Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Pulmonary *Lophomonas blattarum* infection in patients with kidney allograft transplantation

Yong Wang,[1] Zheng Tang,[2] Shuming Ji,[2] Zhen Zhang,[2] Jinsong Chen,[2] Zhen Cheng,[2] Dongrui Cheng,[2] Zhihong Liu[2] and Leishi Li[2]

1 Life Science Department of Nanjing University, Nan Jing, Jiang Su Province, China
2 Institution of Nephrology of Jin Ling Hospital, Nan Jing, Jiang Su Province, China

Keywords
kidney transplantation, *Lophomonas blattarum*, pulmonary infection.

Correspondence
Zheng Tang, Institution of Nephrology, Jin Ling Hospital, Nan Jing, Jiang Su Province, China. Tel.: 086 025 80860218; fax: 086 025-84801992; e-mail: tdr@public1.ptt.gs.cn

Received: 23 February 2006
Revision requested: 2 April 2006
Accepted: 17 July 2006

doi:10.1111/j.1432-2277.2006.00380.x

Summary

The aim of the study was to analyse the clinical manifestation and management of pulmonary *Lophomonas blattarum* infection in four allograft transplantation recipients retrospectively. Four patients with pulmonary *L. blattarum* infection were diagnosed by using Fiberoptic bronchoscopy (FOB) and bronchoalveolar lavage (BAL) examination. Their clinical manifestation and management are summarized. Four cases of pulmonary *L. blattarum* were found during the period from the second month to the third month after transplantation. Concurring infection by other pathogens was found in three of them. Common initial symptoms included fever (>38 °C) without cough and breathlessness. Lower lobe shadowing could be found on chest X-ray. Body temperature decreased to the normal range in three patients and to 37.5 °C in the other one, after intravenous injection of metronidazole and tapering immunosuppressant. Radiological examination confirmed improved health condition of the patients afterwards. Two patients received repeated FOB and only dead *L. blattarum* was found. Pulmonary *L. blattarum* infection in allograft transplant recipients carry relatively obscure initial symptoms. Possible *L. blattarum* infection needs to be screened in post-transplantation pulmonary infection patients with similar symptoms, especially in those who respond poorly to anti-infection treatment. Microscopic examination of BAL fluid can help to identify pulmonary *L. blattarum* infection and metronidazole is an ideal treatment choice.

Introduction

Renal transplantation has become a well-established therapeutic option for end-stage renal disease. Because of the improved surgical and organ preservation techniques, particularly the advances in immunosuppressant, early mortality after allograft kidney transplantation has decreased significantly [1]. Unfortunately, while the rejection as a cause of graft loss had fallen from 65.7% (1970–1979) to 44.6% (1990–1999), death with a functioning graft had increased from 23.8% (1970–1979) to 37.5% (1990–1999) [2]. Among all the causes of death in cadaveric kidney graft recipients, infection remains the second most common one, which reached more than 20% [3,4]. Infection has become a major determinant of successful transplantation and the good outcome of renal transplants depends on the prevention, early diagnosis, and specific and timely treatment of infectious complications [5].

Transplantation patients are particularly susceptible to infection because of epidemiologic exposure and persistent suppression of immune system. A number of opportunistic pathogens such as cytomegalovirus (CMV), *P. carinii* and *Aspergillus* spp. etc. prevail in post-transplant infection patients. The spectrum of infectious pathogens in transplant recipients has been broadened and relatively scarce pathogens have been reported in the infection of organ transplant recipients [6]. Leishmania and Malaria are the most frequently found protozoa involved in infections of transplant patients and such

infections are mostly found in the tropical area [7]. From November 2004 to December 2005, pulmonary infection caused by a rare protozoa had been found in four cadaveric kidney transplant recipients in Nanjing, which is located far from tropical area. Such infection in transplant recipients has not been previously reported and the clinical manifestation and management of these patients are summarized in following study.

Materials and methods

Patients

From November 2004 to December 2005, 142 cadaveric kidney transplantations were performed in the Institution of Nephrology in Jin Ling Hospital. Seven patients suffering febrile illness were diagnosed as having pneumonia during the period from the second month to the third month after transplantation. There was no cough and breathlessness when they were re-admitted to hospital. All patients gave their written consents for the Fiberoptic bronchoscopy (FOB) examination and their inclusion in the present study. All clinical practices in the present study were in accordance with the regulation of the ethic committee of Jin Ling Hospital and local public health bureaucracies.

