

Original Article

The Use of Sirolimus in Patients with Recurrent Cytomegalovirus Infection after Kidney Transplantation: A Retrospective Case Series Analysis

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ABSTRACT. Cytomegalovirus (CMV) is one of opportunistic infections post solid organ transplant and remains a cause of morbidity and mortality. Mammalian target of rapamycin inhibitors has a theoretical antiviral advantage compared to conventional immunosuppression. The primary outcome was to assess the viremic response and kidney function in a cohort of kidney transplant recipients (KTRs) with difficult to manage CMV infection when converted to sirolimus. We retrospectively analyzed the outcome of substituting sirolimus for mycophenolate mofetil (MMF) or tacrolimus in 18 KTR with difficult to manage, resistant/recurrent CMV viremia unresponsive or intolerant of standard anti-CMV treatment, or immunosuppression reduction. Safety and feasibility of sirolimus conversion were assessed through studying CMV viral loads, creatinine levels, immunosuppression, antiviral therapy, kidney function, and acute rejection episodes before and after starting sirolimus as well as the sirolimus side effects. Data were collected from the hospital filing system. The Wilcoxon matched-pairs signed-rank test and Friedman test were used for statistical analysis. The area under the curve for Log_{10} CMV viral load (log_{10} copies/ml) was significantly higher before than after the sirolimus switch ($P = 0.0156$). The median number of days on antiviral treatment was reduced after conversion to sirolimus [48 days (0–95); vs. 68 days (21–146)]. Acute rejection occurred more commonly before than after starting sirolimus [$n = 5$ (27.7%) vs. $n = 2$ (11.1%)]. Median serum creatinine

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before conversion to sirolimus was 175.5 $\mu\text{mol/L}$ (79–243), and showed no deterioration three months and one year after conversion [148 (69–271) and 162.5 (69–287) $\mu\text{mol/L}$, respectively, $P = 0.002$]. The use of sirolimus, often alongside tacrolimus and after discontinuation of MMF, is a useful strategy in

treating recurrent CMV viremia without provoking rejection.

Introduction

Despite the current advances in its diagnosis and management, human cytomegalovirus (hCMV) remains a significant source of morbidity and mortality after kidney transplantation with a reported incidence of 12.8% for CMV infection and 3.9% for CMV disease.¹

The therapeutic options for CMV infection after solid organ transplantation (SOT) are limited. All approved anti-hCMV agents like the first-line agent, ganciclovir (GCV), its oral prodrug valganciclovir (ValGCV), and the second-line agents, foscarnet (FOS) and cidofovir (CDV), target the viral DNA polymerase (pUL54). However, they are all hampered by dose-related toxicities including neutropenia for GCV and nephrotoxicity for FOS and CDV.^{2,3} In addition, there is emerging GCV resistance resulting from mutations in either pUL97 kinase or pUL54 DNA polymerase.

Reduction in immunosuppression, if feasible, is an important and complementary strategy, especially in severe clinical cases.^{2,4} However, CMV viremia is more common after augmented immunosuppression following organ rejection and in a significant proportion of high immunological risk patients or those who have already experienced rejection; there is limited potential for immunosuppression reduction.

There is growing evidence that mammalian target of rapamycin (mTOR) inhibitors have significant antiviral properties.⁵ mTOR is essential for the viral life-cycle and mTOR inhibition results in reduced production of immediate, early, and late viral proteins.⁶ The anti-CMV properties of mTORi appear to be associated with a clinically relevant reduction in CMV end-points among SOT recipient.⁵ In kidney transplant recipient (KTR), several studies have shown reduced rates of CMV viremia when mTORi are used *de novo* or introduced as a maintenance therapy,⁷⁻¹¹ compared with either mycophenolate mofetil (MMF)¹²⁻¹⁴ or cyclosporine¹⁵-based immuno-

suppression, with a reduction of up to 51% when compared with an antimetabolite.¹⁶ Even after antilymphocyte globulin prophylaxis, patients with sirolimus-based immunosuppression showed a very low CMV infection frequency.^{17,18}

