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Efficacy and safety of universal valganciclovir prophylaxis combined with a tacrolimus/mycophenolate-based regimen in kidney transplantation

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Summary

Background: Immunosuppressive and antiviral prophylactic drugs are needed to prevent acute rejection and infection after transplantation. We assessed the efficacy and safety of the introduction of universal valganciclovir prophylaxis in combination with a tacrolimus/mycophenolate-based regimen in kidney transplantation at our centre.

Methods: We reviewed all consecutive patients who underwent kidney transplantation over a 5.5-year period. Patients transplanted from January 2000 to March 2003 (period 1) were compared to patients from April 2003 to July 2005 (period 2). In period 1 patients were treated with basiliximab, cyclosporine, steroids and mycophenolate (or azathioprine). Prophylaxis with valacyclovir was prescribed in cytomegalovirus (CMV) D+/R- patients, while any R+ patients were managed with a preemptive approach. In period 2, immunosuppression consisted of basiliximab or thymoglobulin induction, tacrolimus, steroids and mycophenolate. Three-month CMV prophylaxis with valganciclovir was used in all at-risk patients.

Results: Data analysis included 73 patients (period 1) and 70 (period 2). Acute rejection was more frequent in period 1 than in period 2 (42% vs 7%, p <0.001). Overall, 30% of patients in period 1 were diagnosed with CMV infection/disease requiring antiviral treatment, compared with 11.4% in period 2 (p = 0.003). Late-onset CMV disease remained a problem in D+/R- patients in both periods. There was no difference in incidence of BK virus nephropathy, fungal infections, PTLD, graft loss or mortality. However, 4 cases (5.7%) of delayed transient asymptomatic agranulocytosis were observed in period 2.

Conclusions: The present analysis indicates that the combined regimen introduced in period 2 improved clinical results with a significant decrease in acute rejection and in CMV infection/disease incidence. However, a unique syndrome of delayed transient agranulocytosis probably due to drug myelotoxicity was observed in a subset of patients.

Key words: valganciclovir; universal prophylaxis; kidney transplantation; outcomes

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Abbreviations

CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
C4d	Complement C4d fragment
D/R	Donor/recipient
MMF	Mycophenolate mofetil
PCR	Polymerase chain reaction
PTLD	Post-transplant proliferative disorder
WBC	White blood cells

Introduction

Calcineurin inhibitors (CNI) remain the cornerstone drugs of immunosuppressive regimens after kidney transplantation. In a recent meta-analysis, tacrolimus was associated with a lower incidence of acute rejection than cyclosporine [1], and recent trends indicate that tacrolimus/mycophenolate is currently the most frequently used maintenance immunosuppressive regimen following kidney transplantation in the U.S. [2].

Cytomegalovirus (CMV) is the most important viral pathogen after organ transplantation in terms of morbidity and mortality [3, 4]. Moreover, CMV infection after organ transplantation has been associated with "indirect effects" such as acute rejection or predisposition to other infectious diseases (i.e. fungal and bacterial infection) [4, 5]. The type of immunosuppressive drug used following transplantation has been involved in the reactivation of CMV. For instance, one study indicated that mycophenolate therapy may increase the incidence of CMV infection [6], while other studies suggested that sirolimus may decrease the risk of CMV infection after transplantation [7]. In addition, antilymphocyte therapy (OKT3, or antilymphocyte globulin) may reactivate CMV as a result of potent immunosuppression and cytokine release [3, 4].

Currently there are two main strategies for prevention of CMV disease after organ transplantation: antiviral prophylaxis and preemptive therapy [3, 8–10]. Antiviral prophylaxis consists in administration of an antiviral drug after transplantation, usually during a period of 3–6 months [8, 10]. Most transplant centres use antiviral prophylaxis only in high-risk patients (targeted prophylaxis), while other centres use it for all patients (universal prophylaxis). Preemptive therapy consists in monitoring for CMV appearance in blood (e.g. with either CMV DNA PCR or antigenaemia) and administering antiviral therapy only when CMV viraemia is detected as a risk marker for impending CMV disease [9]. Drugs which have been used for prevention of CMV infection are oral valacyclovir (approved only in kidney transplant recipients) and intravenous and oral ganciclovir. Recently, oral valganciclovir, a valyl-ester prodrug of ganciclovir with an improved bioavailability with respect to oral ganciclovir, has been approved for CMV prophylaxis in kidney and heart transplantation [10].

