NDT Plus (2008) 5: 349–353
doi: 10.1093/ndtplus/sfn061
Advance Access publication 28 May 2008

NDT PLUS
Nephrology Dialysis Transplantation

Teaching Point
(Section Editor: A. Meyrier)

Diagnosis by inclusion

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Keywords: colitis; diarrhoea; renal transplantation

Introduction

Diarrhoea after renal transplantation is not an uncommon problem [1]. We present the case of a 61-year-old renal transplant recipient on mycophenolate mofetil (MMF) and steroids who presented with chronic diarrhoea and substantial weight loss. The diarrhoea was ascribed to MMF and the patient was converted to enteric-coated mycophenolic sodium (EC-MPS), without effect. Comprehensive workup revealed a surprising aetiology of his diarrhoea. We discuss diarrhoea in renal transplant recipients with an emphasis on differential diagnosis and workup.

Case

A 61-year-old male haemodialysis patient with renal failure due to autosomal-dominant polycystic kidney disease (ADPKD) received a cadaveric renal transplant in October 2003. He had previously undergone left-sided native nephrectomy. Immunosuppresion was induced with daclizumab and maintained with cyclosporin A (Neoral®, Novartis) and prednisolone. A participant of a randomized trial with cyclosporin withdrawal, he eventually received MMF (Cellcept®, Roche) 1 g b.d. and 10 mg prednisolone daily. The patient sustained no rejection episodes and maintained good graft function (serum creatinine 140 μmol/L). Co-morbidity included vascular disease and abdominal aortic aneurysm, for which the patient had undergone implantation of an aorto-iliac dacron graft.

He presented elsewhere in summer 2007 with a history of persistent diarrhoea and lassitude. Stool cultures grew *Campylobacter jejuni* and the patient made an uneventful recovery with antibiotic treatment. Diarrhoea recurred and the patient underwent gastroenterological evaluation. Sigmoidoscopy revealed patchy inflammatory changes, and biopsies confirmed inflammation with focal vasculitis. Stool cultures were negative. MMF was replaced with enteric-coated mycophenolate sodium (Myfortic®, Novartis 720 mg b.d.) with no improvement. The patient was transferred to our unit without a definitive diagnosis.

On admission, the patient was not pyrexial but was clinically markedly dehydrated and hypotensive (BP 95/60) and appeared chronically ill; he had by now lost 10 kg in weight. His abdomen was slightly tender in the left iliac fossa; the remainder of the clinical examination was unremarkable. Laboratory tests revealed a marked deterioration in transplant function with serum creatinine 370 μmol/L. There was metabolic acidosis (serum bicarbonate 14 mmol/L, anion gap 11) with an elevated serum lactate of 3.05 mmol/L (normal <2.2 mmol/L). Liver function tests were also abnormal (ALT 64 Units/L, Gamma GT 370 Units/L). White blood count was normal except for mild thrombocytopenia (platelet count 111 $\times$ 10^9/L). The patient was hydrated aggressively. Another ultrasound showed multiple liver cysts, a large right polycystic kidney with one dominant cyst that showed signs of previous bleeding. The transplant was normal in size, with good parenchyma and marked medullary pyramids in keeping with acute renal failure. The renal resistive index of the distal segmental arteries was elevated at 0.9. There was no renal transplant artery stenosis and no evidence of urinary tract obstruction. High-resolution ultrasound demonstrated a thickened wall of the left-sided colon with scattered mesenteric lymph nodes; there was no intra-abdominal fluid. Duplex ultrasound showed an aortobiiliac prosthesis and patent coeliac trunc and superior mesenteric artery. The inferior mesenteric artery could not be demonstrated. An abdominal computed tomography (CT) with angiography confirmed a large cyst of the right polycystic kidney with signs of previous haemorrhage and excluded significant stenosis of the coeliac trunc and superior mesenteric artery; again, the inferior mesenteric artery could not be demonstrated. Stool cultures and assays for *Clostridium difficile* toxin remained negative.

At this point, we asked the pathologist to exclude cytomegalovirus (CMV). Another meticulous examination of the colonic biopsy specimens revealed a solitary atypical inclusion body on haematoxylin–eosin stained specimens (Figure 1). A tentative diagnosis of CMV colitis was made.