Despite the theoretical advantage of mTORi in difficult to manage CMV infections, very few published studies show the impact of introducing a mTORi in these patients. Ozaki et al described nine patients with GCV-resistant CMV infection with DNA-proved point mutations, in whom sirolimus was introduced in place of a calcineurin inhibitor (CNI) or an anti-metabolite and in combination with GCV. In eight of the nine patients, undetectable CMV antigenemia was achieved within a median of 20.3 ± 10.1 days.¹⁹

For five years, our unit has introduced sirolimus in KTR with problematic CMV viremia unresponsive or unamenable to immunosuppressive reduction. The primary outcome of this study was to assess the viremic response and kidney function in a cohort of KTR with difficult to manage CMV infection when converted to sirolimus.

Subjects and Methods

We retrospectively studied a cohort of 18 consecutive adult patients who were transplanted from 2009 to 2015 at the Royal Free London NHS Foundation Trust with recurrent CMV infection after kidney transplantation. Safety and feasibility of sirolimus conversion were assessed through studying CMV viral loads, creatinine levels, immunosuppression, antiviral therapy, kidney function, and acute rejection episodes before and after starting sirolimus as well as the sirolimus side effects.

The study population was identified by having the following inclusion criteria: (1) Recurrent (3 or more) or prolonged episodes of CMV viremia, before the sirolimus switch, despite appropriate antiviral therapy and implementing strategies to combat clinically suspected CMV resistance,²⁻⁴ (2) Active CMV viremia at or immediately before and after sirolimus introduction with or without CMV

disease or end-organ disease.²⁰ Twenty-three patients were originally identified, of whom five were excluded from the analysis because sirolimus was introduced after the resolution of CMV viremia in four and one was intolerant to sirolimus and discontinued it.

Immunosuppression

According to the in-house immunosuppression protocols, all patients had basiliximab induction (20 mg IV on day 0 and day 4), tacrolimus (at 0.15 mg/kg/day, target trough level: 8–12 ng/mL within the first 3 months of transplantation and 6–8 ng/mL from month 4–12), MMF (1 g twice daily for the 1st month, reduced to 750 mg twice daily thereafter with a gradual reduction at 12 months to 500 mg twice daily), and early steroid withdrawal (methylprednisolone: 500 mg single dose at induction and 40 mg once daily for the first 3 days, followed by prednisolone 20 mg for the next 7 days and reducing to 5 mg thereafter aiming to wean off by 2 weeks).²⁰ One patient (number 9) required pre-transplant rituximab and plasma exchange for ABO incompatibility.

Viral monitoring and antiviral therapy

Whole blood samples for CMV surveillance were collected twice a week for the first 60 days posttransplant, then once a week with a targeted minimum follow-up of the first 90 days after transplantation. Post 90 days, whole blood samples for CMV PCR were obtained at every clinic visit or if CMV syndrome/disease was suspected.²⁰ CMV DNA in whole blood was quantified using a real-time PCR approach. The Applied Biosystems Taqman 7500 is used for CMV viral load assay. As a part of the assay quality control, we run a positive control with known CMV copy number to control for the interassay variation and to ensure that the results are comparable.

At our unit, preemptive therapy was adopted for every patient regardless of donor or recipient CMV serostatus. CMV treatment [ValGCV (900 mg BID) with dose adjusted for renal function] is initiated if CMV copies are >200 copies/mL of blood in CMV naïve patients. However, in recipients who are CMV

IgG positive, therapy is only started when CMV copies are over 3000 copies/mL of blood. In CMV viremic patients, whole blood samples for CMV PCR were collected twice weekly to follow episodes through to resolution. Therapy was discontinued following two consecutive samples where CMV DNA was undetectable (assay cutoff 200 genomes/mL) and after a minimum of two weeks.²⁰ Additional therapeutic options are implemented when CMV resistance is clinically suspected and include immunosuppression reduction (reduction of MMF to a minimum of 500 mg once daily with a switch to sirolimus if CMV viremia is still prevalent or rejection occurs. Tacrolimus is reduced at the discretion of the treating nephrologist), intravenous GCV, and FOS when appropriate.

