

Systemic Fungal Infections after Renal Transplantation

MEHMET RIZA ALTIPARMAK¹, SUHEYLA APAYDIN¹, SINAN TRABLUS¹,
KAMIL SERDENGECTI¹, REZZAN ATAMAN¹, RECEP OZTURK² and EKREM EREK¹

From the Departments of ¹Internal Medicine, Division of Nephrology and ²Clinical Bacteriology and Infectious Disease, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey

In a retrospective evaluation, the incidence of systemic fungal infections (SFIs) in 296 kidney graft recipients admitted to our center between 1986 and 1999 was found to be 4%. Eighteen percent of 28 recipients transplanted in India and 8% of 12 recipients transplanted in Russia developed SFI. In contrast, SFI was encountered in only 2% of recipients transplanted at our center. The median time of diagnosis of SFI was 5 months after transplantation. The lungs and central nervous system were the most frequently affected sites. The most common etiologic agent was *Aspergillus fumigatus* ($n=7$) but *Candida* spp. ($n=1$), *Rhizopus* spp. ($n=1$) and *Cryptococcus neoformans* ($n=1$) were also encountered. In 2 patients, 2 different pathogens were isolated at the same time: *A. fumigatus* and *Rhizopus* spp. in 1 patient and *Candida* spp. and *A. fumigatus* in another. In order to determine predisposing factors for SFI, patients admitted immediately before and after those with SFI were used as controls: long-term hospitalization, long-term antibiotic use and post-transplant diabetes mellitus were found to be predisposing factors. Eight patients were treated with antifungal drugs and a good response to liposomal amphotericin B therapy was obtained in 3/5. Nine patients (75%) with SFI died. As SFIs are associated with a high mortality rate in renal transplant recipients, antifungal therapy, especially with liposomal amphotericin B, should be started whenever fungal infection is suspected, even before the results of microbiologic and/or histologic examinations are known.

Suheyila Apaydin, Turan Güneş Bulvarı, Denizatı Kent sitesi A1, Blok Daire 27, TR-34770 Zeytinburnu, Istanbul, Turkey.
E-mail: suheyilaapaydin@yahoo.com

INTRODUCTION

Systemic infectious diseases continue to be a major cause of morbidity in renal transplant units. As a consequence of immunosuppressive therapy, renal transplant recipients are prone to opportunistic infection by bacteria, fungi, viruses and protozoa. Systemic fungal infections (SFIs) following renal transplantation are associated with high morbidity and mortality rates despite their lower incidence compared to viral and bacterial infections (1). The incidence of fungal infections after renal transplantation is reported to range from 1% to 14% depending on the hygiene and sanitation conditions of the transplantation center. Although all other types of fungal infection have been reported, those involving *Candida* spp., *Aspergillus* spp. and *Cryptococcus neoformans* are the most frequently encountered (1, 2). *Candida* spp. mainly present as fungemia related to catheter or urinary tract infections, *Aspergillus* infection primarily affects the lungs and central nervous system (CNS) and *C. neoformans* mainly affects the CNS (1–3).

The major risk factors reported to predispose towards fungal infections in renal transplant recipients are increased age, anti-rejection therapy (pulse steroids, antilymphocytic globulins), prolonged antibiotic treatment, diabetes mellitus, CMV disease, concomitant bacterial infections and neutropenia (1–3).

Delay in the diagnosis of SFI and/or late institution of therapy results in a high mortality (1). Amphotericin B is still the first-line drug for the treatment of SFI (1, 2) but imidazole compounds may be used as alternative agents to treat infections caused by *Candida* spp. and *C. neoformans* (1).

We report herein our experience with SFIs in renal graft recipients who were transplanted and/or followed in our transplantation unit between 1986 and 1999. We aimed to elucidate the incidence, clinical patterns and outcomes of SFIs, as well as determining the risk factors involved in their development.