Pathogen screening

All patients were re-admitted to hospital within 2 days of fever. Upon the re-admittance, blood samples and urine samples were collected successively for at least two times. Phlegm samples were collected repeatedly if available.

All samples of blood, phlegm and bronchoalveolar lavage (BAL) were cultured semi-quantitatively for bacteria and fungi. Polymerase chain reaction (PCR) examination for CMV, tubercle bacillus (TB) and *P. carinii* were performed in all these blood, urine, phlegm and BAL specimens. Phlegm and BAL samples were observed under the microscope.

Fiberoptic bronchoscopy and bronchoalveolar lavage fluid examinations

Fiberoptic bronchoscopy (Pantax FB, EB150, Pantax, Japan) examination was performed in all the seven patients for at least once and BAL sample was collected in each patient. BAL collection was performed by wedging the tip of the bronchoscope into the segmental or subsegmental bronchus with the greatest radiological abnormality. About 40–100 ml of sterile physiologic saline warmed to the body temperature was instilled in 50 ml aliquots. Gentle manual suction was applied to retrieve the saline. Bronchoalveolar lavage fluid was collected in sterilized containers and brought to the laboratory within 30 min. Fresh BAL fluid was observed under the microscope. All BAL fluid samples were stained with Giemsa silvermethenamine.

Fiberoptic bronchoscope was sterilized with glutaric dialdehyde for more than 2 h before each examination. The outer wall and lumen of the fiberoptic bronchoscope, oxygen pipeline and medical utensils used for these patients were washed with physiologic saline before the examination. All the wash solutions were collected in sterilized containers for microscopic examination after centrifugation.

All BAL samples were observed under the microscope and sent for a culture examination for bacteria and fungi. On the day of FOB examination, urine, blood and faecal samples were collected. Secretions of nasal cavity and throat were collected with sterilized tampon for microscopic examination at the same day.

Immunosuppressant

Maintenance therapy consists of mycophenolate mofetil (Cellcept, Roche Pharmaceuticals Inc., Palo Alto, CA; initial dose 1.5 g/day, orally), cyclosporin A (CsA, Neoral, Novartis AG, Basel, Switzerland; initial dose 5 mg/kg/day, adjusted to trough level) or tacrolimus (FK506, Fujisawa Inc., Deerfield, IL; initial dose 0.1 mg/kg/day, adjusted to trough level) and prednisone (20 mg/day). Immunosuppressants except prednisone (10 mg/day) were stopped when pneumonia was diagnosed. After the patients were cured, the immunosuppressant dosage was adjusted gradually to the previous dosage level.

Antimicrobe medication

Empirical treatment consisted of ceftizoxime or cefoperazone. The latter was used when there was no improvement in febrile disease and/or radiological examinations after treatment with Ceftizoxime. In patients with the initial symptoms of breathlessness or interstitial shadowing on X-ray, gancyclovir (500 mg/day) was prescribed.

Specific antibiotics were used according to the results of the culture examination. Repeated positive results of the CMV-PCR exam led to gancyclovir (1000 mg/day) treatment for 2 weeks. Metronidazole was prescribed (500 mg, three times/day) when *L. blattarum* infection was diagnosed.

Follow-up

The follow-up lasted for at least 6 months after the infection. Two patients were followed up for 1 year thereafter. During the follow-up, serum creatinine, body temperature and chest X-ray were monitored intermittently.

Results

Lophomonas blattarum observations

Of the seven patients receiving FOB examination, similar microbes (Fig. 1a) were observed in the BAL fluid from four. The common characteristics of these microbes included round body and approximately 15–20 flagella on one side. Air-dried BAL was stained with Giemsa silver-methenamine(Fig. 1c and d). Photographs (Fig. 1a–d) of this unknown microbe were sent to the Institution of Parasite Disease of Chinese Center for Disease Control and Prevention. Live microbes were identified by the Parasitology Department of Nanjing Medical University. The demographic and clinical data of these four patients are listed in Table 1.

This unknown microbe was identified as *L. blattarum* by the Parasitology Department of Nanjing Medical University. This microbe was further confirmed by the Institution of Parasite Disease, Center for Disease Control and Prevention. It has outer plasmalemmas and internal nuclei (Fig. 1). In fresh BAL specimens, *L. blattarum* waved its flagella rhythmically. The body shape changed from round to oval or turned olive shaped after death or Giemsa silver-methenamine staining. Dead *L. blattarum* could be easily distinguished by immotile flagella and changed body shape.