Data collection

Patients' data were collected from the hospital filing system. For each patient we analyzed CMV viral loads for the period from the first positive test (>200 genomes/mL of whole blood; equivalent to IU/mL)²¹ until the commencement of sirolimus and then for an equal period following commencement of the drug. Creatinine levels before sirolimus conversion, at three months and at one year were recorded. Demographic data, immunosuppression, antiviral therapy, and acute rejection episodes were also recorded.

Statistical Analysis

All categorical data were reported as number (percentage) and numeric data as median (range). We used Wilcoxon matched-pairs signed-rank test to compare areas under the curve for Log₁₀ CMV viral load (log₁₀ copies/mL) before and after starting sirolimus. Friedman test was used for comparing serum creatinine levels before, three months after and one year after starting sirolimus. $P = 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences software version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Demographic data

The study was conducted on 18 KTR. Thirteen were males (72%) and five were females (28%) with median age at transplantation of 53 years (range: 20–73). Seven patients received kidneys from live donors and 11 from deceased ones. Fourteen patients (77.8%) had primary CMV infection, whereas the remaining four (22.2%) had either CMV reactivation or reinfection. Patient characteristics are illustrated in Table 1.

Immunosuppression and CMV infection data

The first viremic episode occurred after a median transplantation period of 28 days (range: 10–54). The median time to starting sirolimus from transplantation was 114.5 days (range 67–204). In most patients, sirolimus was started where there is ongoing CMV viremia, defined as a persistent count of >200 copies/mL CMV copies in CMV naïve patient and >3000 copies/mL of CMV IgG positive patient despite appropriate interventions and regardless of the presence of CMV disease or EOD (15/18). However, in three patients, we started sirolimus with low viral load or immediately after viremia resolution to avoid prolonged excessive immunosuppression reduction and as prophylaxis against CMV recurrence. Those patients had recurrent viremia while on sirolimus and thus were included in the analysis (patients 4, 5, 6). Sirolimus was used to replace MMF in all our patients, and in three of them, tacrolimus was discontinued as well (patients 1, 2, 3). The median tacrolimus trough levels were generally lower after than before starting sirolimus [8.9 (7.1–11.1) vs. 5.5 (0–7.9) ng/mL].

GCV resistance was tested in six patients. However, resistance mutations could be identified in three only (patients 6, 9, 15).

Impact of sirolimus conversion

The area under the curve for Log_{10} CMV viral load (log_{10} copies/mL) was significantly higher before than after the sirolimus switch ($z = 2.417$, $P = 0.0156$, Wilcoxon matched-pairs

signed-rank test) (Figure 1).

Acute rejection occurred more commonly before starting sirolimus in the context of immunosuppression reduction (5 patients, 27.7%), while only two patients had rejection after sirolimus (11.1%). All patients received ValGCV before sirolimus conversion, and one had FOS, while after sirolimus, 14 patients received ValGCV, and three of them had FOS as well. The remaining four patients could be managed without receiving antiviral therapy after the sirolimus switch (patients 4, 8, 14, 16). The median number of days on anti-viral treatment was reduced after conversion to sirolimus (48 days, range 0–95) as compared to before conversion (68 days, range 21–146). Median serum creatinine before conversion to sirolimus was 175.5 $\mu\text{mol/L}$ (range 79–243), and showed no deterioration at three months after conversion at 148 $\mu\text{mol/L}$ (range 69–271, $P = 0.002$) and 162.5 $\mu\text{mol/L}$ (range 69–287, $P = 0.002$) at one year (Figure 2). The serious side effects of sirolimus observed in our cohort were pneumonitis in four patients, due to either infection or sirolimus, and mild neutropenia in two patients.

Discussion

CMV infection continues to represent a clinical challenge in a subset of SOT recipients. We describe a cohort of 18 patients with either prolonged or recurrent (3 or more) episodes of CMV viremia. All possible treatment options, although limited, were exhausted before introducing sirolimus; in particular, immunosuppression reduction was either not possible because of immunological risk or resulted in rejection and had to be reversed. Furthermore, hCMV from three patients developed drug resistance, severely limiting therapeutic options. Facing such difficult to manage CMV infections, the choice was between CMV disease and losing the transplant through rejection precipitated by excessive immunosuppression reduction, particularly in high immunological risk patients. As such, introducing sirolimus, with its potential anti-CMV effect was a reasonable therapeutic option.

