NDT Plus (2011) 4: 342–345
doi: 10.1093/ndtplus/sfr074
Advance Access publication 18 July 2011

NDT PLUS
Nephrology Dialysis Transplantation

Teaching Point
(Section Editor: A. Meyrier)

Cytomegalovirus in the transplanted kidney: a report of two cases and review of prophylaxis

Shuchi Anand[1], Julie M. Yabu[1], Marc L. Melcher[2], Neeraja Kambham[3], Zoltan Laszik[4] and Jane C. Tan[1]

[1]Department of Medicine, Stanford University School of Medicine, Palo Alto, CA, USA, [2]Department of Surgery, Stanford University School of Medicine, Palo Alto, CA, USA, [3]Department of Pathology, Stanford University School of Medicine, Palo Alto, CA, USA and [4]Department of Pathology, University of California San Francisco, San Francisco, CA, USA

Correspondence and offprint requests to: Shuchi Anand; E-mail: sanand2@stanford.edu

Keywords: cytomegalovirus infection; renal allograft biopsies; valganciclovir prophylaxis

Cytomegalovirus (CMV) disease is a leading cause of infectious morbidity and mortality in patients with a kidney transplant. Before the advent of specific prophylaxis and therapy, more than half of the patients who had not been exposed to CMV prior to kidney transplantation developed primary disease and ~20% of patients with prior evidence of exposure developed reactivation disease [1]. Mortality estimates from CMV disease exceeded 30% [2]. Although use of prophylaxis or preemptive treatment of detectable viral load has drastically reduced its burden, CMV remains a common clinical problem with a wide variety of presentations.

To highlight the importance of clinical vigilance for CMV even in the current era of effective prophylaxis, we describe two adult kidney transplant recipients with invasive CMV disease. Both patients were diagnosed with CMV disease after they underwent allograft biopsy, which demonstrated the rare finding of CMV inclusions in the kidney allograft.

Case 1

A 64-year-old man with end-stage kidney disease (ESRD) of unknown etiology, gout and nephrolithiasis underwent deceased donor kidney transplantation 5 years after initiating hemodialysis. Serology testing immediately prior to transplant indicated presence of immunoglobulin G (IgG) antibodies to CMV, Epstein–Barr virus (EBV) and Varicella Zoster virus. The donor tested negative for CMV antibody.

As his calculated panel-reactive antibody level was 68%, the recipient received rabbit anti-thymocyte globulin (rATG) induction therapy. He was begun on tacrolimus, mycophenolate mofetil and prednisone for maintenance immunosuppression. Except for mild suppression of his white blood cell count to 2.5 k/μL, he had an uneventful postoperative course with prompt allograft function immediately post rATG induction. He initially received valganciclovir for prophylaxis against CMV disease. He was switched to acyclovir 800 mg three times daily on postoperative Day 4 as the patient would have incurred a high out-of-pocket cost for valganciclovir.

On discharge, his white blood cell count recovered to 9.5 k/μL and his serum creatinine (SCr) improved to 2.1 mg/dL. However, ~3 weeks postoperatively, his SCr rose to 2.4 mg/dL. Prerenal azotemia was suspected in the setting of relatively poor fluid intake. He was encouraged to increase oral intake, and acyclovir was discontinued in order to minimize potential nephrotoxicity. His SCr improved thereafter to 1.8 mg/dL. Two weeks later, his SCr again increased to 2.3 mg/dL in conjunction with a drop in his white blood cell count to 2.9 k/μL. Blood levels of mycophenolic acid, the active metabolite of mycophenolate mofetil, had been routinely measured and were largely within our target range of 2–4 μg/mL (1.7–4.6 μg/mL). The patient reported a poor appetite without much nausea or dysphagia. He denied any concurrent diarrhea, abdominal discomfort, fevers or flu-like symptoms. Physical examination was unremarkable. Urinalysis demonstrated trace protein.

A transplant kidney biopsy was performed and is shown in Figure 1 panels A and B. The biopsy revealed mild patchy interstitial inflammation associated with scattered CMV inclusions. Characteristic intracytoplasmic and intranuclear inclusions were identified in endothelial cells of peritubular capillaries and also blood vessels in perirenal adipose tissue. Chronic tubulointerstitial damage was minimal (<5% of cortex sampled). There was no evidence of acute or chronic rejection. Immunohistochemical confirmation of CMV infection was obtained using a mouse monoclonal antibody (anti-CMV antibody, clone CCH2+DDG9; dilution 1:100; DAKO, Carpinteria, CA).