Clustered *L. blattarum* were found adjacent to white blood cells and some *L. blattarum* closely adhered to white blood cells (Figs 2 and 3). BAL specimens were kept in a sterilized container (without culture medium) at room temperature. Alive *L. blattarum* with decreased number were found 60 h after the collection. No *L. blattarum* was found in the samples of blood, urine, faeces, throat and nasal secretion. There was no similar protozoa in the wash solution of medical utensils.

Phlegm specimen was available in the first patient 6 days after hospitalization. Repeated microscopic examinations of her phlegm specimens did not reveal *L. blattarum*.

Maintenance therapy and immune function

All the four patients had almost normal renal function (around 1.4 mg/dl) after transplantation. Main-

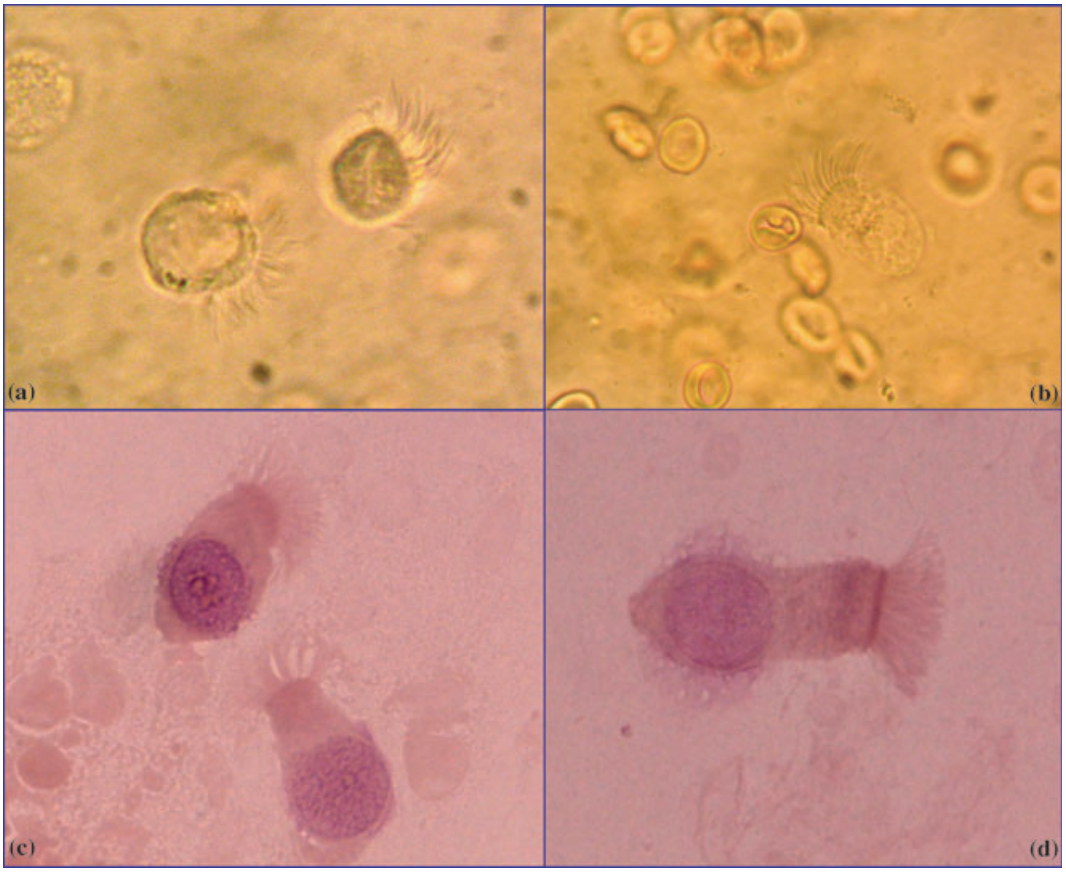

Figure 1 *Lophomonas blattarum* in BALF. (a) Alive *Lophomonas* and its round body with waving flagellum on one side (40 × 10). (b) Dead *Lophomonas* and its oval shape body with silent flagellum (40 × 10). (c) and (d) Metamorphic body of dead *Lophomonas* and its immotile flagellum (Gram's 100 × 10).

Table 1. Demographic and clinical data of the patients with pulmonary *Lophomonas blattarum* infection.