Table 1. Clinical characteristics of the study population.

No.	Gender	Primary renal disease	Age at Tx, years	Type of CMV infection	Time to 1 st viremic episode, days	Tx period before Sirolimus, days	Creatinine, µmol/L			Sirolimus serious side effects	Rejection		Antiviral therapy ^a , days		Median tacrolimus trough levels (C ₀ , ng/mL)	
							Before	3 M	1 Y		Before	After	Before	After	Before	After ^c
1	Male	Dysplasia	72	Re	21	112	189	174	189	Pneumonitis		+	103	24 ^b	10.4	0
2	Male	MN	61	1ry	22	119	189	174	189	Proteinuria Pneumonitis			90	48	9.9	0
3	Male	Traumatic renal loss	65	1ry	41	107	155	144	154				88	62 ^b	7.1	0
4	Female	Unknown	73	Re	11	98	87	131	165	Pneumonitis		+	55	0	7.9	7.9
5	Female	Interstitial nephritis	56	1ry	35	87	79	69	69		+		55	48	7.7	7.9
6	Male	Dysplasia/hypoplasia	20	1ry	38	77	174	130	131				67	47	8.7	4.2
7	Male	Renal dysplasia with reflux	28	1ry	22	165	119	111	120				109	79	7.1	3.5
8	Male	Pyelonephritis (congenital obstructive nephropathy)	27	1ry	54	199	192	187	217		+		146 ^b	0	7.5	4.1
9	Male	ADPKD	39	1ry	41	94	126	121	123	Pneumonitis			69	72	11.1	4.7
10	Male	Dysplasia	23	1ry	46	204	196	238	224		+		94	16	9.0	4
11	Female	DN	51	1ry	34	117	206	271	287		+		55	68	8.9	6.6
12	Female	MN and hypertensive renovascular disease	49	1ry	17	122	231	217	248	Neutropenia and thrombocytopenia			56	95	86	6.1
13	Male	ADPKD	33	1ry	28	67	153	141	146				21	73	9.2	4.6
14	Female	SLE	56	Re	10	150	199	162	160		+		49	0	8.4	6.6
15	Male	IgAN	51	1ry	32	182	123	107	103				94	8	9.8	6
16	Female	Renal TB	55	Re	27	170	137	97	121				52	0	8.9	5.5
17	Male	APKD	63	1ry	25	102	243	235	243	Neutropenia			52	68	10.5	6.2
18	Male	Obstructive uropathy	65	1ry	17	80	177	152	177				70	67 ^b	9.0	4.5
overall characteristics ^d	M: 13 (72%) F: 5 (28%)		53 (20–73)	1ry: 14 (77.8%) Re: 4 (22.2%)	28 (10–54)	114.5 (67–204)	175.5 (79–243)	148 (69–271)	162.5 (69–287)		5 (27.7%)	2 (11.1%)	68 (21–146)	48 (0–95)	8.9 (7.1–11.1)	5.5 (0–7.9)

MN: Membranous nephropathy, DN: Diabetic nephropathy, IgAN: IgA nephropathy, 1ry: Primary CMV infection, Re: Reactivation of CMV, ADPKD: Autosomal dominant polycystic kidney disease, SLE: Systemic lupus erythematosus, TB: Tuberculosis, ^aAll patients who received antiviral therapy had Valganciclovir, ^bPatients who had Foscarnet, ^cIn patients 1, 2, 3: tacrolimus stopped when sirolimus started, ^dData are presented as number (%) or median (range).