A recent meta-analysis showed that both strategies (prophylaxis and preemptive) decreased the incidence of CMV disease after organ transplantation, but only antiviral prophylaxis seemed to reduce bacterial infection, fungal infection and also death [11]. However, the two strategies have not been compared in large-scale prospective randomised clinical trials.

At our transplantation centre we introduced in April 2003 a new universal antiviral prophylaxis regimen (a 3-month course of oral valganciclovir in all at-risk patients) together with a new immunosuppressive strategy consisting in induction therapy with basiliximab (or thymoglobulin in immunologically high-risk patients) associated with tacrolimus, mycophenolate and prednisone. In this study we analyse the efficacy and safety of this new combined regimen and compare it with the previous period (2000–2003).

Patients and methods

We retrospectively analysed the clinical records and electronic database of all consecutive patients undergoing kidney transplantation at the Centre Hospitalier Universitaire Vaudois (University Hospital of Lausanne, Switzerland) over a 5.5-year period (2000–2005) who completed a follow-up of at least 12 months. Patients transplanted from January 2000 to March 2003 (period 1) were compared to patients transplanted from April 2003 to July 2005 (period 2).

Immunosuppressive regimen

In period 1, most patients (55/73, 76%) were treated with basiliximab, 20 mg at day 0 and day 4, as induction therapy. Maintenance therapy consisted in cyclosporine (targeted plasma levels 150–200 mg/l), steroids (daily bolus of 500 mg methylprednisolone on the first 3 days post-transplantation, followed by tapered steroids to a dose of 5 mg prednisone per day at 6 months post-transplantation) and mycophenolate mofetil (MMF) (initially 2 g per day) or azathioprine (1–2 mg/kg per day). Nine patients (12%) received an immunosuppressive regimen consisting of sirolimus (targeted plasma levels between 8–12 mg/l), cyclosporine and steroids, without induction therapy.

In period 2, induction therapy was basiliximab for de novo kidney transplants (51/70, 73%). In the event of retransplant or if panel-reactive antibody was >50%, thymoglobulin at 1.5 mg/kg/day for 4 days was given. Maintenance therapy consisted in tacrolimus (targeted plasma levels 8–10 mg/l), steroids (daily bolus of 500 mg, 250 mg, and 125 mg methylprednisolone for the first 3 days post-transplantation, followed by tapered steroids to a dose of 5 mg prednisone per day at 3 months post-transplantation), and MMF (initially 2 grams per day). In hepatitis C-infected patients, cyclosporine was given instead of tacrolimus (4/70, 6%).

Antiviral prophylaxis

The CMV antibody status of donors and recipients was determined by enzyme-linked fluorescent assay for CMV IgG (Vidas, Biomérieux, Marcy l'Etoile, France).

In period 1, antiviral prophylaxis with a high-dose regimen (8 g per day, adapted to kidney function) of valacyclovir was prescribed in CMV donor positive/recipient

negative (D+/R-) patients [12]. Otherwise a preemptive antiviral approach based on intravenous ganciclovir was used, as described [13]. Patients were monitored weekly for 6 weeks and then biweekly for another month and a half after organ transplantation (preemptive strategy) or after cessation of valacyclovir prophylaxis (monitoring), for a 3-month period. In patients receiving sirolimus, prophylaxis with cotrimoxazole (1 single-strength capsule per day) was given for 6 months to prevent *Pneumocystis* infection.

