	0
Candida	4	2	0	1	1	3
Aspergillus	2	2	0	2	0	0
Phycomycete	2	2	0	1	0	1
Chromomycete	1	1	0	0	1	0
Phialophora	1	0	0	0	1	0

Fig 1.—Left, Chest roentgenogram of patient 1 before operation showing multiple bilateral nodular densities; center, roentgenogram 11 months after transplantation with progression to cavitation; right, roentgenogram three years after transplantation showing resolution.



dominated: nine patients had headaches, seven had other neurologic or psychiatric disturbance, seven had pulmonary infiltrates (five of which were shown to be cryptococcal), and one patient had a paraspinal abscess secondary to *Cryptococcus* and *S aureus*. Cerebrospinal fluid cultures were positive in nine patients, with the diagnosis made by serologic findings in the other. The cerebrospinal fluid pressure was measured in eight patients and was greater than 20 cm H_2O in six; the glucose value was less than 60 mg/100 ml in eight of ten (range, 20 to 63 mg/100 ml); the protein value was 60 mg/100 ml or greater in ten of ten (range, 60 to 222 mg/100 ml); and the white blood cell (WBC) count was greater than 10/cu mm in eight (range, 3 to 1,400), with greater than 50% lymphocytes in four.

Tests for cryptococcal antigen were performed on cerebrospinal fluid from eight patients and were positive in each case. Complement fixation titers ranged from 1:1 to 1:16 and latex agglutination titers from 1:2 to 1:64. Concomitant sera were positive for antigen by complement fixation in four of five patients (1:4 to 1:256). Cryptococcal antibody was not detected in the sera of six patients tested. One patient who had fever, headaches, and chronic meningitis had the diagnosis of cryptococcal meningitis made on the basis of a cerebrospinal fluid complement fixation titer for antigen of 1:1 and latex agglutination titer of 1:16. He responded to therapy with amphotericin B and flucytosine, with cultures remaining negative throughout his course.

Six of the ten patients died, three within 11 days of diagnosis, while two others received inadequate therapy and died within three months. The remaining patient received 1,500 mg of amphotericin B and died of unknown causes four months after diagnosis. Four patients survived following therapy with amphotericin B, three of these in combination with flucytosine. Two patients, including the patient with the negative culture, had uncomplicated courses and are in remission 12 and 18 months following therapy. The other two survivors had complicated courses that are summarized as follows.

Patient	Age (yr) at Transplantation/ Sex	Class	At Infection Onset: Onset, mo	At Infection Onset: Azathioprine Dosage, mg/day	At Infection Onset: Prednisone Dosage, mg/day
1 D, A	47/F	2	7	100	35
2	21/M	2	3	0	15
3 D	56/M	3	5	100	40
4 D, A	43/M	2	48	85	25
5	46/M	2	16	75	40
6	24/M	2	31	100	25
7	30/M	2	[illegible]	[illegible]	[illegible]
8 D, A	41/F	3	18	50	30
9 D, A	44/M	3	61	100	20
10 D	28/M	3	11	100	45
11 D, A	30/M	3	3	25	40
12	42/M	2	27	100	60
13 D, A	21/M	2	6	100	60
14	18/M	2	8	75	35
15 D, A	47/M	3	5	70	40
16	43/M	3	4	125	50
17 D, A	31/M	3	7	100	35
18	19/F	2	4	75	40
19 D, A	20/M	2	5	†	40
20	37/M	2	33	100	35
21 D, A	49/M	3	3	75	60
22 D, A	46/M	3	16	50	25

PATIENT 7.—A 30-year-old man was first noted to be uremic in June 1970, and four months later, he received a renal transplant from his father. Although he had had a normal chest roentgenogram two months previously, a chest roentgenogram at the time of surgery disclosed bilateral pulmonary infiltrates (Fig 1, left). Sputum cultures and smears were uninformative, and the lesions partially cleared with no specific therapy. Prednisone dosage was tapered from 200 mg/day, and he was discharged on a regimen of azathioprine, 100 mg, and prednisone, 120 mg/day.