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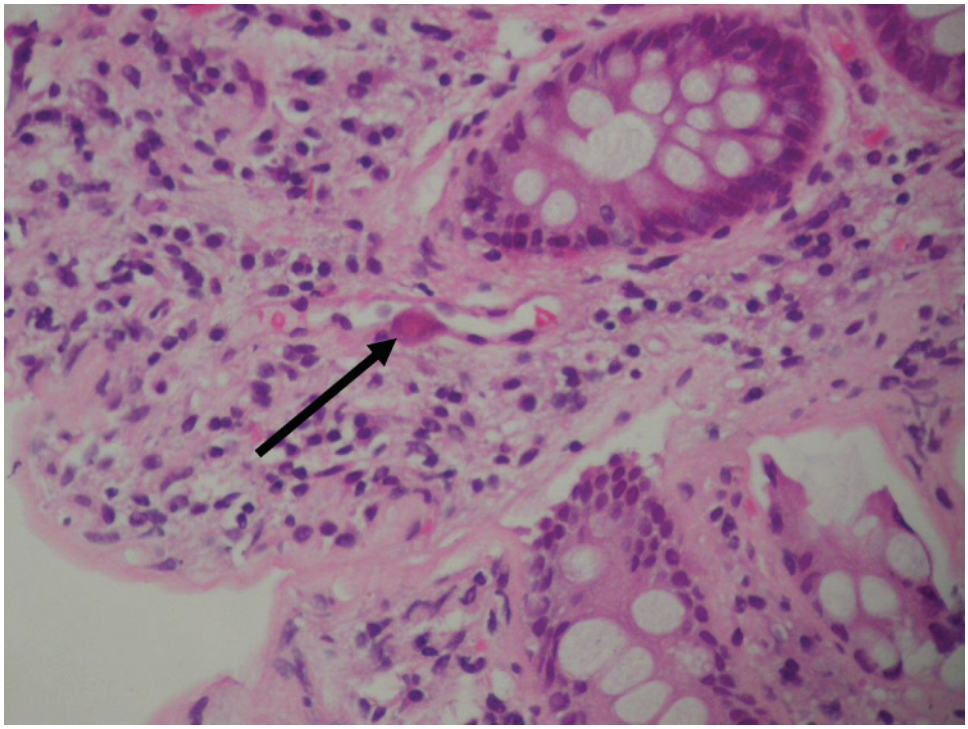

Fig. 1. Colonic biopsy with nucleus of a diseased endothelial cell with inclusion body (arrow) (haematoxylin/eosin stain, 400×)

Immunohistochemistry was strongly positive for CMV antigen (Figure 2). The CMV polymerase chain reaction (PCR) in serum was positive with 65 434 copies per milliletre. Intravenous gancyclovir therapy was commenced, resulting in dramatic improvement with abatement of diarrhoea. Renal function returned to baseline and the patient was discharged on valganciclovir. His diarrhoea resolved and he started to gain weight when seen in March 2008.

Discussion

Diarrhoea is not uncommon amongst renal transplant recipients [1]. The differential diagnosis is broad and chiefly includes co-morbidity, drugs and infection. It is also worthwhile to remember that diarrhoea influences the absorption of immunosuppressive drugs. Lower serum levels must be anticipated with cyclosporine while diarrhoea increases serum levels of tacrolimus. Co-morbid causes of diarrhoea include diabetic autonomic neuropathy, ischaemic colitis, inflammatory bowel disease and other gastrointestinal disorders. More than 700 drugs can also cause diarrhoea (Table 1) [2]. MMF and Sirolimus are most frequently implicated in transplant recipients. MMF is an ester prodrug of the active immunosuppressant mycophenolic acid. It is a noncompetitive, selective and reversible inhibitor of inosine monophosphate dehydrogenase, an important enzyme in the *de novo* synthesis of guanosine nucleotides in lymphocytes [3]. MMF is frequently used in renal transplant recipients, and a very recent study demonstrated the superiority of low-dose calcineurine inhibitors together with MMF over conventional immunosuppression [4]. MMF is often used as a supposedly more effective substitute for azathioprine although its superiority has been disputed [5]. Gastrointestinal side effects, such as nausea, diarrhoea and abdominal pain following ingestion, have been reported ever since MMF became available and their incidence has been reported to be as high as 45% in some reports [6]. Direct as well as indirect effects of the active metabolite, mycophenolic acid, have been implicated [7]. Dose reduction and ingestion with food are often recommended to alleviate gastrointestinal side effects of MMF, but, to our knowledge, these measures have not been evaluated in a double-blind fashion. Enteric-coated mycophenolate sodium (EC-MPS, Myfortic^{TM}, Novartis) is marketed as a superior formulation with regard to diarrhoea and other gastrointestinal side effects. This issue is fiercely disputed and beyond the scope of our little teaching point, suffice to say that current data are probably inadequate to prove or disprove the superiority of EC-MPS over generic MMF. In our case, MMF had been replaced with EC-MPS and the only other suspicious drugs, the proton pump inhibitor and allopurinol, were stopped, all to no avail.