MATERIALS AND METHODS

A total of 296 renal recipients (229 living, 67 cadaveric donors) transplanted and/or followed in our unit between 1986 and 1999 were retrospectively evaluated. Twenty-eight recipients underwent renal transplantation from living unrelated donors in 3 different centers in India and were admitted to our unit between 4 and 21 d post-transplantation. Twelve recipients were transplanted from cadaveric donors in different centers in Russia. The female:male ratio of the overall sample was 98:198 and the median age was 29 y (range 16–59 y). A total of 211 recipients received triple therapy with prednisolone, azathioprine and cyclosporin A. Eighty-five recipients received double therapy with prednisolone and azathioprine. Eleven cadaveric graft recipients also received antithymocytic globulins for induction therapy. In addition, trimethoprim-sulfamethoxazole (80/400 mg/d), nystatin (4 MU/d) and acyclovir (3 × 600 mg/d) were used in all recipients for prophylaxis. Fifty-five patients died during the period 1986–99 and 39 of these deaths (71%) were caused by infectious diseases.

Fungal infections encountered in these recipients were evaluated on the basis of their frequency, clinical presentation, microbiologic and histopathologic findings, clinical course and response to antifungal therapy. The presence and frequency of predisposing factors were also determined in these patients. In our recipients, a diagnosis of SFI was made in the presence of 1 of the following 4 criteria (4):

1. Histopathologic evidence of tissue invasion on tissue biopsy and/or autopsy;

2. Positive culture from a deep tissue specimen such as blood, cerebrospinal fluid (CSF), peritoneal fluid or biopsy specimen;
3. Positive cultures from ≥ 3 sites (BAL, urine, wound, etc.); and
4. Positive sputum smear for pseudohyphae and budding yeast and/or positive sputum culture with clinical findings consistent with pneumonitis resistant to broad-spectrum antibiotics.

Diagnostic tests for fungal infection used in our patients included examination of sputum ($n = 8$), BAL fluid ($n = 5$) and CSF ($n = 1$), transbronchial biopsy ($n = 2$), abscess material smears and cultures ($n = 1$), bone biopsy and culture ($n = 1$) and lymph node biopsy ($n = 1$). For all specimens a direct microscopic examination was performed using KOH preparation, Gram stain, Giemsa stain and India ink preparation (CSF only). After processing the material, the appropriate culture medium (Sabouraud's dextrose agar or brain–heart infusion agar) was used according to the source of the specimen.

In order to determine predisposing factors for SFI, patients admitted immediately before and after those with SFI were used as controls. Age, gender, donor source, creatinine level, hospitalization time, leukocyte count, pulse steroid treatment, antibiotic use and duration, CMV disease and diabetes mellitus were considered as probable predisposing factors. Specific antifungal therapy was begun whenever fungal infection was suspected or diagnosed. The type of antifungal therapy and outcome were also evaluated. A total of 11 variables representing predisposing factors for fungal infection were subjected to Fisher's exact test for statistical analysis.

RESULTS

SFIs developed in only 12/296 (4%) renal graft recipients (5 females, 7 males; mean age 40 ± 11 y). All 12 patients were receiving triple therapy with prednisolone, azathioprine and cyclosporin A and none of them were receiving antilymphocytic therapy. Five of these patients had been transplanted from unrelated living donors in India and 1 had been transplanted from a cadaveric donor in Russia. The other recipients (1 from a living donor and 5 from cadaveric donors) had been transplanted in our unit. It is of interest that 17.9% of 28 recipients transplanted in India and 8.3% of 12 recipients transplanted in Russia developed SFI, whereas it was encountered in only 2.3% of recipients transplanted in our center.

The features of patients with SFI are shown in Table I. The median time of diagnosis was 5 months following transplantation (range: 1–15 months). Eleven patients were diagnosed within the first post-transplant year. The most common symptoms were antibiotic-unresponsive prolonged fever ($n = 10$), cough and sputum production ($n = 8$) and dyspnea ($n = 8$). Eight patients had pathologic findings on their chest X-rays. In addition, a fungus ball was observed in 1 patient on pulmonary CT. Bacterial, protozoal and viral infections were detected in 8 patients before diagnosis of the fungal infection (Table I). No clinical response was obtained upon treatment with specific agents and therefore SFIs were suspected in these patients.

The lungs were the most common site of involvement ($n = 9$). Meningitis ($n = 1$), cerebral abscess ($n = 1$) and nasofacial involvement ($n = 1$) were also observed. In 3

patients with lung involvement, CNS dissemination had also developed. Hyphae and yeast were seen in sputum and/or BAL smear in 6 patients. Fungi were grown in the appropriate culture medium in samples obtained from sputum ($n = 2$), BAL ($n = 3$), CSF ($n = 1$), abscess material ($n = 1$) and bone specimens ($n = 1$) of different patients. In 2 patients, typical fungal osteomyelitis in maxillary bone biopsy (hyphae without septae and focal giant cells) and granuloma formation without necrosis surrounded by giant cells in transbronchial biopsy, respectively were considered as histopathological evidence of SFI. In 3 patients, diagnosis was first made at autopsy. In the other 3 patients, autopsy confirmed the clinical and laboratory diagnosis of fungal infection.