One month later, the sputum was reported to be growing *Cr neoformans*, and he was rehospitalized. Spinal fluid examination disclosed a positive India ink preparation but a negative culture and test for cryptococcal antigen. During the following ten weeks, he received 1,726 mg of amphotericin B intravenously. The serum antigen titer was 1:128 initially and 1:64 at the end of therapy. Prednisone dosage was reduced to 30 mg/day and azathioprine dosage to 25 mg alternating with 50 mg/day.

Seven months after transplantation, the chest roentgenogram worsened and cavitation developed. One month later, headaches developed and lumbar puncture showed an elevated pressure with 187 leukocytes/cu mm (57% lymphocytes), protein value of 63 mg/100 ml, glucose level of 36 mg/100 ml, and positive India ink and culture for *Cryptococcus*. The antigen titer was 1:16 by complement fixation. Therapy was begun with oral doses of flucytosine, 4 to 6 gm/day, which produced adequate cerebrospinal fluid and serum levels. The chest roentgenogram and cerebrospinal fluid showed improvement on this regimen, although spinal fluid cultures were intermittently positive and the antigen titer was unchanged.

Nine months after transplantation, pulmonary and meningeal relapse occurred while the patient was still receiving flucytosine (Fig 1, center). He was rehospitalized and received amphotericin B intravenously (2,084 mg) and intrathecally

Table 4.—Clinical Data*

Type of Infection	Sites of Infection: Central Nervous System	Lung	Skin	Genito-urinary Tract	How Initial Diagnosis Was Made	Concomitant Infection
ococcus	+	−	−	−	I, culture of CSF	None
ococcus	?+	−	−	−	Antigen CSF	None
ococcus	+	+	−	−	Culture of sputum, culture of CSF	*S aureus*
cardia	−	+	−	−	Culture of sputum	
ococcus	+	−	−	−	Culture of CSF	*P carinii*
ococcus	+	+	−	−	Culture of CSF, sputum	*M kansasii*
ococcus	+	−	−	−	Culture of CSF	None
ococcus	+	+	−	−	Culture of sputum, I, culture of CSF	None
ococcus	+	+	−	−	Culture of CSF	*K pneumoniae*
ococcus	+	+	−	−	I, culture of CSF	*E coli*
omycetes	−	−	+	−	Autopsy	
ococcus	+	−	+	−	I, culture of CSF, culture of skin	*S aureus, ?P carinii*
cardia	−	+	−	−	Gram stain of sputum	*S aureus,* ?influenza
cardia	?+	+	−	−	Culture of sputum	None
cardia	+	+	−	−	Culture of sputum	*S pneumoniae, P carinii*
cardia	?+	+	+	−	Culture of wound, lung	*Sa typhimurium, S aureus, E coli*
oides	−	−	+	−	Culture of wound	*E aerogenes, Bacteroides*
cardia	−	+	−	−	Culture of sputum	None
comycetes	−	+	−	−	Autopsy	
	−	−	+	−	Gram stain, culture of skin	*S aureus*
cardia	−	+	−	−	Autopsy	*S aureus, E coli*
onica	−	−	−	+	Gram stain, culture of urine	*P aeruginosa, Bacteroides*
onida	−	−	−	+	Culture of urine, nephrectomy	*P aeruginosa, K pneumoniae*
comycetes	−	−	−	+	Nephrectomy	
ylflora	−	−	+	−	Culture of skin	*S aureus*
gillus	−	+	−	−	Autopsy	None
gillus	−	+	−	−	Culture of sputum, autopsy	*E coli*

*D indicates death; A, autopsy; I, India ink procedure; CSF, cerebrospinal fluid; ?, infection presumed.
†Cyclophosphamide (75 mg/100 ml) was given instead of azathioprine.

(7.3 mg) during the next five months as well as an additional one-month course of flucytosine. The cerebrospinal fluid antigen titer fell to 1:2, cultures became negative, and the chest roentgenogram gradually showed progressive improvement (Fig 1, right). During this hospitalization, azathioprine therapy was discontinued, and prednisone dosage was reduced to 10 mg/day. The creatinine clearance declined to 40 ml/min during therapy but later rose to near pretreatment levels of 55 ml/min. The patient returned to full employment and was asymptomatic.

