

# A Case of Intestinal Microsporidiosis in a Renal Transplant Recipient

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## Abstract

Post-renal transplant diarrhea is a common clinical presentation. An extensive list of potential etiology adds to the diagnostic dilemma. In cases of prolonged or intractable diarrhea, invasive tests are often performed. Intestinal microsporidia can be diagnosed with simple non-invasive stool polymerase chain reaction (PCR). Based on this case, we propose an easy to follow flow chart and present a literature review on post-renal transplant diarrhea. Further multicenter validation testing is required for the proposed flow chart.

**Keywords:** Microsporidiosis; Renal transplant recipient; Diarrhea

## Introduction

This is a unique case which was managed in a regional hospital in Australia. Diagnosis of microsporidiosis is often delayed. A series of investigations are undertaken before the diagnosis is reached. Often the cause of diarrhea is empirically presumed to be immunosuppressants. Modification of immunosuppressant has risk of resulting in rejection of the transplant.

In literature it has been cited that the onset of symptom occurs within 6 to 12 months post-transplant, but in our case it occurred 6 years after the transplant. Microsporidiosis is still under-recognized resulting in delayed diagnosis and increased morbidity through heightened risk of transplant rejection due to immunosuppression being adjusted. Non-invasive stool polymerase chain reaction (PCR) test should be considered early in the diagnostic workup. Diarrhea is a very common presentation in solid organ transplant (SOT), despite that there is no

universally agreed or region-specific guidance. We have come up with a unique flow chart that is easy to understand, can be cost effective and reduce morbidity. We suggest that validity of the flow chart be tested in larger multicenter studies in future.

## Case Report

A 58-year-old man received a deceased donor renal transplant in 2014. The cause for his end-stage renal failure was unknown. Prior to renal transplant, he had 6 years of hemodialysis. Post-transplant, he was prescribed standard immunosuppressive therapy including tacrolimus, mycophenolate mofetil and prednisolone. His other comorbidities were hypertension treated with ramipril and gout that was well controlled with allopurinol. He was of Caucasian origin and worked as a tradesman.

Three years post-transplant, he presented to the outpatient renal clinic with symptoms of diarrhea for 2 weeks. He was afebrile and had no abdominal pain. Stool was described as watery with a frequency of about 10 to 14 times per day. There was no mucus or blood present in the stool. On clinical examination, he was tachycardic to 110 per min and blood pressure was 92/68 mm Hg. His tongue was moderately dry. His abdomen was soft and non-tender. Respiratory and cardiac examination was unremarkable. He was admitted for initial investigations and fluid resuscitation. His inflammatory markers were normal and renal function showed urea of 8.3 mmol/L, creatinine 138  $\mu$ mol/L and glomerular filtration rate (GFR) 49 ml/min (baseline GFR 60 - 62). Potassium level was 3.4 mmol/L. Tacrolimus drug level was within the target range. Fecal assay was negative for routine bacterial, viral and protozoan pathogens.

The dose of mycophenolate mofetil was split from 720 mg BD to 360 mg QID. Following improvement in his symptoms and return of renal function to baseline, he was discharged home.

At outpatient review 2 weeks post-discharge, he described persistent symptoms. His stool frequency had increased to 16 per day. He had maintained his hydration level with increased fluid intake. He was hemodynamically stable. To expedite further investigations, he was re-admitted and referred for inpatient colonoscopy and biopsy. Biopsy result showed parasitophorous vesicles containing finely granular eosinophilic structures, suspicious for microsporidia. Subsequent test for microsporidia on stool PCR was positive.

After nearly 6 weeks of investigations and invasive tests,

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**Table 1.** Differential Diagnosis of Diarrhea in Renal Transplant Recipients

Drug related
Mycophenolate, tacrolimus, antibiotics, proton pump inhibitors, metformin
Infective causes
Bacterial: salmonella, <i>C. difficile</i> , campylobacter, shigella, yersinia
Viral: CMV, norovirus and rotavirus
Parasitic: cryptosporidium, giardia, entamoeba, microsporidia
Surgical causes
Intra-abdominal sepsis, mesenteric ischemia and colorectal carcinoma
Miscellaneous
Coeliac disease, irritable bowel syndrome, malabsorption syndrome, post-transplant lymphoproliferative disorder

CMV: cytomegalovirus.

he was diagnosed with intestinal microsporidiosis. Subsequently, he was commenced on albendazole 400 mg twice daily. Within 48 h of albendazole treatment, stool frequency reduced to six per day. He was observed for 1 more day and then was discharged home.