	Patients			
Exams	First	Second	Third	Fourth
Background				
Gender	F	M	M	M
Age	45	19	53	51
Profession	Teacher	Student	Official	Businessman
Hometown	He Nan	An Hui	Jiang Su	GuangDong
Operation date	Aug 2004	Sep 2004	Aug 2004	Oct 2004
Infection date	Nov 2004	Dec 2004	Nov 2004	Dec 2004
Blood routine				
Whit blood cells ($\times 10^9$)	3.1	7.5	5.1	7.6
Neutrophil (%)	66.5	75	68	78
Lymphocyte (%)	17.4	19	23	13
Eosinophil (%)	7.4	1.3	1.3	2
Liver function				
TBIL (μmol/l)	4.5	4.2	9.2	6.7
DBIL (μmol/l)	1.1	1.2	2.6	2.2
AST (U/l)	32	23	30	20
ALT (U/l)	26	5	22	51
LDH (U/l)	ND	308	ND	173
Albumin (g/l)	37	37.4	33	38
Globulin (g/l)	19 g/l	18	19	22.6
Renal function				
Bun (mg/dl)	32.7	29.47	15.59	21.56
Scr (mg/dl)	1.24	1.32	1.18	1.02

TBIL, total bilirubin; DBIL, direct bilirubin; AST, aspartate amino transferase; ALT, alanine transaminase; LDH, lactate dehydrogenase; Bun, blood urea nitrogen; Scr, serum cretinine.

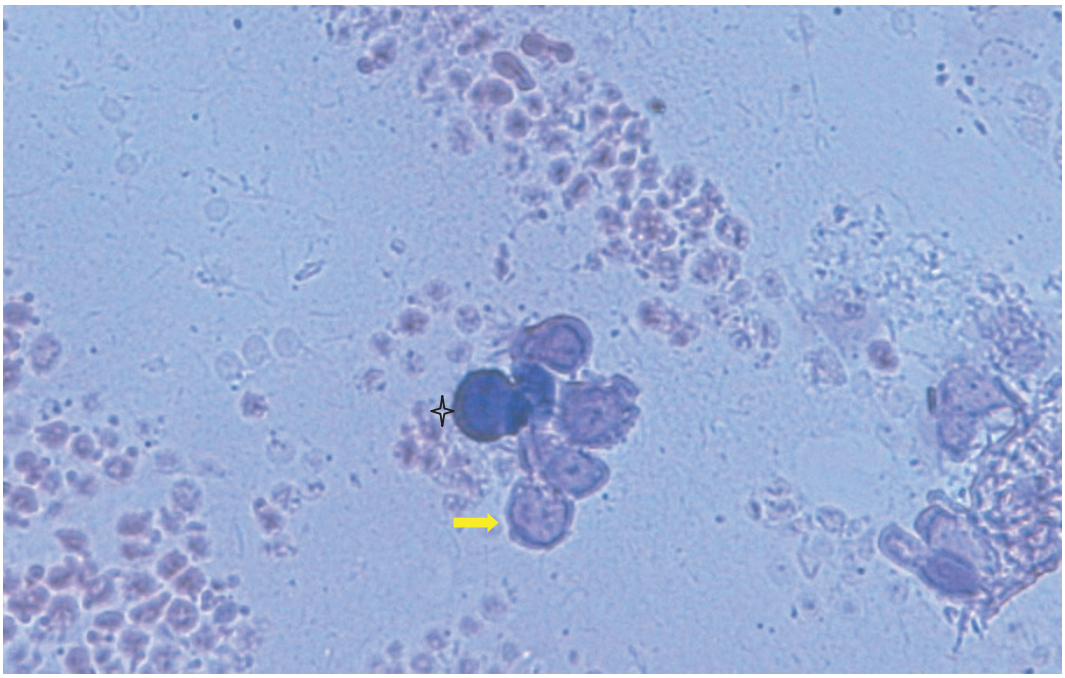

Figure 2 *Lophomonas blattarum* closely attached to white blood cells (HE 40 × 10). ✧, white blood cell; ⇨, *L. blattarum*.