Two years ofter transplantation, nephrotic syndrome developed. Urinary protein excretion rose to 14 gm/24 hr and the creatinine clearance fell to 40 ml/min. Renal biopsy demonstrated membranoproliferative glomerulonephritis, and a tapering course of alternate-day dosages of prednisone was instituted. The creatinine clearance rose to 55 ml/min, and protein excretion fell to 0.5 gm/24 hr. He remains well 28 months after discontinuation of amphotericin B therapy and 42 months after transplantation.

Comment.—This patient underwent transplantation with unrecognized active pulmonary cryptococcosis. Dissemination to the meninges and relapse of pulmonary disease occurred despite amphotericin B therapy. Treatment with approximately 75 mg/kg/day of flucytosine produced a temporary remission with relapse occurring during therapy; however, pulmonary and meningeal infections were apparently cured by a second course of amphotericin B. Despite a total dose of 3,810 mg of amphotericin B, discontinuation of azathioprine therapy, and the development of nephrotic syndrome associated with membranoproliferative glomerulonephritis, creatinine clearance has been well maintained and he is fully rehabilitated.

PATIENT 5.—A 49-year-old man first noted fever, myalgias, and dark urine at age 16 years with a recurrence at age 20 years. He was well until age 45 years when severe myalgias and arthralgias occurred following an upper respiratory tract infection. During the next few months, renal function deteriorated rapidly, requiring dialysis. He subsequently received a renal transplant from his son. The period after transplantation was complicated by ureteral obstruction that required surgical repair. Rejection occurred at six weeks and was treated with prednisone, 200 mg/day, and irradiation of the kidney. The creatinine clearance, which had fallen to 16 ml/min, rose to prerejection levels of 50 to 60 ml/min. Azathioprine therapy was maintained at 75 to 100 mg/day during the ensuing three months.

Five months after transplantation, a cavity developed in the upper lobe of the right lung (Fig 2). *Mycobacterium kansasii* and normal flora were cultured from sputum. The cavity improved transiently on therapy with isoniazid and ethambutol hydrochloride. Three months later, pulmonary emboli occurred and he received anticoagulant therapy. Multiple episodes of pulmonary edema and infarction occurred

