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Title: Very late onset Cytomegalovirus disease with ganciclovir resistance >15 years following renal transplantation

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Abstract:

Cytomegalovirus (CMV) infection is a significant cause of morbidity and mortality after solid organ transplantation. There has been a significant shift in disease epidemiology with the introduction of antiviral prophylaxis, with CMV disease occurring later and clinical presentations more atypical. We describe two cases of very late onset CMV disease where first disease occurred 15 and 18 years post renal transplantation, with both cases complicated by antiviral drug resistance. We subsequently review the published cases and literature of very late onset CMV disease (onset >10 years post solid organ transplantation) as an increasingly recognised phenomenon which is emerging as an important aspect in improving long term patient outcomes in the current era of renal transplantation.

Introduction

Cytomegalovirus (CMV) infection is a significant cause of transplant-related morbidity and mortality[1-3]. Advances in the diagnosis, monitoring, and therapeutics have resulted in significantly improved outcomes from CMV disease[1-3]. However, with these advances there has been a change in the epidemiology of CMV disease with an increasing trend towards 'late onset' CMV disease, more atypical disease presentations, as well as increasing rates of antiviral resistance[3-12]. Due to widespread use of valganciclovir prophylaxis, the timing of CMV disease has shifted to after the cessation of antiviral prophylaxis, with "delayed" onset CMV occurring most frequently in the 3-12 months post solid organ transplantation (SOT)[3-9]. "Late" onset CMV has been defined as occurring more than 12 months after transplantation[3-9] and is becoming increasingly important as 10 year graft survival rates for renal transplant are approaching 75%[13].

We describe two cases of "very late" onset CMV disease due to viral reactivation, with first episode of CMV disease presenting more than 15 years after renal transplantation [Figure 1 & 2]. Both cases did not have antecedent changes in immunosuppression, and both cases developed drug resistant CMV disease.

Case 1:

A 68 year-old CMV seropositive male with IgA nephropathy received a renal transplant from a live-related, CMV seropositive, donor in 1996. No CMV prophylaxis was given. He experienced acute cellular rejection one month after transplant which was treated with pulse methylprednisolone and muromonab. Asymptomatic CMV pp66 antigenaemia developed one month later and was managed with 10 days of intravenous ganciclovir. Oral aciclovir was continued (800mg three times daily) for three months afterwards. Long term immunosuppression consisted of prednisolone 5mg and mycophenolate mofetil (500mg mane, 250mg nocte). Graft function was stable with a creatinine of 180 micromol/L (reference range 60-110 micromol/L) due to interstitial fibrosis, tubular atrophy and glomerulosclerosis. There were no subsequent episodes of acute rejection.

The patient presented 15 years post transplantation with a 2 week history of worsening chest pain associated with 10kg weight loss. There had been no changes to his immunosuppression in the preceding year. Gastroscopy revealed multiple oesophageal and gastric ulcers, with positive immunohistochemistry for CMV. A CMV viral load was detectable in plasma, but below the lower limit of quantification of the assay at <400 IU/mL. Mycophenolate was reduced to 250mg twice

daily, and the patient completed 3 months of valganciclovir (recommended treatment dose of 450mg daily for CrCl 32mL/min) with symptomatic improvement.

The patient experienced recurrences of biopsy proven CMV oesophagitis and persistent ulceration over the subsequent 2 years. CMV treatment and events are summarised in Figure 1. Mycophenolate was changed to azathioprine in November 2013. Repeated attempts at CMV resistance testing were unsuccessful due to the low viral load.

In March 2014, the patient presented with haematemesis. Gastroscopy showed severe oesophagitis and duodenitis with extensive ulceration due to CMV. A CMV viral load was 418 IU/mL and resistance testing demonstrated two UL54 polymerase mutations: a N408K mutation conferring ganciclovir and cidofovir resistance [16], and a L773V mutation conferring both foscarnet and ganciclovir resistance [17]. There were no UL97 mutations detected. CMV viral load continued to rise despite ganciclovir, leflunamide, CMV immunoglobulin and cessation of azathioprine. Three further upper gastrointestinal bleeds occurred with gastroscopy demonstrating gastro-duodenal fistula formation with persisting gastritis and duodenitis. Donor-derived CMV specific T cells were administered with a CMV viral load at the time of 1377 IU/mL. Antiviral treatment was ceased one week after CMV specific T cells and the patient was monitored. Subsequent events are summarised in Figure 1.

A CMV viral load (8 months post T cell infusion) showed a CMV viral load of 47 IU/mL, and a repeat gastroscopy showed resolution of the duodenitis and gastritis, improving ulceration, and a healing fistula. There was no histological evidence of CMV. Throughout this period the patient experienced 25kg weight loss and severe malnourishment requiring a percutaneous endoscopic jejunostomy (PEJ) tube.