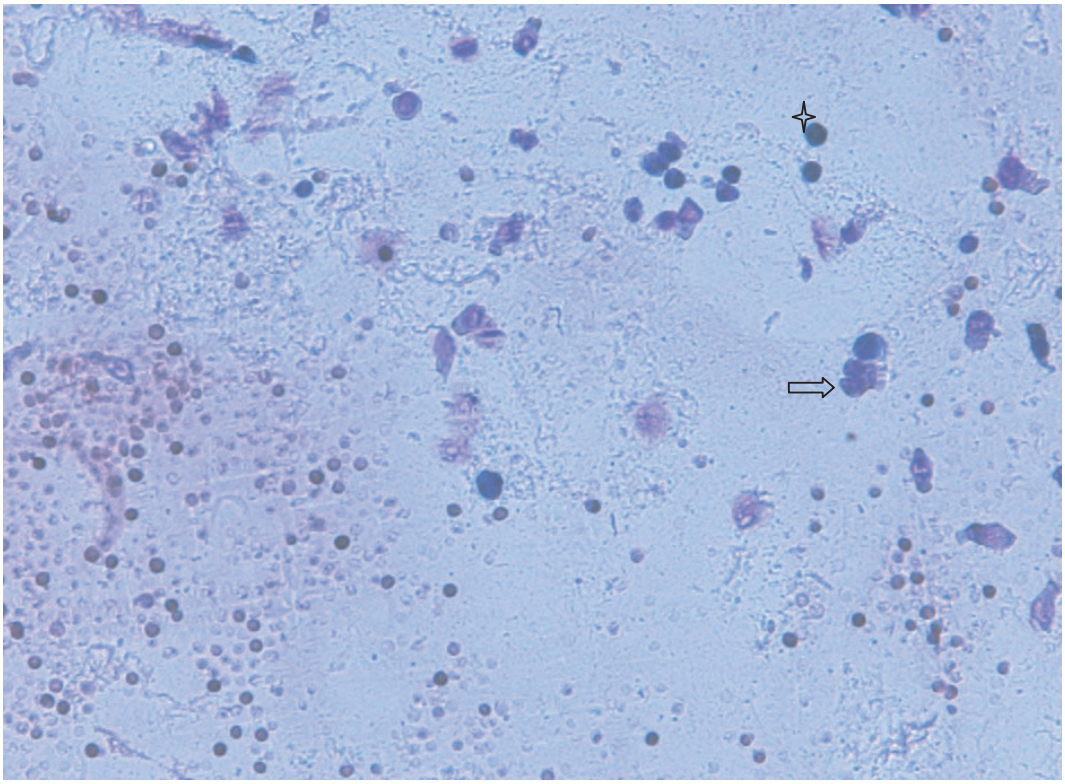

Figure 3 *Lophomonas blattarum* clustered with white blood cells (HE 40 × 10). ✧, white blood cell; ⇨, *L. blattarum*.

Table 2. Immunosuppressant and immune function of the four patients with *Lophomonas blattarum* infection.

		Trough level		1 month post-transplant		Onset of infection	
	MMF	CsA	Tac	$CD4^+$cell*	$CD8^+$cell*	$CD4^+$cell	$CD8^+$cell
1	1.25 g/day		3.2 ng/ml			170	263
2	1.25 g/day	110 ng/ml				298	337
3	1.25 g/day		5.5 ng/ml	620	615	296	466
4	1.25 g/day		5.27 ng/ml	622	207	340	276
Target	1.25 g/day	120–150 ng/ml	5–7 ng/ml	651.3 ± 273.6	452.6 ± 210.8	651.3 ± 273.6	452.6 ± 210.8

*Peripheral $CD4^+$ and CD8+$^+$ cell quantification are expressed as number per microlitre.
CsA, Cyclosporine; Tac, Tacrolimus.

tenance therapy was followed within target levels (Table 2) by monitoring Cellcept dosage and CsA/FK506 trough levels. There was a significant decrease in peripheral $CD4^+$ and $CD8^+$ lymphocytes when the symptom of fever and abnormal radiological change presented.

Clinical manifestations

Pulmonary infection was found during the period from the second month to the third month after transplantation. These four patients suffered febrile illness (higher than 38 °C) but no cough, breathlessness, or tachypnoea in the initial period. Lower lobe shadowing was found on chest X-ray or computed tomography (CT) in the initial stages of fever. Afterwards, there were different radiological changes, such as solitary nodules (in the first patient) and an upper lobe shadow (in the second patient).