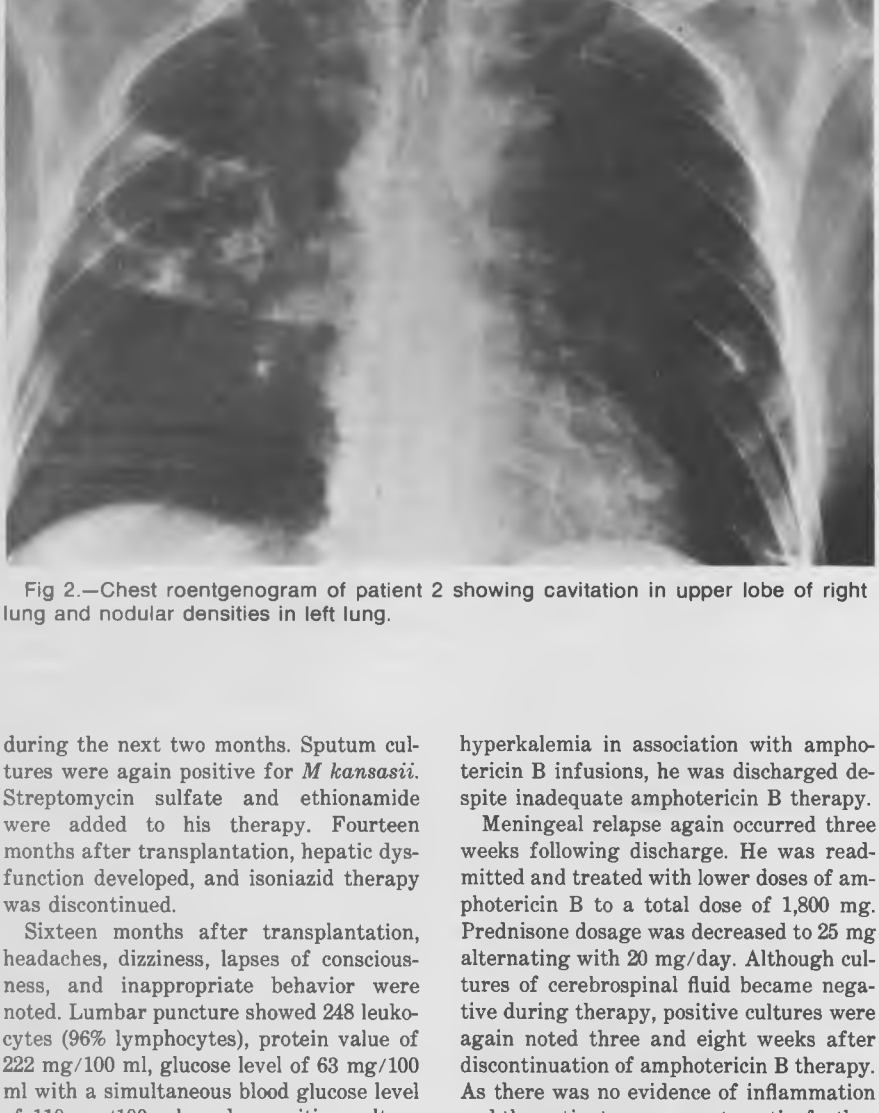


Fig 2.—Chest roentgenogram of patient 2 showing cavitation in upper lobe of right lung and nodular densities in left lung.

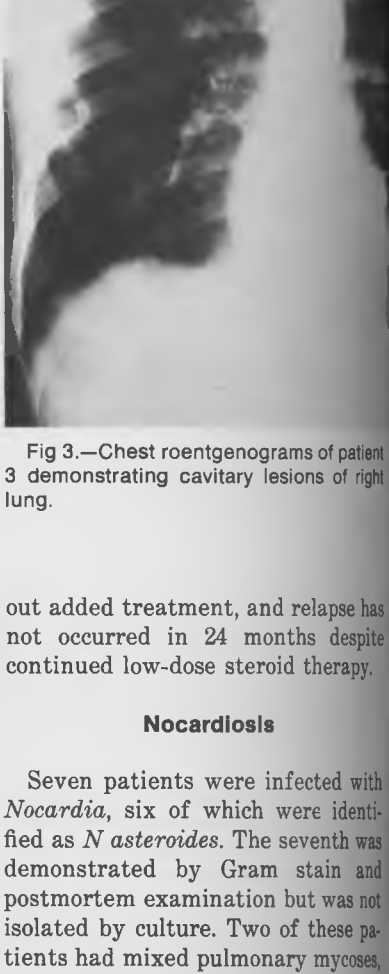


Fig 3.—Chest roentgenograms of patient 3 demonstrating cavitary lesions of right lung.

during the next two months. Sputum cultures were again positive for *M kansasii*. Streptomycin sulfate and ethionamide were added to his therapy. Fourteen months after transplantation, hepatic dysfunction developed, and isoniazid therapy was discontinued.

Sixteen months after transplantation, headaches, dizziness, lapses of consciousness, and inappropriate behavior were noted. Lumbar puncture showed 248 leukocytes (96% lymphocytes), protein value of 222 mg/100 ml, glucose level of 63 mg/100 ml with a simultaneous blood glucose level of 110 mg/100 ml, and a positive culture for *Cr neoformans*. He responded well to a six-week course of flucytosine, 6 gm/day (cerebrospinal fluid levels ranged from 20 to 40 mg/ml). Relapse occurred while receiving therapy, however, and he was treated with intravenous (800 mg) and intrathecal (13 mg) doses of amphotericin B. A pulmonary infiltrate developed during this admission and one sputum culture was positive for *Cr neoformans*. Cerebrospinal fluid cultures remained negative throughout this time. The creatinine clearance fell from 50 to 20 ml/min. Azathioprine therapy was discontinued, and prednisone dosage was reduced to 30 mg/day. Cryptococcal antigen titers in spinal fluid fell from 1:16 to 1:4. After three months of hospitalization, complicated by multiple episodes of ventricular tachycardia, acidosis, and hyperkalemia in association with amphotericin B infusions, he was discharged despite inadequate amphotericin B therapy.