On follow-up phone call at 7-day post-discharge, he described stool consistency as formed and improvement in frequency of bowel motion to two per day. At further 6-week follow-up, he was symptom-free. Repeat test for microsporidia was negative on stool PCR. Albendazole was stopped at the end of 4 weeks of therapy.

## Literature Review and Discussion

World Health Organization defines diarrhea as three or more loose stools per day and persistent diarrhea as symptoms of more than 14-day duration [1]. Symptoms lasting longer than a month are defined as chronic. Diarrhea is a frequent complication after renal transplant. The incidence of diarrhea is highest in the first-year post-renal transplant. It has been estimated that one in 10 patients are affected with diarrhea in the first year post-renal transplant [2-4].

Acute diarrhea in a post-renal transplant recipient has two-fold risk of graft rejection due to variable absorption of immunosuppressants. In post-renal transplant diarrhea (PRTD), there is reduction in p-glycoprotein enzyme activity with a resultant higher tacrolimus level and potential subsequent renal toxicity [2]. Chronic diarrhea can result in hyperoxaluria, which causes inflammation and graft rejection [2].

In renal transplant patients, up to 64% of diarrheal cases are thought to be infectious in origin [2]. *Clostridium difficile* (*C. difficile*), cytomegalovirus (CMV) and norovirus are the commonest pathogens in PRTD [3]. However, diarrhea may also be due to factors not related to immunosuppressive therapy such as oral hypoglycemic agents, broad-spectrum antibiotics or inflammatory bowel disease (Tables 1 and 2) [4, 5].

Microsporidia have been known to cause infection in HIV patients [6-8]. Over the last 20 years, 31 cases of intestinal and disseminated microsporidiosis have been cited in renal transplant recipients [9-14]. It has been estimated that microsporidia accounts for approximately 3.5% of all post-transplant diarrhea [12, 13]. In 2012, George et al reported the first case of disseminated microsporidiosis in a non-HIV renal transplant recipient in Australia [8]. In the last decade, microsporidia has been recognized as the emerging opportunistic pathogen that causes diarrhea in a SOT recipient as well as in other immunosuppressed patients such as those who have diabetes, hematological malignancy, rheumatological conditions receiving anti-tumor necrosis treatment, in elderly and in patients who are on long-term corticosteroids [13, 14].

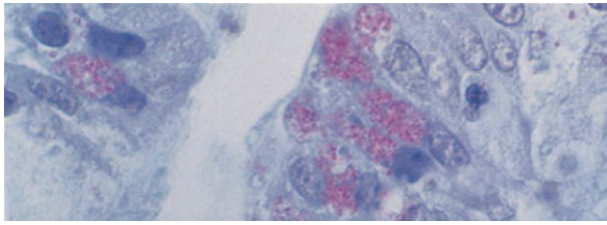
Microsporidia are a group of obligate intracellular parasites [15]. Fourteen species have been identified that cause diarrhea in humans [8, 15]. Amongst the microsporidia species, *Enterocytozoon bieneusi* (*E. bieneusi*), *cuniculi*, *intestinalis* and *hellen* are the commonest human pathogens [16-18].

Transmission of spores occurs through feco-oral route from infected humans, animals or contaminated food and water. It has been cited that preceding high-dose corticosteroid treatment, concurrent CMV and giardia infection can predis-

**Table 2.** Diagnostic Investigations for Diarrhea in a Renal Transplant Recipient

Non-invasive
Stool: PCR, microscopy
Blood (serum): blood culture, CMV PCR and quantitative assay, immunosuppressive drug serum assays, immunological test for endomysial antibodies and transglutaminase antibodies
Invasive
Biopsy: colonic, terminal ileum, gastric, duodenal

CMV: cytomegalovirus.