Nine months later, the patient presented in respiratory extremis due to the formation of an oesophageal-pleural fistula resulting in a tension pneumothorax. Due to the need for dialysis, the patient requested withdrawal of active care and palliation, and subsequently passed away. Biopsy of the ulcer recurrence and fistula demonstrated histopathological evidence of active CMV disease.

Case 2:

A 18 year old CMV seronegative male underwent a renal transplant from a deceased, CMV seropositive donor in 1996 for congenital renal hypoplasia. Antiviral prophylaxis consisted of 3 months of oral aciclovir (800mg three times daily) and weekly CMV immunoglobulin for 2 months. Asymptomatic CMV pp65 antigenaemia developed two months post transplantation and was treated with one week of ganciclovir, followed by return to oral aciclovir. Nine years after transplant his kidney function declined with a creatinine of 294 micromol/L (reference range 60-110 micromol/L) and biopsy showed chronic allograft nephropathy with interstitial fibrosis and tubular atrophy. Maintenance immunosuppression consisted of prednisolone 5mg and mycophenolate sodium 360mg twice daily. There were no episodes of acute rejection or changes in immunosuppression in the preceding year.

The patient presented in 2014 (18 years post transplantation) with an acute onset of fevers, bloody diarrhoea, and acute kidney injury (creatinine 380 micromol/L). A plasma CMV viral load was elevated at 5003 IU/mL. Colonoscopy demonstrated ulceration in the colon, with viral inclusion bodies and positive CMV immunohistochemistry, consistent with CMV colitis.

The patient received 2 weeks of IV ganciclovir with clinical and virological response by day 15 of therapy and continued on valganciclovir 450mg daily (recommended treatment dose adjusted for CrCl 25 mL/min by Cockcroft-Gault equation) for the following 6 months with suppression of CMV viraemia. The patient was subsequently changed to valganciclovir prophylaxis (450mg every 2nd day) throughout 2015, during which the patient developed asymptomatic, low level CMV viraemia (<400 IU/mL).

The patient experienced further episodes of biopsy proven CMV colitis through 2016, with major events and treatment summarised in Figure 2. Notably, chronic abdominal pain and viraemia persisted throughout this period. CMV resistance testing on blood was unsuccessful due to the low viral load.

Virus was subsequently extracted from colon biopsy tissue and CMV genotype resistance testing resulted in complete sequencing of the UL97 gene and partial sequencing of the UL54 gene. H520Q and A591V mutations were detected in the UL97 gene and the T5031 mutation in the UL54 polymerase gene was detected, conferring resistance to ganciclovir (high level) and cidofovir [15]. No resistance mutations to foscarnet were detected within the limitations of sequencing.

Figure 2 summarises further events and changes to CMV treatment. Mycophenolate was ceased with the initiation of leflunamide.

Progressive allograft dysfunction necessitated initiation of haemodialysis in 2018. Leflunamide was ceased after 6 months, and IVIG was stopped with initiation of haemodialysis. The patient remains asymptomatic from CMV with an undetectable viral load, and has been weaned off all immunosuppression.

Discussion:

These two cases describe very late-onset CMV gastrointestinal disease, occurring 15 and 18 years following renal transplantation respectively, in recipients on stable low-grade immunosuppression but with chronic allograft dysfunction. Both recipients experienced early (<100 days) post-transplant CMV reactivation which was successfully treated with a short duration of anti-CMV therapy without further CMV complications. Following a long period where the patients were managed for chronic allograft dysfunction without additional increase in immunosuppression, both patients presented with symptoms of CMV gastrointestinal disease at a time period where CMV infection is not classically suspected. Notably, both cases demonstrated persistent low-level viraemia following initial treatment for CMV disease, had received prolonged low-dose valganciclovir, and had genotypically confirmed antiviral resistance. Despite multiple anti-CMV therapies and a reduction in immunosuppression, one of the patients died as a direct result from a CMV-related complication.

"Very late" onset CMV disease, which we have defined as occurring more than 10 years following SOT, has been reported in the literature and is summarised in Table 1. Including both cases presented here, a total of 14 cases were found in the published literature. Thirteen out of 14 cases were in renal transplantation, probably related to inherent biases from renal transplantation being

the most common organ transplant and also having the highest rates of long term graft survival. Using the case report data available [See Table 1], the median age was 52 years (range 44-69 years) and the majority had chronic renal impairment. The majority had no antecedent increases in immunosuppression and did not develop resistant CMV disease. All of the clinical manifestations included some form of CMV related gastrointestinal disease. Notably none of the cases were on mTOR inhibitors such as sirolimus, which have been shown to have antiviral activity against CMV[1,17].