Infections are another important cause of diarrhoea after solid-organ transplantation [7], and a comprehensive discussion is provided elsewhere [8]. Table 2 provides a list of common infectious causes of diarrhoea. CMV represents the most common opportunistic viral infection after solid-organ transplantation, and the incidence of clinically apparent disease varies between 20% and 60%; different immunosuppressive regimes are thought to be responsible. The mortality of untreated CMV disease in renal transplant recipients is high. Common clinical manifestations include malaise and low-grade fever, elevated liver function

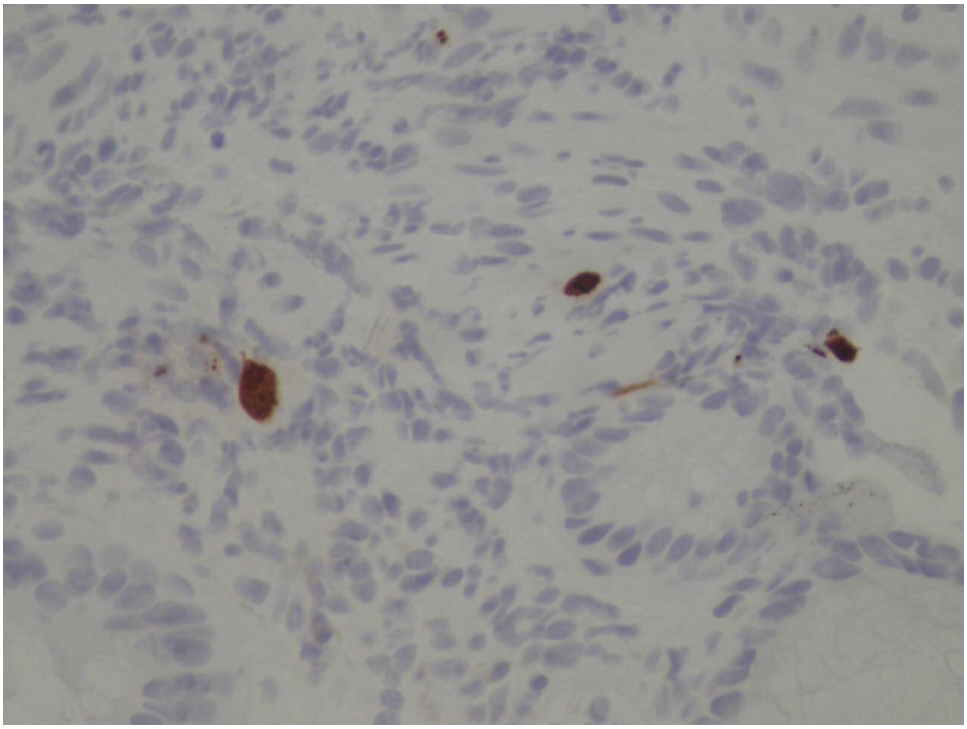

Fig. 2. Colonic biopsy with several CMV positive cells (CMV immunohistochemistry, 400×)

Table 1. Drug-induced diarrhoea [2,7]

Allopurinol	Lipase inhibitors
Antineoplastic chemotherapy	Mycophenolate mofetil
Biguanides	NSAIDs
Broad-spectrum antibiotics (via disturbance of the gastrointestinal flora)	Prokinetic agents (metoclopramide,cisapride, domperidone)
Carbamazepine	Proton-pump inhibitors
Cholinergic agonists and choline esterase inhibitors	Quinidine
Cimetidine	Simvastatin
Clonidine	Sirolimus
Colchicine	Sulphonylureas
Enteral feeding and nutritional supplements	Tacrolimus
Everolimus	Tetracycline
Ferrous sulphate	Theophylline
5 HT3-antagonist antiemetics	Ticlopidine

tests, leucopenia and retinitis. CMV pneumonitis is rare but feared. Late cases have been described [9] although most cases are seen in the first 6 months post-transplantation. These late cases are also more likely to present in an atypical manner, often with the absence of classical symptoms.