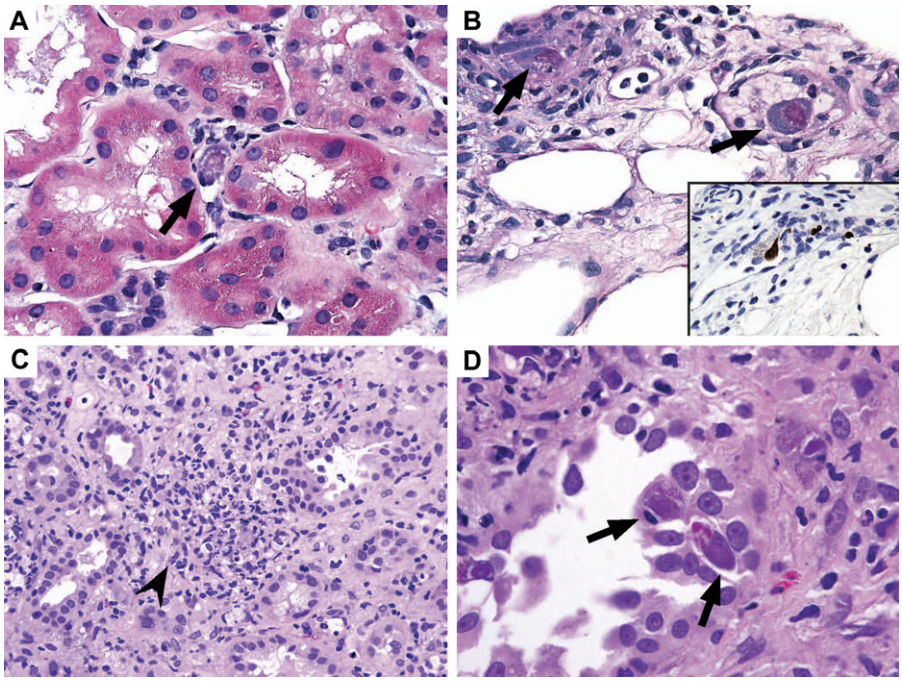

Fig. 1. The renal allograft biopsy in Case 1 shows (panel **A**) mild interstitial inflammation and a CMV inclusion (arrow) in a peritubular endothelial cell; similar inclusions (panel **B**, arrows) were identified in the capillary endothelial cells within adjacent adipose tissue. The inclusions stain brown with CMV immunostain (panel B, insert). Case 2 allograft biopsy (panel **C**) shows severe interstitial inflammation (arrow head) composed of lymphocytes, histiocytes and occasional eosinophils; (panel **D**) tubular epithelial cells have CMV inclusions characterized by enlarged eosinophilic nuclei and granular cytoplasm (arrows).

After the biopsy results were obtained, serum was sent for CMV polymerase chain reaction (PCR), which showed the presence of 20 300 viral copies/mL. Valganciclovir was obtained through our institution's emergency drug provision program and later through a compassionate program from the drug manufacturer. The viral load fell to 1650 copies/mL after 2 weeks of treatment with valganciclovir 450 mg twice daily and became undetectable within 4 weeks. The patient's white blood cell count recovered to 4.3 k/μL. The SCr reached a plateau at 2.0 mg/dL from a peak of 2.3 mg/dL and has remained at this level for several months.

Case 2

A 68-year-old woman with ESRD presumed secondary to hypertension underwent deceased donor kidney transplantation 13 years after initiating hemodialysis. Her pre-transplant serologies were positive for IgG antibodies to CMV and EBV. Donor serologies were positive for CMV antibody. The patient's panel-reactive antibody was 49% prior to transplant. She received rATG for induction and tacrolimus, mycophenolate mofetil and prednisone for maintenance immunosuppression. Her postoperative course was notable for rapid atrial fibrillation. She had excellent allograft function with an SCr of 0.8 mg/dL by Postoperative Day 8. For post-transplant prophylaxis of CMV, she received valganciclovir for 3 months.