Meningeal relapse again occurred three weeks following discharge. He was readmitted and treated with lower doses of amphotericin B to a total dose of 1,800 mg. Prednisone dosage was decreased to 25 mg alternating with 20 mg/day. Although cultures of cerebrospinal fluid became negative during therapy, positive cultures were again noted three and eight weeks after discontinuation of amphotericin B therapy. As there was no evidence of inflammation and the patient was asymptomatic, further therapy was not instituted and he has remained well for the following 25 months, 52 months after transplantation. Cerebrospinal fluid antigen titers have remained negative as have subsequent cultures.

Comment.—This patient has had a long course of recurrent infection, pulmonary emboli, and numerous complications of steroid and amphotericin B therapy. As with patient 7, meningeal dissemination was present with pulmonary cryptococcosis, initially responded to flucytosine, but relapsed during therapy. Despite a second course of amphotericin B, cultural relapse occurred; however, cultures subsequently became negative, without added treatment, and relapse has not occurred in 24 months despite continued low-dose steroid therapy.

Nocardiosis

Seven patients were infected with *Nocardia*, six of which were identified as *N asteroides*. The seventh was demonstrated by Gram stain and postmortem examination but was not isolated by culture. Two of these patients had mixed pulmonary mycoses, one having pulmonary cryptococcosis and meningitis and the other pulmonary phycomycosis. Six patients had pulmonary infiltrates, five of which progressed to cavitation. Pleural effusion was present in two of these patients. The seventh patient had fever and pulmonary infiltrates from which no pathogens were isolated. One month later, a paraspinal abscess developed, from which *N asteroides* and *S aureus* were isolated.

Three patients had symptoms referable to the central nervous system. Two had alterations of consciousness; one had multiple nocardial brain abscesses at autopsy while the other had abnormal cerebrospinal fluid (glucose

level, 35 mg/100 ml; protein value, 108 mg/100 ml; WBC count, 500/cu mm with 95% neutrophils and negative stains and cultures), which resolved following therapy with trisulfapyrimidines, ampicillin, and gentamicin sulfate. The third patient developed a hemiparesis with abnormal brain scan that resolved with multiple antibiotics including ampicillin and trisulfapyrimidines.

In general, the response to antibiotics was good. Four of the seven patients received adequate therapy with probable cure. Three of these four received therapy with penicillins or ampicillin in addition to sulfonamides. One died of other causes with no evidence of *Nocardia* at postmortem examination. Two patients died within seven days without having received adequate therapy. Another received no therapy for *Nocardia* and died of unknown cause following amphotericin B therapy for cryptococcal meningitis. This patient had a pulmonary abscess and his sputum grew *S aureus, Cr neoformans,* and *N asteroides.* The lesion cleared with isoniazid, amphotericin B, and oxacillin therapy. *Nocardia* was accompanied by other pathogens in six individuals, with delay in diagnosis in three. *Staphylococcus aureus* was the most common and was present in four patients. Open lung biopsy was required for diagnosis in one of the patients who survived.

PATIENT 14.—A 20-year-old man developed acute glomerulonephritis with nephrotic syndrome at age 16 years and progressed to renal failure. Cadaveric transplantation was performed, but rejection ensued and the graft was removed after two months. Five months later, he received a renal transplant from his sister. Seven months after transplantation, a large left flank abscess developed and *E coli, En aerogenes,* and *Sa typhimurium* were cultured. The blood also grew *Sa typhimurium.* Fungal cultures were not obtained. He was treated with cephalothin sodium and gentamicin sulfate for 14 days and discharged on a regimen of ampicillin. A large abscess in the lower lobe of the right lung had been noted, but no causative agent was determined.

He was readmitted one month later with fever, headache, dizziness, and nonproductive cough. Medications on admission were azathioprine (75 mg/day) and prednisone (35 mg/day). The temperature was 39 C (102.2 F), oral thrush was noted, a continuous murmur was present over the third left intercostal space, and purulent material was draining from the abdominal wound. The WBC count was 10,000/cu mm with 88% segmented neutrophils. The chest roentgenogram (Fig 3) showed several abscesses in the right lung. A urine culture grew *Pseudomonas aeruginosa.* The flank abscess grew *E coli, S aureus, Sa typhimurium, B oralis* and *melaninogenicus, Fusobacterium* species, *N asteroides,* and *Candida* species. A right thoracotomy disclosed an abscess that grew *Sa typhimurium* and *N asteroides.* Therapy was initiated with gentamicin sulfate, clindamycin, ampicillin trisulfapyrimidines, and nystatin, orally. Azathioprine therapy was discontinued. Fecal drainage was noted in the flank abscess and a diverting colostomy was performed.