CMV infection of the gastrointestinal tract is less well appreciated. The first cases were reported in patients infected with the human immunodeficiency virus (HIV) in the early 1990s [10]. CMV infection of the gastrointestinal tract is also seen as a super-infection in patients with ulcerative colitis [11], but remains exceedingly rare in previously healthy, immunocompetent individuals. The case presented here illustrates CMV colitis as a diagnostic pitfall >4 years after renal transplantation. Maes and colleagues have suggested an algorithm for evaluation of diarrhoea in renal transplant recipients [12] and screening for CMV is included in step 1 of this algorithm. CMV should indeed be top of the list when a transplant patient presents with diarrhoea and weight loss and inflammatory changes on biopsy. *De novo* inflammatory bowel disease in renal transplant recipients is exceedingly rare [13]. It is also worthwhile remembering that lack of CMV pp 65 antigen in peripheral blood does not exclude CMV infection of the gastrointestinal tract [14]. We would therefore suggest to include CMV immunohistochemistry of colonic biopsies into the algorithm proposed by Maes and colleagues [12]. It is tempting to speculate as to the relevance of previous *Campylobacter jejuni* infection in our patient, since expression of pro-inflammatory cytokines by macrophages is known to facilitate CMV infection [15]. We were also keen to exclude a vascular aetiology of the diarrhoea, since the patient had previously undergone abdominal aortic aneurysm repair. CT angiography showed no stenosis of the celiac trunk and superior mesenteric artery (the inferior mesenteric artery is usually ligated in aneurysm repair). We cannot exclude that low-grade ischaemia played a role in this case although the colonic biopsies did not show any signs of ischaemia.

In summary, our little teaching point serves to remind us that drug-induced diarrhoea due to MMF remains a diagnosis of exclusion, i.e. other causes of diarrhoea in the transplant recipient need to be ruled out before this diagnosis can be made with confidence. Another teaching point is that pathologists find it easier to pick up inclusion bodies if they are alerted to the possibility of CMV disease. In our case, an inclusion body made the diagnosis. We emphasize the importance of close liaison with the pathologist and

Table 2. Common microbial aetiologies of diarrhoea

Infectious agent	Epidemiology/risk factors
Bacteria	
Bacillus cereus (toxin)	Ingestion of contaminated starch food (rice)
Campylobacter jejuni	Ingestion of undercooked contaminated poultry
Clostridium difficile	Previous use of antibiotics, contact with infected patients
Clostridium perfringens (toxin)	Ingestion of contaminated food (meat, poultry, gravy)
Enterohemorrhagic E. coli	Ingestion of contaminated meat
Enterotoxigenic E. coli	Ingestion of contaminated food/water
Listeria monocytogenes	Ingestion of contaminated un-pasteurized dairy products
Salmonella species	Ingestion of contaminated meat, egg or chicken
Shigella species	Ingestion of contaminated food/water (developing world), contact with infected patients (developed world)
Staphylococcus aureus (toxin)	Ingestion of contaminated food, improper handling (meat)
Vibrio species	Consumption of contaminated raw seafood (shellfish)
Yersinia species	Consumption of contaminated food (milk); thalassaemia
Viruses	
Noro-, Astro-, Sapo-, Rota-, Adenovirus	Contact with infected patients
Fungi	
Candida, Aspergillus	Rare; neutropenia, steroid use
Parasites	
Blastocystis hominis	Contact with infected patients, water or food
Cryptosporidium parvum	Contact with infected patients or animals or contaminated swimming water, poor sanitation, HIV
Cyclospora cayetanensis	Consumption of contaminated food, particularly fruit
Entamoeba histolytica	Consumption of contaminated food or water (developing countries); fecal-oral contact in homosexual patients (developed countries)
Giardia lamblia	Contact with infected patients, animals, or water, poor sanitary conditions and insufficient water treatment

assume that more of these inclusion bodies lurk undetected in the mucosa of transplant recipients with diarrhoea.

Teaching points

(i) Gastrointestinal side effects of MMF are common and include abdominal pain following ingestion, nausea and diarrhoea.
(ii) It may be tempting to ascribe diarrhoea in a renal transplant recipient to drugs such as MMF but the differential diagnosis is broad and a complete workup is essential.
(iii) Infectious causes of diarrhoea in renal transplant recipients are common and potentially dangerous.
(iv) CMV colitis is a recognized cause of diarrhoea in immunosuppressed patients. It is dangerous and potentially fatal, as well as difficult to diagnose. The key to diagnosis is a high degree of suspicion, endoscopy with biopsy and demonstration of inclusion bodies in mucosa together with immunohistochemistry for CMV.
(v) Inclusion bodies in histological specimens are less than obvious, and CMV immunocytochemistry is neither cheap nor routine; hence pathologists appreciate it if they are alerted to the possibility of CMV.

Conflict of interest statement. Dr Woywodt participated in the Myqol study (A study of the effect of changing to Myfortic on quality of life in patients with gastrointestinal symptoms related to Mycophenolate Mofetil therapy after kidney transplantation) by Novartis. He has no other involvement with Novartis, nor its competitor Roche and has not received honoraria, grant support or any other monies from either of the companies. The Renal Unit at Lancashire Teaching Hospitals NHS Foundation Trust has received reimbursement for the Myqol study on a per-patient basis. All other authors declare no conflict of interest.

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Received for publication: 30.4.08
Accepted in revised form: 6.5.08