Her allograft function remained stable until 3 months post-transplant at which time her SCr increased to 1.1 mg/dL. By 5 months post-transplant, the SCr had risen to 2.0 mg/dL. She also developed anemia and leukopenia with a hematocrit of 23% and white blood cell count of 3.8 k/μL. She reported fatigue and dyspnea on exertion but denied any gastrointestinal or urinary symptoms. On physical examination, she was pale, afebrile and normotensive, and her allograft was non-tender to palpation. Urinalysis showed 21–50 white blood cells/high power field, no hematuria and 3+ protein. Urine culture did not indicate any infection. Spot urine protein-to-creatinine ratio was 16.5 g/g, suggesting massive proteinuria.

After transfusion with 2 units of packed red blood cells, a transplant kidney biopsy was performed (Figure 1 panels C and D). The light microscopy revealed CMV nephropathy with extensive mixed interstitial inflammation and focal ill-defined granulomas with epithelioid histiocytes. Several tubular epithelial cell nuclei had intranuclear and intracytoplasmic inclusions characteristic of CMV infection. Focal segmental glomerulosclerosis was also identified in two glomeruli. The CMV infection was confirmed by immunohistochemistry. The serum CMV antigen test returned positive with 66 positive cells per 200 000 cells.

Based on the biopsy results, mycophenolate mofetil was discontinued and valganciclovir 450 mg twice daily was started for treatment of CMV. Three weeks later, the SCr improved to 1.6 mg/dL, and a repeat CMV test was negative. Two weeks later, mycophenolate mofetil was restarted at 250 mg twice daily and increased to 500 mg twice daily by 8 months post-transplant.

Discussion

CMV of the allograft kidney, particularly with classic intranuclear inclusions as seen in the cases we presented, is a rare presentation of invasive CMV disease. In the few available reports, the pathologic features of CMV in the allograft have been variably described and clinical significance remains uncertain [3]. Richardson *et al.* [4] first proposed in 1981 that CMV-related kidney disease presented primarily as a glomerulopathy with necrosis of endothelial cells and tubular infiltration by mononuclear cells. No evidence of direct CMV invasion was noted. However, a strict association between CMV infection and this pathologic lesion was not reproducible, and it is postulated that Richardson *et al.*'s findings may have been part of the spectrum of transplant glomerulopathy.

A more recent study by Liapis *et al.* [5] described a variety of lesions associated with a positive result for CMV PCR of allograft tissue, including no changes, interstitial nephritis, intranuclear inclusions and acute rejection. Evidence of CMV in the allograft as detected by tissue PCR was noted in half of cases with CMV viremia where biopsy was performed for elevated SCr. Inclusions in epithelial or endothelial cells were present in only 2 of the 10 cases. A few other reports have highlighted the rarity of CMV inclusions in epithelial or endothelial cells as definitive evidence of CMV infection of the allograft kidney [5, 6].

In contrast to the widely accepted adverse effects of BK virus infection in the allograft kidney, it is not clear whether CMV infection creates a direct and/or reversible injury to the allograft. In a report of 10 patients with CMV inclusions in the allograft who were subsequently treated with ganciclovir, Kashyap *et al.* [6] found that although nearly all patients had cleared the virus, only a subset (40%) had some posttreatment decline in SCr, with one patient recovering to the prior nadir level. In our patients, although treatment with valganciclovir appeared to eradicate the virus, allograft function remained impaired. CMV viremia may cause indirect injury to the allograft. One postulated mechanism of glomerular injury is upregulation of major histocompatibility antigens and subsequent stimulation of the immune system, resulting in increased risk for rejection [7]. Some prospective observational studies in kidney transplant recipients have identified CMV infection as a risk factor for episodes of rejection [8, 9].

Recent research has focused on prophylaxis or preemptive treatment of early disease in high-risk patients not only to reduce disease-related mortality but also to prevent this indirect route of allograft injury. Among the most consistently identified risk factors for symptomatic or hospitalized CMV disease in the post-transplant period is donor CMV IgG positive, recipient negative (D+/R−) serostatus. In an analysis of data from over 33 000 kidney transplant recipients collected in the United States Renal Data System, CMV D+/R− pairs experienced a 5-fold increase in the odds of hospitalization due to CMV disease, compared to the lowest risk group of seronegative donors and recipients (D−/R−) [10]. The clinical scenario discussed in this report, that is, D+/R+, ranks second highest for risk of disease, with odds for hospitalization being double that of the lowest risk group. Induction therapy with lymphocyte-depleting antibodies as was utilized in our cases has been reported to increase the risk for disease as well [11].