A mild right hemiparesis developed, and a brain scan disclosed increased tracer uptake in the left occipital region. Lumbar puncture, electroencephalogram, and brachial arteriogram were normal. Fever persisted despite the addition of methicillin sodium. Subsequently, all antibiotic therapies, except for ampicillin and sulfa, were discontinued, and he gradually became afebrile. Although sulfonamide levels ranged from only 3.3 to 4.4 mg/100 ml, his pulmonary and neurologic status improved. He gained 9 kg (20 lb) and was discharged after three months in the hospital. Sulfonamide levels rose to 15 to 20 mg/100 ml, and he was maintained on therapy for nine months. Azathioprine therapy was restarted three months later and he has returned to work.

Comment.—This patient demonstrates many of the complexities of disseminated nocardial infection. He is typical of many of our patients in that other pathogens were present and initially obscured the diagnosis of nocardiosis. This underscores the necessity of obtaining fungal cultures in all transplant patients with suppurative infection. Central nervous system involvement was presumed on the basis of neurological abnormalities and abnormal brain scan, both of which improved during therapy. In addition, he represents the only instance in this series of patients in which diagnostic thoracotomy was performed. Because of this experience and that of others, we have been more aggressive in the diagnosis of pulmonary disease of unknown origin, especially by fiberoptic bronchoscopy and thoracotomy.

Candidiasis

Four patients had visceral candidiasis, and two died. Patient 17 had severe tracheobronchitis and bronchopneumonia from which *Candida* was not recovered antemortem. Patient 19 had renal papillary necrosis and a small focus of phycomyosis, both discovered only at transplant nephrectomy, although on one occasion prior to surgery, a urine culture had disclosed 50,000 *Candida* organisms per milliliter. Patient 18 had severe cystitis complicating a vesicocutaneous fistula and long-term Foley catheter drainage. Yeast and pseudomycelia were noted in the urine, which yielded *Candida* on culture, and fever responded to bladder irrigation with amphotericin B. Patient 14 had a flank abscess that grew *Candida* species from two cultures in association with *Nocardia* and multiple other pathogens. This resolved following colostomy, drainage, and antibacterial therapy. Many patients had mucosal lesions and transient urinary colonization, but they were not included in this group.

Aspergillosis

Two patients had pulmonary aspergillosis. Both had diffuse involvement of pulmonary parenchyma with invasion and thrombosis of blood vessels noted at autopsy. In one case (patient 21), *Aspergillus* was not cultured from sputum, and the diagnosis was made at autopsy following death from acute myocardial infarction and pneumonia. The other infection developed in the setting of chronic berylliosis manifested by bronchiectasis with broncholithiasis and hemoptysis (patient 22). Eight sputum cultures were positive for *A flavus* in a one-year period. Specific therapy was not given, as the organism was believed to be a saprophyte colonizing a diseased bronchial tree, although, during this interval a complement fixation titer of 1:8 to *A flavus* was demonstrated. Seventeen months after transplantation, pneumonia and fatal pulmonary invasion occurred, with

negative sputum cultures immediately prior to death.

Phialaphora

Patient 20 developed extensive subcutaneous abscesses of the right forearm that grew *S aureus*. These lesions responded poorly to therapy with oxacillin and recurred intermittently in a 24-month period, progressing to multiple draining sinuses with fluctuant nodules. A culture for fungus yielded *P gougeroti*. Initial therapy consisted of incision and drainage, topical and intravenous doses of amphotericin B, and oral doses of iodide. After 275 mg of amphotericin B was given intravenously, this and the iodide therapy were discontinued due to patient intolerance. The arm worsened through a five-month period, until therapy with flucytosine was begun with continuation of topical applications of amphotericin B. In vitro sensitivity to flucytosine showed a minimal inhibitory concentration of 0.037 mg/ml. After ten weeks of therapy, the lesions regressed, and the arm remained healed 27 months later.