**Figure 1.** Jejunal biopsy containing microsporidia in an enterocyte.

pose to microsporidia infection [9]. In 2015, Hocevar et al reported cluster of three microsporidia cases in SOT recipients, acquired through a common infected donor [19].

The onset of symptoms varies from 6 to 60 weeks post-transplant; however, the majority present within 6 to 12 months. The symptoms usually last from 1 to 40 weeks, with a median period of 2 weeks [14]. In an immunocompromised host, microsporidia primarily infect enterocytes, though several case reports of disseminated disease involving respiratory, renal, central nervous systems and eyes have been described [8, 20-25].

Several laboratory tests are available for identification of spores and confirmation of infection [26]. Traditional methods such as light microscopy using modified trichrome stains or Giemsa (special stains) are inexpensive methods but lack species identification. Serological tests using enzyme-linked immunosorbent assay (ELISA) lack sensitivity in differentiating recent versus past infection and therefore are not recommended for routine diagnostic use [27]. Immunofluorescent techniques using reagents are only available in research laboratories. Spores can be detected in centrifuged specimen from duodenal aspirate, colony-stimulating factor (CSF), bronchoalveolar lavage and bile. In disseminated microsporidiosis, renal involvement is relatively common and testing of urine specimen for spores is recommended [26, 27].

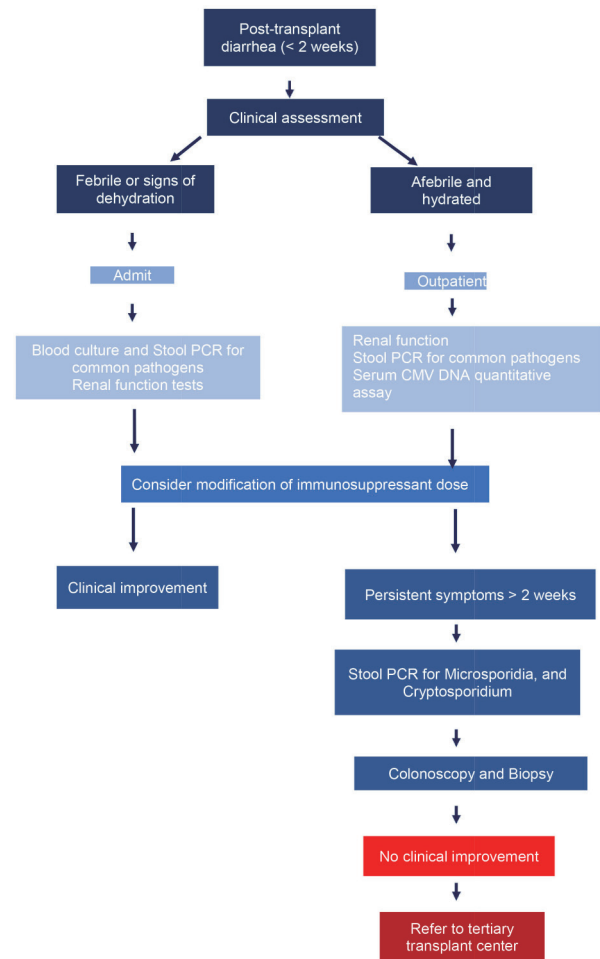
The molecular diagnostic tests using PCR detects microsporidia in stool, urine and various biopsy specimens [2]. In comparison with light microscopy, molecular testing is more sensitive and provides additional benefit of species identification. This information has therapeutic advantage in initiating species-specific treatment [23, 28].

In 2013, Coste et al studied severe diarrhea in 49 renal transplant recipients [4]. Detection rate of pathogens was only 23% using classic microbiological methods, whereas using multiplex PCR the detection of pathogens increased to 72% [2]. In clinical practice routine tests are often negative, leading to modification of immunosuppressive medications [2, 5, 29].