A significant proportion of the previously reported cases of very late onset CMV disease are de novo infection [Table 1]. It is important to differentiate de novo CMV infection compared to reactivation, as these are distinct in terms of risk factors and relation to immune function. Key interventions in preventing de novo CMV infection revolve around behavioural modifications to reduce exposure, similar to preventing CMV in seronegative pregnant women, and the use of leukocyte deplete and/or CMV seronegative blood products. The limited literature around very late CMV disease demonstrates vast variation in clinical presentation[1,4-9], and should always remain in the differential diagnosis in any illness post transplantation.

Previous studies have not been able to establish risk factors to predict late onset CMV disease after transplantation. Cummins et al describes two cases of late CMV colitis post renal transplantation, associated with marked deficiencies in the number and functionality of CMV specific CD8+ T cells[1]. Due to the nature of very late onset CMV this kind of information is not routinely known. Routine monitoring for CMV specific immunity or other surrogate immune markers in the very late transplantation period is not usually performed and long term, stable patients are seen infrequently for follow-up. The specific host factors contributing to the development of very late CMV reactivation warrants further research, as well as the cost-benefit of regular assessments of surrogate immune markers and assessments of CMV specific immunity in long term follow up.

The prevention and management of late and very late onset CMV is a fundamental component in the battle to improve long term transplant outcomes, but best practice is yet to be determined. There is a paucity of evidence to guide either duration of therapy or antiviral prophylaxis in this cohort, especially in the “very late” post transplantation period. The routine use of prophylaxis would seem unjustified in this very late transplantation period on the basis of overall very low incidence of CMV disease, potential for developing antiviral resistance, drug toxicity, and costs of

antiviral prophylaxis. Pre-emptive therapy with frequent viral load monitoring is inconvenient long-term and there is still a need to understand which transplant recipients are at the highest risk of viral complications, particularly when immunosuppression is stable. Additional guidance from CMV immune monitoring may be one way to identify patients requiring ongoing viral monitoring[2]. The drawbacks of prolonged antiviral prophylaxis may also include a delay in onset of CMV disease (especially within the 12 months post cessation) rather than elimination of risk[5]. Finally, the development of late onset CMV has been associated with poorer long-term outcomes of graft and patient survival, further highlighting the need for clinical vigilance, prevention, and optimised management[3,7,22].

Both of these cases were complicated by drug resistant CMV, including demonstrable UL54 polymerase mutations. Whether very late onset CMV disease is an independent risk factor for drug resistance, in particular CMV polymerase mutations, is not able to be established from these two cases alone. One case required tissue amplification for diagnosis of resistance due to a very low plasma viral load precluding amplification. Additional risk factors for developing CMV drug resistance present in both cases included previous prolonged exposure to antiviral agents (especially maintenance dosing with possible suboptimal drug levels), immunosuppression, and renal failure [10-12]. This also highlights the importance of resistance testing in the setting of disease, especially in refractory or relapsing cases of CMV.

Conclusions:

We describe two cases of very late onset CMV gastrointestinal disease with multi-drug resistant virus following renal transplantation. This highlights numerous important learning points about late CMV disease, with significant clinical heterogeneity differing from the traditional teachings around CMV disease post transplantation. Whether very late onset CMV disease represents a distinct clinical entity is yet to be fully elucidated, as well as the risk factors for developing very late onset reactivation and disease. We discuss some common characteristics among very late (>10 years post transplantation) CMV disease in solid organ transplantation, but further research is required before strong conclusions can be made. De novo CMV disease is pathophysiologically different to very late reactivation, but highlights an important area for patient education and risk factor modification. How patients post transplantation are monitored in the long term need to be revisited, with specific

consideration of the cost-benefit in routine assessments of CMV specific immunity and whether surrogate immune markers are of benefit. Continuous re-evaluation and improvement in the strategies for prevention and management of CMV post transplantation is essential to further improve long-term graft outcomes and reduce patient morbidity and mortality.

Declarations and Conflicts of Interests:

None

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None

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Author Contributions

SFK contributed to data collection, data analysis and drafting of manuscript with review of literature.

MKY contributed to conception, data collection, data analysis, and critical revision and coordination of manuscript.

MAS contributed to conception and critical revision of manuscript

PH contributed to data analysis and critical revision of manuscript

JS contributed to data collection, data analysis, critical revision and coordination of manuscript.

All authors gave final approval of the version to be published.

Tables & Figures:

Figure 1. See attached figure.

Timeline of important events, therapeutics, and relevant investigations for Case 1.

Figure 2. See attached figure.