Treatment and prognosis

Mycophenolate mofetil and CsA/FK506 were stopped when pneumonia was confirmed (2–3 days after the

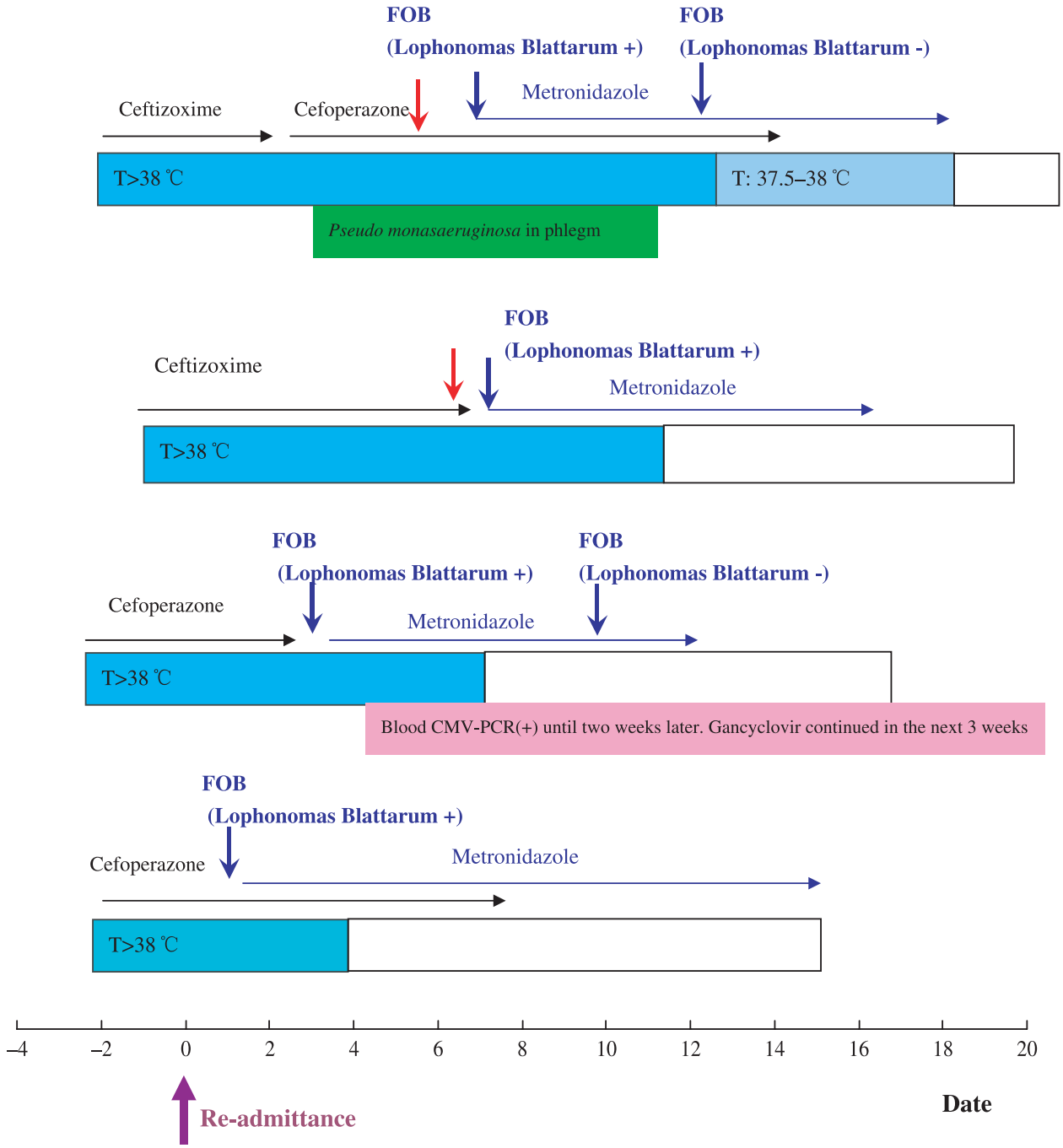

Figure 4 Clinical manifestation and treatment of the kidney transplant recipients with *Lophomonas blattarum* infection. Lined bars show the time of hospitalization. Different blue areas show the dates of febrile illness and the white bar indicates the dates of normal body temperature. Bars without encompassed line show the dates in which different pathogens were identified. Minus signs indicate negative results and plus signs positive results. Red arrows indicate the date in which symptom of breathlessness appeared and the SpO_2 began to decrease. The negative data show the time before re-admittance.

beginning of fever). Treatment and prognosis of these four patients are listed in Fig. 4.