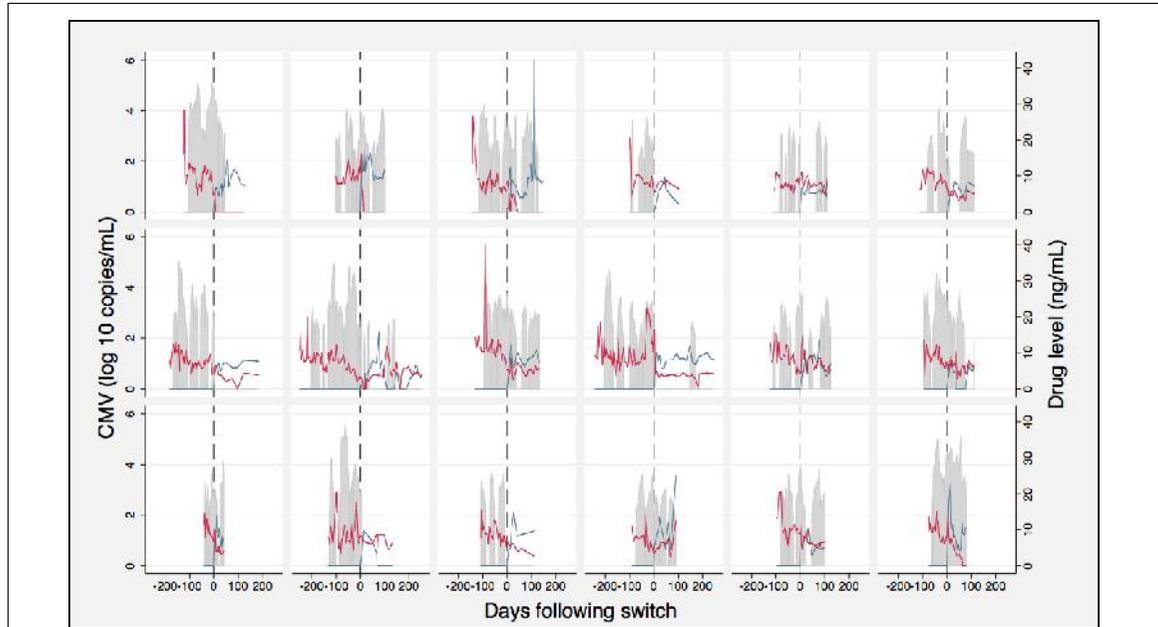


Figure 1. Log₁₀ CMV viral load (log₁₀ copies/mL), Sirolimus and tacrolimus levels (ng/mL) over the same period before and after starting sirolimus in 18 kidney transplant recipients who had prolonged and/or recurrent CMV.

Gray: CMV; Blue: Sirolimus; Red: Tacrolimus.

Although there are theoretical advantages to late conversion. The CONCEPT²² and ZEUS²³ studies recorded no significant difference in immunosuppression in patients with CMV viremia and CMV incidence in those converted late to mTORi compared to conventional treatment. However, all CMV-negative recipients who received a kidney from a CMV-positive donor evidence of benefit is controversial following

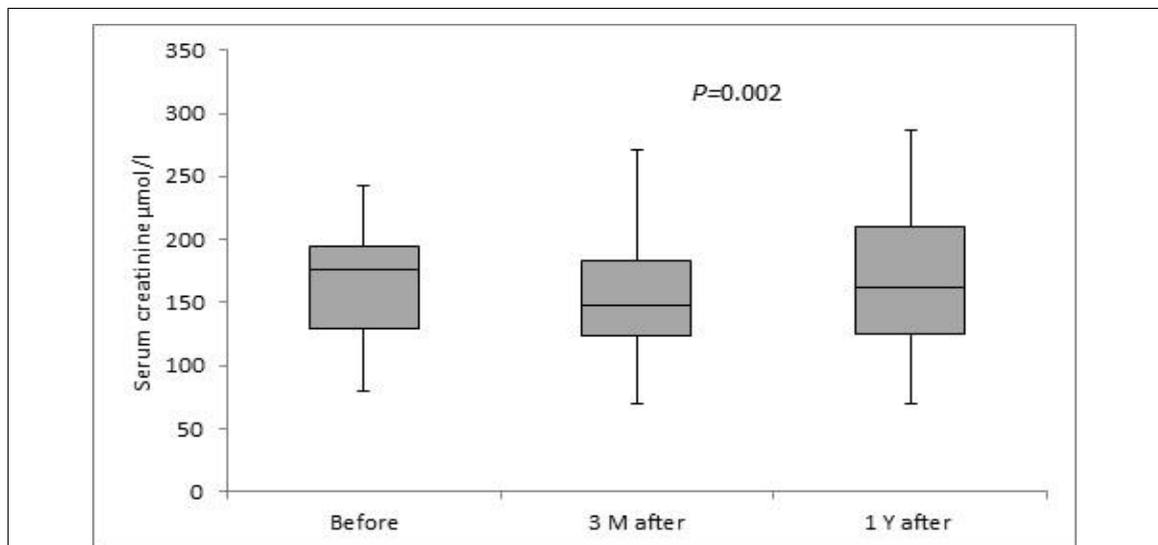


Figure 2. Serum creatinine levels before, 3 months after, and 1 year after switching to sirolimus ($n = 18$). The box blots depict the median and interquartile range.