Timeline of important events, therapeutics, and relevant investigations for Case 2.

Table 1. See attached document for table and caption.

Table 1. Summary table of published cases of "very late" onset CMV disease, defined as date of first onset of disease greater than 10 years post solid organ transplantation. Medication doses are stated if known. Cases were classified as de novo if stated in the case report, or if CMV infection developed in a patient with previously known negative CMV serology. *CMV - cytomegalovirus; D - donor; R - recipient; Tx - transplant; Cr - creatinine (normal range <100 micromol/L); pred - prednisolone; MMF - mycophenolate; aza - azathioprine; cyclo - cyclosporin; AIDP - acute inflammatory demyelinating polyradiculopathy; ? - unknown.*

	Age	**Sex**	**Organ**	**CMV D/R Status**	**CMV Prophylaxis**	**Time of onset post Tx**	**Recent Rejection**	**Resistant Disease**	**Renal Function**	**Clinical Disease**	**Immuno-suppression**	**CMV related Mortality**
Case 1	47	M	Kidney	D+/R-	None	18 years	No	Yes	Cr 294 micromol/L	Colitis.	Pred 5mg daily MMF 360mg BD	No
Case 2	68	M	Kidney	D+/R+	None	15 years	No	Yes	Cr 170 micromol/L	Oesophagitis.	Pred 5mg daily MMF 500/250mg	Yes
Boobes et al.	57	M	Kidney	D?/R+	Unknown	12 years	No	No	Cr 220 micromol/L	Nephritis & colitis.	Cyclo MMF 1g BD Pred	No
Slifkin et al.	45	F	Kidney	D-/R-	None	22 years	No	No	Cr 90 micromol/L	CMV syndrome with hepatitis & colitis. **De novo**	Aza 50mg BD	No
Burgan et al.	69	M	Kidney	D-/R-	None	12 years	Humoral 5 years prior.	No	eGFR 15 ml/min	CMV syndrome with Gastritis. **De novo**	MMF 500mg BD Cyclo Pred 5mg daily	No
Hodowanec et al.	44	M	Heart	D?/R-	Unknown	14 years	Humoral 4 years prior.	No	Not stated	CMV Syndrome with AIDP & Colitis. **De novo.**	MMF Cyclo Pred	No
Browne et al.	Describes 8 patients with CMV disease occurring >10 years post renal transplantation (i.e. very late disease). No further clinical details are available. This occurred within a cohort of 77 patients who developed late CMV disease defined as >1yr post transplant. Generalisations from the entire cohort noted the incidence of CMV											

	disease was more frequent closer to time of transplantation and incidence decreased with time. Acute rejection was reported to have preceded almost all cases of late CMV. 15% of overall CMV disease were de novo.

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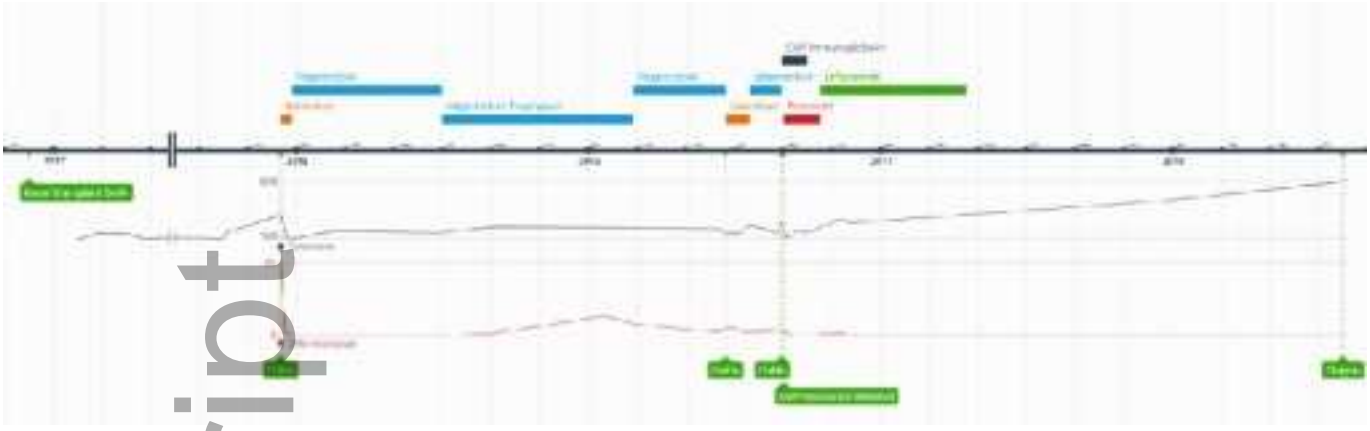

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