The most frequently encountered etiologic agent was *Aspergillus fumigatus* ($n = 7$), but *Candida* spp. ($n = 1$), *Rhizopus* spp. ($n = 1$) and *C. neoformans* ($n = 1$) were also observed. In 2 patients, 2 different pathogens were isolated at the same time (*A. fumigatus* and *Rhizopus* spp. in 1 and *Candida* spp. and *A. fumigatus* in the other).

Increased age ($p = 0.01$), long-term hospitalization ($p = 0.03$), post-transplant diabetes mellitus (PTDM) ($p = 0.01$) and long-term antibiotic use ($p = 0.01$) were found to be predisposing factors for SFI (Table II).

Eight patients were treated with antifungal drugs. Three patients treated with oral itraconazole were unresponsive and died. Liposomal amphotericin B was used in 5 patients (starting dose 1–2 mg/kg): in 3, a good response was achieved and no drug toxicity was noted but 2 others died. No antifungal therapy was given to the 3 patients who were first diagnosed at autopsy.

Because of the severity of the fungal infections, we had to reduce immunosuppressive doses in 5 patients. Management of fungal infections also included aggressive nutritional and metabolic support, treatment of superinfection and surgical debridement when indicated. Nine patients with SFIs (75%) died. Fungal infection was responsible from 16% of all deaths and for 23% of deaths due to infection encountered at our center between 1986 and 1999.

DISCUSSION

In developed countries the overall incidence of SFI following renal transplantation has been reported to have significantly decreased (to 1–2%) during the last decade (5, 6). However, in developing countries post-transplant SFI continues to have a high incidence rate (11–14%). Chugh et al. (2) reported a high incidence (14%) of SFI after renal transplantation in India and suggested that this finding might be related to poor hygiene and sanitation. Our series reveals a slightly higher incidence rate (4%) than those reported from other developed countries and this was mainly due to some of our recipients having been transplanted in India and Russia. When these recipients were excluded from the group, the incidence rate of fungal

Table I. Features of patients with SFIs

Patient No. (year)	Age (y) Sex	Site	Pathogen	Concomitant infection	Diagnostic procedure	Diagnostic criteria ^a	Antifungal therapy	Outcome
Patients transplanted in Turkey								
1 (1988)	48/F	Meninx (CNS)	C. neoformans	–	Culture of CSF	2	–	Died
2 (1992)	27/M	Lung and CNS dissemination	A. fumigatus	Lobar pneumonia (S. pneumoniae)	Direct microscopic examination, culture of sputum and autopsy	4, 1	Itraconazole	Died
3 (1992)	34/M	Lung	A. fumigatus	Gram-negative pneumonia	Direct microscopic examination, culture of BAL and autopsy	3, 1	Itraconazole	Died
4 (1992)	40/F	Lung	A. fumigatus	–	Direct microscopic examination, culture of sputum and autopsy	4	Itraconazole	Died
5 (1992)	40/F	Lung (fungus ball)	A. fumigatus	–	Direct microscopic examination, culture of BAL	3	L-Ampho B	Remission
6 (1995)	38/F	CNS	A. fumigatus	Gram-negative pneumonia	Direct microscopic examination, culture of abscess material	2	Surgical procedure, L-Ampho B	Died
Patients transplanted in India or Russia								
7 (1994)	25/F	Lung	A. fumigatus	Nocardiosis in lung	Direct microscopic examination, culture of BAL	3	L-Ampho B	Remission
8 (1994)	48/M	Nasofacial	Mucoracea	–	Tissue biopsy and tissue culture	1, 2	Surgical procedure, L-Ampho B	Remission
9 (1995)	59/M	Lung	A. fumigatus	M. tuberculosis	Direct microscopic examination, culture of BAL, TBB	1, 3	L-Ampho B	Died
10 (1995)	37/M	Lung and CNS dissemination	A. fumigatus + Candida spp.	CMV + Pl. falci- parum	Autopsy	1	–	Died
11 (1995)	57/M	Lung and CNS dissemination	A. fumigatus + Mucoracea	CMV	Autopsy	1	–	Died
12 (1994)	29/M	Lung	Candida spp.	M. tuberculosis	Autopsy	1	–	Died

According to the criteria defined in Materials and Methods.