In period 2, 3-month universal CMV prophylaxis with 450 mg per day of valganciclovir was given starting on postoperative day 3, except in D-/R- patients, for whom valacyclovir (1 g per day) was used (for herpes prophylaxis). Patients were thereafter monitored by CMV blood culture (until April 2004), or by CMV DNA PCR [13], every 15 days for 3 months after cessation of valganciclovir prophylaxis. Cotrimoxazole (1 single-strength capsule per day) was given to all patients for 6 months to prevent *Pneumocystis* infection. In the event of allergy, cotrimoxazole was replaced by atovaquone and levofloxacin.

Definitions and management of CMV infection

The definition of infectious diseases, including CMV infection and disease, appearing after transplantation followed the recently published American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation [14]. Briefly, CMV active infection was defined by the detection of CMV in blood or in appropriate specimens, and CMV disease was defined by the evidence of CMV infection with attributable symptoms. CMV disease was classified as tissue-invasive disease, if there was evidence of localised CMV infection in a biopsy or another appropriate specimen, or as CMV syndrome if there was no such evidence [14]. Since two techniques with different sensitivity measuring CMV on blood were used during the study period, in order to avoid selection bias we separately analysed patients with high-level viraemia or DNAaemia deemed to require antiviral therapy and patients with "low-grade viraemia" (a positive test for CMV DNA with levels below 10.000 copies/10^6 cells or a blood culture with fewer than 10 infectious units / 10^6 WBC).

Treatment with intravenous ganciclovir (5 mg/kg bid, adapted to renal function) was started for 2 or 3 weeks, if patients developed significant CMV infection (CMV disease and/or high-level viraemia) [13, 15]. After stopping ganciclovir administration, CMV DNA was monitored in whole blood by real time PCR every two weeks for three additional months. Since November 2002, in the context of a pilot study, intravenous ganciclovir was replaced by oral valganciclovir (900 mg bid, adapted to renal function) to treat recipients with CMV infection or disease. Our experience in the treatment of CMV with valganciclovir has been published previously [16].

Definitions and management of rejection

In cases of acute renal dysfunction renal biopsy was performed. Acute rejection was defined by the Banff 97 working classification of renal allograft pathology [17]. Since 2003, C4d staining has been performed on all renal allograft biopsies and correlated with the presence of donor-specific antibody in recipient's serum.

Treatment for acute rejection consisted of 3 days of high-dose methylprednisolone (500 mg daily boluses), followed by a prednisone taper. Additionally, steroid-resistant cases were treated with antilymphocyte globulin for 4–7 days.

Statistical analysis

Discrete variables were compared using a χ^2 or a Fischer exact test. Continuous variables were compared using a Student's or a Mann-Whitney's test. All analyses were performed using STATA software (Intercooled STATA 8.2) and a p value <0.05 was considered to be statistically significant.

Results

Patients

Seventy-six patients were transplanted in period 1. Three patients received a double transplantation (2 liver and kidney, and 1 heart and kidney) and were excluded from the present analysis. Thus 73 kidney transplantations were included in period 1. 70 patients were transplanted in period 2. Baseline clinical characteristics of both groups are shown in Table 1, and were comparable. There was a trend towards more advanced age in period 1 compared to period 2, but it did not reach statistical significance.

In period 2, one patient developed renal artery thrombosis at day 1 post-transplantation (primary non-function) and returned to dialysis, and one patient received a non-functional graft that was explanted on the day of transplantation. Thus, in period 2, 97% of patients had a functioning graft after a year's follow-up, compared to 89% of patients in period 1 (p = 0.09). As shown in Table 2, median serum creatinine 12 months after transplantation was 156 mmol/l in period 1 compared to 137 mmol/l in period 2 (p = 0.10).

After a year of follow-up there were no deaths in period 2. In period 1 a 60-year-old patient died of *Pneumocystis jirovecii* pneumonia 7 months after transplantation; a 50-year-old patient died of cardiorespiratory arrest 1 month after transplantation, and a 19-year-old patient died of probable invasive aspergillosis 19 days after transplantation. This latter patient had concomitant active CMV infection at the time of death.