Within 2 days after empirical treatment of ceftizoxime and cefoperazone, four patients were re-admitted to hospital. FOB examination was carried out 1–8 days after re-admittance and metronidazole was prescribed afterwards. Phlegm was available in only one (the first one) patient. Repeated culture of phlegm specimens showed

growth of *Pseudomonas aeruginosa* (sensitive to ceftizoxime and cefoperazone). Positive result of TB-PCR was obtained from the blood samples of the second patient. CMV-PCR (blood samples) of the third patient yielded a positive result. Anti-TB treatment (including isoniazid 0.3 g/day, rifampicin 0.45 g/day and ethambutol 0.75 g/day) and gancyclovir (1000 mg/day) were given.

The first and the second patient complained of breathlessness at the fifth day and at the eighth day respectively, after re-admittance. Peripheral SpO_2 dropped to around 90% after initial empirical treatment (which was sensitive treatment to *P. aeruginosa* in the first one) and the fever continued along with the aggregating radiological abnormality. After metronidazole treatment, body temperature decreased in 4–5 days and the breathlessness was alleviated. FOB and metronidazole were given 1–3 days after re-admittance in the third patient and the fourth patient. A rapid decrease in body temperature was observed and there were no symptoms of breathlessness (Fig. 4).

Normal body temperature and improved radiological examination results were obtained in all the four patients after metronidazole treatment. Peripheral $CD4^+$ and $CD8^+$ lymphocytes recovered to near-normal levels after the treatment. Maintenance therapy was adjusted back to the previous dosage after recovery. Four patients were followed for at least 6 months and no relapse of similar infection was found.

Two patients (the first and the third) received repeat FOB, 7 days after metronidazole treatment. Only dead and metamorphic *L. blattarum* was found in the BAL fluid (Fig. 1b–d). At all nine times of FOB examination, there were no complications such as haemorrhage, breathlessness or obstruction.

Discussion

Although there have been tremendous improvements in transplantation techniques, mortality with functioning graft is still high in kidney transplantation recipients [3]. Among all the reasons resulting in the death of recipients, cardiovascular disease remains the major one [8]. Infectious diseases are the second (21.8%) most common cause of death among kidney graft recipients, especially in the early post-transplant period [3].

There is a timetable for post-transplantation infections [9]. Between the second month and the sixth month after transplantation, the immunosuppression reaches maximum levels, allowing microorganisms like *P. carinii*, *C. neoformans*, *Nocardia* spp., Legionella and mycobacteria, *S. stercoralis*, *Aspergillus* spp. and protozoa to produce varied infection complications, especially pulmonary infections [5]. Malaria and Leishmania are the most common protozoa causing post-transplant infection [7]. *Lophomonas blattarum* infection in transplant recipients has not been reported before.

The importance of *L. blattarum* in these patients can be best seen in the fourth patient, in whom *L. blattarum* was the only affirmed pathogen producing febrile disease. Moreover, active *L. blattarum* in the first BAL sample were found dead in the second BAL sample after metronidazole treatment, along with the recovery from fever and abnormal radiological change.

Although there was concomitant activity of other pathogens such as TB (in the second patient) or CMV (in the third patient), there were no signs or symptoms of TB/CMV pneumonia in these two patients. On the other hand, after metronidazole treatment, the febrile diseases ceased within 4 days, which was a too short period for the concomitant gancyclovir or anti-TB treatment to produce enough effect. On the bases of these clues, TB and CMV might have produced continuous reproduction but not active pulmonary infection in these two patients.

The treatment of the first patient provides us with a better understanding of the detrimental effect of *L. blattarum*. In this patient, pneumonia continued to deteriorate even after specific antibiotic treatment, but was quickly ameliorated after metronidazole treatment. Similarly, repeated FOB found only dead *L. blattarum* afterwards. *Lophomonas blattarum* infection was thus an important reason for the quickly aggregating pneumonia and deteriorating pulmonary function, just as the *P. aeruginosa*.

Pulmonary *L. blattarum* infection is characterized by fever and radiological abnormality. Symptoms such as dry cough and breathlessness were absent in the initial phase, which was contrary to CMV and *P. carinii* infection [10,11]. In the present study we found *L. blattarum* only in BAL fluid but not in phlegm and nasal/throat secretion, which indicates that it might have resided exclusively in the deep parts of the pulmonary system.

Fiberoptic bronchoscopy with BAL examination is a well-established procedure in the diagnosis of infections, rejection, and airway complications in lung transplant and heart–lung transplant recipients [12,13]. In the present study, FOB and BAL examination proved to be a very useful and safe tool in pulmonary *L. blattarum* infection patients and in pneumonia patients without definite pathogen diagnosis.