TBB = transbronchial biopsy; L-Ampho B = liposomal amphotericin B; Pl. falci-parum = Plasmodium falciparum.

Table II. Predisposing factors for SFIs

Risk factor	Patients (n = 12)	Controls (n = 23)	p
Mean age (y)	40 ± 11	29 ± 10	0.01
Male:female ratio	7:5	16:7	NS
Cadaveric/living donors	6:6	9:14	NS
Serum creatinine (mg/dl)	1.9 ± 0.56	1.6 ± 0.65	NS
Duration of hospitalization (d)	75 ± 44	40 ± 13	0.03
Leukocyte count (per µl)	5,900 ± 2,300	6,000 ± 1,800	NS
Antibiotic use (No. of patients)	11	17	NS
Duration of antibiotic use (d)	40	23	0.01
Pulse steroid/antilymphocytic globulin use	6/0	12/0	NS
PTDM	5	3	0.01
CMV disease (No. of disease)	2	0	NS

infection fell to 2.3%, a figure much closer to the incidence rates encountered in developed countries.

It was reported that intense immunosuppression could be a major predisposing factor responsible for the high incidence rate of fungal infectious diseases in transplant recipients (1, 2, 7). However, in our patients no statistically significant difference was found with regard to the intensity of immunosuppression between those who developed fungal infectious disease and those who did not.

Long-term hospitalization has also been reported to be another important risk factor associated with the development of fungal infection (1, 3, 8, 9) and was significantly associated with the incidence of fungal infections in our recipients ($p = 0.01$). We think that long term-hospitalization may enhance fungal colonization in the respiratory system in particular.

It is known that use of antibiotics, especially broad-spectrum antibiotics, is an important risk factor for colonization with *Candida* spp. (1, 3, 5, 8, 10). In our study, antibiotic use was found to be a predisposing factor only when antibiotics were administered for longer than usual. It should be borne in mind that the empirical use of antibiotics for febrile episodes is a double-edged sword in graft recipients. For this reason, invasive procedures (including fiberoptic bronchoscopy with BAL, transbronchial biopsies and tissue biopsies, etc.) would seem to be necessary for the proper diagnosis and documentation of specific infectious agents and to enable the appropriate antimicrobial therapy to be initiated.

According to some recent studies increasing age (especially > 40 y) is a risk factor for SFIs (2, 3) and increasing age was also found to be a predisposing factor in our study.

Recipients with coexisting diabetes mellitus are reported to be prone to fungal infection and to mucormycosis in particular (2, 3, 8, 11). In our group, we also found that PTDM is significantly associated with the development of fungal infection.

A. fumigatus was the most commonly isolated pathogen in our patients with fungal infections. The hospital environment might be a source of *Aspergillus* spores (9). On the other

hand, the source of the aspergillosis may have been subclinically infected organ donors from India or Russia (12, 13).

It is known that CMV may lead to superinfection with fungi (1, 5, 8, 10, 14). Prolonged use of antibiotics for the treatment of bacterial infections may be an important risk factor for colonization with fungal species (1, 3, 5, 8, 10). In our patients, fungal infections were mostly seen following CMV or bacterial infections.

In our renal transplant recipients, fungal infection is associated with a high mortality rate despite having a lower incidence than bacterial and viral infections. The difficulty of early diagnosis and the lack of effective therapy may be the causes of the high mortality rate. The insidious nature and protean manifestations of fungal infections and the difficulty of obtaining suitable material for proper diagnosis often delay the diagnosis and the institution of appropriate antifungal treatment. A high index of suspicion, appropriate investigations and early treatment are vital in order to reduce the mortality rate.

Although the number of patients in this study was too small to enable definitive conclusions to be drawn, liposomal amphotericin B seems to be more effective than itraconazole for the treatment of SFIs. As SFIs are associated with a high mortality rate in renal transplant recipients, antifungal therapy, especially with liposomal amphotericin B, should be started whenever fungal infection is suspected, even before the results of microbiologic and/or histologic examinations are known.

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