Widespread use of ganciclovir and then valganciclovir prophylaxis or preemptive treatment of CMV has significantly attenuated the incidence of disease. Intravenous ganciclovir was first evaluated as a prophylactic treatment in CMV seropositive recipients in concordance with lymphocyte-depleting antibody therapy [12]. The incidence of CMV disease in the treatment group (15%) was about half that of the placebo-treated group. A later trial comparing the efficacy of oral ganciclovir with the more bioavailable valganciclovir (in high-risk group of D+/R−) reported equal rates of reduction in CMV disease, with a slightly lower rate of CMV viremia in the valganciclovir-treated group during the 100 days of prophylaxis [13].

The duration of prophylaxis remains under investigation. The Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines advocate at least 3 months of prophylaxis in high-risk groups. However, due to an increasing recognition of delayed-onset CMV, a longer duration of prophylaxis for high-risk groups (for example, those with D+/R− serostatus or high degree of immunosuppression) has been proposed. In the initial trial of valganciclovir prophylaxis for 100 days post-transplant, ~20 and 30% of patients underwent treatment for active CMV disease by 6 and 12 months, respectively [13].

The patient in our second case likely had delayed-onset CMV disease with primary infection with a new strain or reactivation, clinically evident at ~5 months post-transplant (or 2 months post prophylaxis). The recent IMPACT study, a multicenter randomized controlled trial of 326 high risk (D+/R−) patients, reported that the 12-month incidence of CMV disease was halved (16%) in the group receiving 6 months of prophylaxis, compared to the 3-month prophylaxis (36%) group [14].

The benefits for extended prophylaxis must be balanced against its side effects as well as cost of treatment. Most commonly reported side effects of valganciclovir include leukopenia, thrombocytopenia and diarrhea. Leukopenia in particular is a major and clinically consequential side effect, occurring in 8–20% of patients treated with valganciclovir. Patients receiving higher doses of valganciclovir, a second kidney transplant or simultaneous kidney and liver transplant are at higher risk. Treatment withdrawal with or without a dose reduction in mycophenolate mofetil is the typical management strategy, although correction of leukopenia with use of granulocyte colony stimulating factor has been reported as well.

Valganciclovir costs $43/450 mg tablet or $2600 for a 1-month supply at the typical prophylaxis dosage. The patient in our first case fell in the Medicare coverage gap in the United States, affectionately known as the 'doughnut' hole. In the Standard Medicare Plan, an enrollee could have to pay up to $3850/year when the total retail costs of his or her medications exceed ~$2135. This out-of-pocket cost is additive to the typical monthly premium, until drug costs exceed $5541. With the addition of his transplant immunosuppressive drugs, our patient fell in this coverage gap immediately after transplant and could not afford the valganciclovir. This scenario is of critical importance, on an aggregate level, as an

extended six-month duration of CMV prophylaxis may be cost effective due to prevention of disease and related hospitalization or mortality [15]. However, the practical issue of direct costs to the patient must be addressed before this practice can be widely adopted.

In summary, we report two unusual cases of CMV disease with evidence of CMV in the allograft kidney. Pathologic features of CMV in the allograft include intracytoplasmic inclusions and interstitial nephritis; the clinical significance of its presence remains uncertain. Prophylaxis with valganciclovir can significantly reduce the incidence of CMV disease, but delayed onset CMV disease continues to be a significant entity requiring clinician awareness. The duration of prophylaxis is currently under investigation and may be limited in part by costs incurred by patients as well as side effects of the drug of choice.

Teaching points

(1) Unlike BK virus nephropathy, evidence of CMV in the allograft kidney likely does not cause direct injury to the kidney allograft. However, it is postulated that CMV upregulates major histocompatibility antigens, leading to an increased risk of rejection.

(2) Patients at highest risk for CMV include recipients without prior exposure who are receiving an allograft from a donor with prior evidence of CMV exposure and those that have received lymphocyte-depleting therapy for induction.

(3) Delayed onset CMV disease is an increasingly recognized clinical entity. Prolonging duration of prophylaxis may reduce the incidence of CMV disease but its benefits must be weighed against the side effects and cost of valganciclovir.

Conflict of interest statement. None declared.

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Received for publication: 16.3.11; Accepted in revised form: 26.5.11