Lophomonas blattarum is sensitive to metronidazole treatment. After 4–5 days of metronidazole treatment (500 mg, three times per day), body temperatures of these patients decreased significantly and their radiological abnormality improved as well. Repeated FOB in the first patient and the third patient (7 days after metronidazole treatment) further confirmed the effect of metronidazole by the presence of dead *L. blattarum* in BAL fluid.

It is worth noting that pulmonary *L. blattarum* infection can be safely controlled only if diagnosed in time. In the two patients (the first and the second one) receiving metronidazole treatment relatively later, refractory fever lasted for a longer time and there were breathlessness and a decrease in SpO_2. Two patients receiving metronidazole got a better recovery and there were no symptoms of breathlessness. The detrimental effect of *L. blattarum* derives from not only epidemic exposure but also the inefficiency of the immune system. A similar dramatically accelerated infection by opportunistic pathogens has also been reported in immunocompromised patients such as kidney or heart transplant recipients [8,9] and this phenomenon has been attributed to the disruption of host immunity [7].

The epidemiology of *L. blattarum* infection remains unknown. Besides our preliminary study, there have been a few case reports of *L. blattarum* infection in recent years in China. These cases were all in the southern parts of China and in the area near the Yang Zi River and the Zhu Jiang River. Environmental factors seem to be a potential risk for *L. blattarum* infection [14,15].

Conclusion

In the present study, we reported *L. blattarum* to be a newly discovered infectious pathogen in kidney transplantation recipients for the first time. It is detrimental but easy to control by metronidazole. FOB examination combined with BAL fluid microscopic examination proved to be useful in diagnosing such an infection. In post-transplant pneumonia patients, especially those with fever and radiological abnormality, possible *L. blattarum* infection should be considered. In patients who are resistant to specific medications and with similar clinical manifestations, empirical or prophylaxis treatment of metronidazole might be helpful.

References

1. Djamali A. Outcomes in kidney transplantation. *Semin Nephrol* 2003; **23**: 306.
2. Richard J, Howard PR, Alan IR, *et al.* The changing cause of graft loss and death after kidney transplantation. *Transplantation* 2002; **73**: 1923.
3. PorażB T, Boratynska M, Patrzaek D. Causes of death among cadaver kidney graft recipients between 1983 and 2000. *Transplant Proc* 2002; **34**: 2066.
4. Snydman R. Infection in solid organ transplantation. *Transpl Infect Dis* 1999; **1**: 21.
5. Splendiani G, Cipriani S, Tisone G. Infectious complications in renal transplant recipients. *Transplant Proc* 2005; **37**: 2497.
6. Sampathkumar P, Paya CV. Fusarium infection after solid organ transplantation. *Clin Infect Dis* 2001; **32**: 1237.
7. Rashad S, Barsoum RS. Parasitic infections in organ transplantation. *Exp Clin Transplant* 2004; **2**: 258.
8. Palau LA, Pankey GA. Strongyloides hyperinfection in a renal transplant recipient receiving cyclosporine: possible *Strongyloides stercoralis* transmission by kidney transplant. *Am J Trop Med Hyg* 1997; **57**: 413.
9. Schaeffer MW, Buell JF, Gupta M, *et al.* Strongyloides hyperinfection syndrome after heart transplantation: case report and review of the literature. *J Heart Lung Transplant* 2004; **23**: 905.
10. Manske CL, Wilson RF, Wang Y, *et al.* Atherosclerotic vascular complications in diabetic transplant candidates. *Am J Kidney Dis* 1997; **29**: 601.
11. Jay AF, Ishman M, Rpber H, *et al.* Infection in organ transplant recipients. *N Engl J Med* 2003; **338**: 1741.
12. Chan CC, Abi-Saleh WJ, Arroliga AC, *et al.* Diagnostic yield and therapeutic impact of flexible bronchoscopy lung transplant recipients. *J Heart Lung Transplant* 1996; **15**: 196.
13. Baz MA, Layish DT, Govert JA, *et al.* Diagnostic yield bronchoscopies after isolated lung transplantation. *Chest* 1996; **110**: 84.
14. Guozhog Y. Bronchia Lophomona Infection (one case report). *Zhonghua Jie He He Hu Xi Za Zhi* 1999; **08**: 25.
15. Dong HF, Wang RF, Yang YP, *et al.* One case of patients with *Lophomonas* in phlegm. *Journal of Shanghai Examination* 2000; **01**: 32.