Miscellaneous Infections

Two had minimal renal (patient 19) and pulmonary (patient 15) phycomycosis and one had cutaneous chromomycosis (patient 9). These lesions were discovered at transplant nephrectomy or postmortem examination and were apparently of little clinical consequence.

COMMENT

Recipients of renal transplants are subjected to a variety of alterations in anatomy and physiology that increase susceptibility to infections. These include disruption of normal cutaneous barriers; cytotoxic, steroid, and antibiotic therapy; and renal failure, acidosis, and catabolism associated with failure of transplant function. These factors result in abnormal phagocytic function, depressed humoral and cellular immunity, and susceptibility to pathogenic and opportunistic organisms. These and other circumstances predisposing to infection have been reviewed by others.[13-16]

The patients in this series were primarily infected by opportunistic fungi.[17] In no instance was there a case of reactivation of so-called primary pathogenic fungi, eg, *Histoplasma* or *Blastomyces*. In addition, there have been no cases of tuberculosis, with the exception of a concomitant *M kansasii* infection in one of the patients with cryptococcosis. Our patients are not routinely treated with prophylactic doses of isoniazid. The low incidence of tuberculosis has been commented on by others[10,18] and perhaps is related to a decreasing incidence of tuberculosis in the general population.[19]

Cases reported by other transplant groups have been consistent with ours with one major exception, an unusually high incidence of cryptococcosis in this series. In a review of 17 reports, we could identify only eight cases of cryptococcal infection in more than 950 transplants.[3,5-10,20-29] However, these figures probably underestimate the true incidence, as only fatal cases were reported in some series. The epidemiology of cryptococcosis in North Carolina has not been investigated, but cryptococcal infections are not uncommon in our hospitals. Also, the incidence of candidiasis in our series was low; however, this may partially reflect the exclusion of several cases of esophagitis. Many patients received oral or vaginal applications of nystatin (or both) as soon as mucocutaneous lesions were identified. In addition, we generally use mycostatin prophylactically when broad spectrum antibiotics are given.[30]

Patients were analyzed with respect to renal function and immunosuppressive therapy in an attempt to identify risk factors. In general, the closer the transplant match, the better was the renal function and the lower the immunosuppressive dosages. No major differences were found between infected patients and other members of their respective groups; however, in many instances these groups were small. No fungal infections occurred among patients receiving HL-A identical transplants. This most likely reflects lower prednisone doses coupled with excellent renal function and fewer episodes of rejection. In a recent series,[31] a review of 194 renal transplant recipients showed that leukopenia, hyperglycemia, and moderate to severe renal failure were the most consistent findings present among patients with fatal infections. In addition, the use of high doses of immunosuppressive agents was more common in infected patients. Leukopenia at the onset of infection was present in only three of our patients, and only one of these died.

This series again confirms the propensity of male patients for developing fungal infection. The incidence in infected males was two to three times that expected from the total male-to-female ratio. This finding has been commented on by others,[10,17] and it would seem unlikely to be related to occupational hazards. Hormonal differences have been suggested as factors, but this has been difficult to evaluate.[10]

Cultures of sputum, cerebrospinal fluid, and other body fluids were generally helpful in making diagnoses. The organism was isolated, seen on smears, or detected by tests for antigen, in 20 of 27 infections (73%). Thoracotomy with lung biopsy was utilized on one occasion to diagnose nocardial infection. In only two patients with severe fungal infection was there no demonstration of organisms prior to death (pulmonary aspergillosis and pulmonary candidiasis). In general, phycomycetes and *Aspergillus* may be difficult to isolate from tissues, blood, and sputum, many times requiring biopsy with subsequent culture, histologic examination, or both.[17]