in the CONCEPT trial received prophylaxis for CMV infection for a minimum of 12 weeks. This is different from the study group while the use of pre-emptive therapy is not mentioned in the methodology of the ZEUS study. In the SMART study^{9,24} however, there was a very significant benefit of mTOR inhibition 7.3% versus 28.2% ($P = 0.0016$), but conversion was early. What is not clear from the literature is whether converting to an mTORi late and in the setting of unresponsive CMV would be beneficial.

In our retrospective study, the total burden of CMV viremia following conversion to sirolimus was significantly lower than observed before. While most of the patients got further viremia the requirement and duration for antiviral therapy postconversion was less. It is possible that resolution of CMV viremia occurred naturally as a result of emerging immunity and it is not possible to exclude this in our study; however, this seems unlikely as all patients converted had prolonged and recurrent CMV up until the point of conversion.

Conversion to sirolimus might also represent a reduction in immunosuppression.^{7,10,25} However, the majority of the patients remained on a CNI (tacrolimus) (15/18) and there was no evidence for an increase in rejection episodes following switch suggesting the antiviral effect of mTORi is not at the expense of increased immunological risk. CMV is cited as a risk factor for acute rejection^{26,27} and it is possible that the reduction in CMV infection post-conversion mitigates against this. The absence of increased rejection rates was reflected in preserved kidney function in short-term follow-up over the year after sirolimus, though it might be argued that glomerular filtration rate is expected to improve with reducing CNIs and continuous recovery of tubular injury in the posttransplant period.

The evidence for switching to mTORi as a line of therapy for patients with proven or suspected CMV resistance is limited to one case series¹⁹ which also involved concomitant immunosuppression reduction, and few case studies.²⁸⁻³⁰ In all, as well as in our study, this

strategy has been considered as salvage therapy for CMV infection when other measures such as immunosuppression reduction, high-dose intravenous GCV, and FOS have been either exhausted or not possible. However, our study represents the largest number of patient series that has been reported in this specific context to date, who mostly are of high immunological risk, and the majority of whom remained on a potent CNI (tacrolimus).

Our study has some limitations including its retrospective nature and the small cohort size. The follow up period on sirolimus was limited to be the same as the period from the first viremic episode to starting sirolimus, thus late CMV recurrence could be missed from the analysis. Indeed, selection bias would have compounded the overall observed effect as it is not a true representation of all patients who suffer from CMV viremia. The improvement of CMV viremia cannot be fully attributed to the use of sirolimus alone. It is not possible to be certain that control of CMV viremia was not part of emerging immunity or an overall reduction in immunosuppression with the introduction of sirolimus. However, our study shows that the introduction of sirolimus facilitated the management of recurrent and resistant CMV in patients with moderate immunological risk despite the occasional side effects which may limit its use in some patients.

Overall, this represents our center's experience in managing difficult cases with CMV infection. It is likely that introduction of sirolimus permitted native immunity to control recurrent CMV viremia without an excess of rejection in a population of KTR who are of moderate-to-high immunological risk. Further multicenter studies are required to prove the therapeutic utility of mTORi as substitutive immunosuppression in patients with recurrent and/or prolonged CMV infections.

Conclusions

Patients with recurrent CMV viremia who are high immunological risk patients or those with anti-viral resistance risk life-threatening infec-

tion or sacrifice of the transplant and as such represent a challenging clinical problem. In our experience, the use of an mTORi often alongside a calcineurine inhibitor (tacrolimus in the current study), and after discontinuation of MMF is a useful strategy in treating recurrent CMV viremia without provoking rejection.

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