Identifying microsporidia on a routine hematoxylin and eosin stain can be challenging and often required an experienced pathologist. Intestinal infection can be patchy and missed on the biopsies. In biopsy specimen, microsporidia are seen within enterocytes and in lamina propria (Fig. 1).

Other histological features include minute ulcer over the duodenal and colonic folds and a necrotic patch over the ulcers [30].

A superficial biopsy may only reveal the necrotic cells. Deeper tissue biopsy carries the risk of perforation. Body fluids and stool examination are equally sensitive, and these



**Figure 2.** Proposed diagnostic algorithm for diarrhea in a renal transplant recipient.

specimens can be repeatedly collected based on clinical presentation. Most authors recommend biopsy as the second-line investigation; however, the yield is no more sensitive than stool and body fluid examination [18, 31].

Often negative initial investigation leads nephrologists to perform invasive tests such as colonoscopy [2]. Modification of immunosuppressive treatment, considered as the cause of diarrhea, has risk of acute or chronic transplant rejection [1, 2, 25].

With stepwise testing strategy, costs can be reduced without compromising diagnostic yields. In the first-stage testing, authors recommend assessment for *C. difficile*, food-borne bacterial and viral pathogens (Fig. 2). For persistent diarrhea, second-stage evaluation should include stool PCR for giardia, cryptosporidium, microsporidia and possibly colonoscopy. In a landmark study it was shown that diarrhea resolved in approximately 50% patients without modification of immunosuppressant drugs [29].

As susceptibility of microsporidia species to different drugs is variable, species identification is recommended for appropriate treatment [32, 33]. Albendazole is efficacious in treating *E. intestinalis* but less effective against *E. bieneusi* [4].

**Table 3.** Summary of Literature Review on Efficacy of Albendazole in Microsporidiosis

Article/reference	Intestinal/disseminated infection	Treatment agent/dose/duration	Outcome
Dacha et al [34]	Intestinal <i>E. intestinalis</i>	Albendazole 400 mg BD for 4 weeks	Effective
Galvan et al [14], patient 1	Intestinal	Initially metronidazole, on relapse switched to albendazole 400 mg BD for 3 weeks	Albendazole effective
Galvan et al [14], patient 2	Intestinal	Immunosuppression withdrawal	
George et al [8]	Disseminated	Albendazole 400 mg BD, duration not defined	Effective
Hocevar et al [26], patient 1	Disseminated, <i>E. cuniculi</i>	Albendazole 400 mg BD for 4 months	Effective
Hocevar et al, 2014 [26], patient 2	Disseminated due to <i>E. cuniculi</i>	Albendazole 400 mg BD for 1 year due to relapse	Effective

Fumagillin, an antibiotic derived from the fungus *Aspergillus fumigatus*, is often efficacious against *E. bienewisi*. Fumagillin use is limited by severe toxicity mainly bone marrow suppression and thrombocytopenia [34, 35].

Two weeks of albendazole treatment is associated with increased risk of relapse. Consequently, in this case, treatment was continued for a total of 4 weeks. To prevent relapses, it is recommended that microbiological clearance should be the end-point of treatment [12, 36-38] (Table 3) [8, 14, 26, 34].

Although species identification was not performed in this case, given the clinical and microbiological response, we believe the infection was likely due to *E. intestinalis*.

In summary, post-transplant diarrhea is associated with high morbidity and mortality. Transplant patients should be considered as high-risk group for microsporidiosis. In absence of common pathogens on initial investigations or poor response to standard treatment, microsporidiosis should be considered in the list of differential diagnosis.

## Conclusions

Microsporidiosis is an emerging disease in non-HIV SOT recipients. Investigation for microsporidiosis should be considered in diagnostic workup for renal transplant patients who present with persistent diarrhea. Authors propose a flow chart for management of diarrhea with a focus on microsporidiosis as an early investigation. This flow chart will require validation in a future multicentre study.

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## Financial Disclosure

None to declare.

## Conflict of Interest

None to declare.

## Informed Consent

Informed consent to publish this case report was taken from the patient.

## Author Contributions

ND is primary author, who was involved in the literature review and final proofreading; ZT and TH contributed to the case report; JM contributed to correction and proofreading.

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