Serologic procedures in the diagnosis of aspergillosis,[32] candidiasis, cryptococcosis, and nocardiosis are currently under investigation by many groups, especially those involved in chemotherapeutic measures for cancer. While precipitins are commonly positive in patients with aspergillomas or bronchopulmonary aspergillosis, controversy exists about their presence or absence in invasive disease, especially in the patient who has had immunosuppressive therapy.[33] *Candida* precipitins, on the other hand, have been demonstrated to be

of value in diagnosing invasive or disseminated candidiasis.[38-40] Cryptococcosis has lent itself best to the detection of circulating fungal antigens, eg, capsular polysaccharide.[11] The presence of cryptococcal antigen in the cerebrospinal fluid may precede the visualization of organisms or positive cultures, allowing the earlier initiation of effective chemotherapy.[41] We had one such patient with positive antigen and negative cultures and subsequent response to amphotericin B and flucytosine. Antibodies to *Cryptococcus* were not detected in six of our patients during their infections. It is possible that antibody might appear following the disappearance of antigenemia, similar to the sequence of events seen in pneumococcal infection.[42] Indirect fluorescent antibody and radioimmunoprecipitin tests have been examined in nocardiosis, but clinical usefulness has not been demonstrated.[43,44] These tests as well as early use of procedures such as fiberoptic bronchoscopy,[45] transbronchial or percutaneous lung biopsy, and thoracotomy[46] may hasten diagnosis in patients with pulmonary disease.

Complicating infections were present in a majority of our patients. Severe bacterial infection accounted for or contributed to death at least in five, and perhaps two additional patients. Concomitant pyogenic infections were especially frequent in association with nocardiosis. The finding of obvious pathogens such as *S aureus* on Gram stains and routine cultures many times obscured the presence of *Nocardia*. In this series, however, fungal cultures, Gram stains, or acid-fast stains helped make an antemortem diagnosis in all seven patients. *Pneumocystis carinii* was demonstrated in two patients at autopsy and was suspected clinically in another.

Therapeutic agents used in our patients were amphotericin B, flucytosine, sulfonamides, and ampicillin, in combination with other antibacterial agents.[47-51] Amphotericin B was successful in eradicating cryptococcal infection in four of ten patients; three of these also received flucytosine. Two of the latter had relapses with flucytosine-resistant organisms; however, a cure was ultimately effected with amphotericin B. Interestingly, a cultural relapse occurred in one of these patients following the second course of amphotericin B. However, cultures spontaneously became negative, and clinical and cultural remissions have been present for 24 months in association with reduced immunosuppressive therapy. All four patients in whom nocardiosis was eradicated received trisulfapyridine therapy. In addition, two of these patients received ampicillin and one received penicillin G potassium. Recent reports have suggested that sulfonamides in combination with ampicillin, erythromycin, or minocycline hydrochloride are most effective.[52-54] We did not have occasion to treat aspergillosis in these patients; however, amphotericin B and flucytosine may be beneficial.[55,56] Preliminary reports have shown that flucytosine is a valuable agent in the therapy of infection due to various *Phialophora* and *Cladosporium* species.[50] This was certainly borne out by in vitro sensitivity and clinical response in our patient with infection due to *P gougeroti*. One patient with severe *Candida* cystitis responded to local bladder irrigation with amphotericin B without systemic therapy. This may be an important therapeutic modality in patients who require long-term catheter drainage for whatever reason.[57]

Reduction or discontinuation of immunosuppressive therapies was necessary on many occasions. We have seen few serious acute rejection episodes in association with discontinuation of azathioprine therapy; however, the nephrotic syndrome developed in two patients, one of whom was known to be nephrotic prior to transplantation.[58,59] One of these patients, who had membranoproliferative glomerulonephritis, responded well to alternate-day steroid therapy and has had no subsequent loss of renal function. Most of our patients, however, were maintained on corticosteroid regimens during therapy. Occasionally, it may be necessary to discontinue all immunosuppression in order to effect a cure of various infectious processes, especially those caused by fungi. Many times this will lead to failure of graft function, necessitating support by dialysis and subsequent retransplantation. Clearly, many of these problems may be solved in the future by more effective antifungal agents, better tissue matching, and more specific methods of immunosuppression.

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Nonproprietary Names and Trademarks of Drugs

Amphotericin B—*Fungizone.*
Azathioprine—*Imuran.*
Cephalothin sodium—*Keflin.*
Clindamycin—*Cleocin.*
Ethambutol hydrochloride—*Myambutol.*
Ethionamide—*Trecator-SC.*
Flucytosine—*Ancobon.*
Gentamicin sulfate—*Garamycin.*
Minocycline hydrochloride—*Minocin.*

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