Acute rejection

Acute rejection was more frequent in period 1 than in period 2 (42% of patients vs 7% respectively, p <0.001). In period 1 there were 38 episodes of acute rejection in 31 patients. 27 acute rejection episodes were treated only with boluses of methylprednisolone. Eleven episodes were additionally treated with antilymphocyte therapy. In period 2 five patients had episodes of acute rejec-

tion: three episodes of cellular rejection were treated with boluses of methylprednisolone only; one episode of acute humoral rejection was treated with boluses of methylprednisolone, OKT3, intravenous immunoglobulin and plasmapheresis; and one episode of acute rejection required both boluses of methylprednisolone and antilymphocyte therapy.

CMV infection

The results concerning CMV infection are shown in Table 3. In period 1, 14 patients were D-/R-. Of the remaining 59 patients, 31 had positive blood cultures for CMV. Nine episodes were identified (per definition) as low-grade viraemia, and thus a total of 22 patients with high-grade viraemia were treated with ganciclovir. Six patients had CMV disease (five patients had CMV syndrome and one patient CMV colitis). All 6 patients were D+/R- and four patients had received antiviral prophylaxis with valacyclovir before CMV disease. Sixteen patients had asymptomatic active CMV infection. Of these, 4 had previously received antiviral prophylaxis. Treatment of CMV disease consisted of 2–3 weeks' intravenous ganciclovir. One patient died of probable disseminated aspergillosis 3 days after starting intravenous ganciclovir. He had asymptomatic active CMV infection but with high-grade viraemia. It was notable that, at the end of period 1 (November 2002 – March 2003), oral valganciclovir was used in 5 patients to treat their active CMV infection (3 with asymptomatic infection and 2 with CMV syndrome). Overall, the mean duration of antiviral therapy was 26 ± 12 days. A total of 12 patients out of 22 (54%) had a relapse of asymptomatic active CMV infection, and 4 patients required a second course of antiviral therapy.

In period 2, 11 patients were D-/R-. Of the remaining 59 patients, 25 had either positive CMV blood culture or detectable CMV DNA after stopping valganciclovir prophylaxis. Seventeen episodes were identified (per definition) as low-grade viraemia. Eight patients (11%) were diagnosed with CMV disease, of whom 7 had CMV syndrome and one probable tissue-invasive disease (acute hepatitis). A point to note was that 3 patients out of the seven with CMV syndrome had diarrhoea, which improved after administration of valganciclovir therapy. Most patients (75%) with CMV disease in period 2 were D+/R-. All patients were treated with oral valganciclovir

Table 1
Clinical characteristics of patients.

	Period 1 Jan 00-Mar 03	Period 2 Apr 03-Jul 05	p value
Number of patients	73	70	
Mean age	48 ± 13	44 ± 14	0.06
Male sex	47 (64%)	50 (71%)	0.37
White race	71 (97%)	67 (96%)	0.67
No. of HLA-antigen mismatches	4.0 ± 1.0	3.8 ± 1.6	0.9
PRA >50%	2 (3%)	3 (4%)	1.0
Previous renal transplantation-no. (%)	12 (16%)	14 (20%)	0.67
CMV serostatus pattern			
D+/R-	13 (18%)	16 (23%)	0.64
D+/R+	27 (37%)	31 (44%)	
D-/R+	19 (26%)	12 (17%)	
D-/R-	14 (19%)	11 (16%)	
Antiviral prophylactic strategy			
Valacyclovir (anti-CMV)	7 (10%)	0 (0%)	<0.001
Valacyclovir (anti-HSV)	0 (0%)	9 (13%)	
Valganciclovir	1 (1%)	61 (87%)	
None	65 (89)	0 (0)	
Induction therapy			
Anti-IL2-receptor	55 (76%)	51 (73%)	0.001
Anti-thymocyte globulin	8 (11%)	19 (27%)	
None	9 (12%)	0 (0%)	
Maintenance immunosuppression			
Prednisone	72 (99%)	70 (100%)	<0.001
Cyclosporin A	57 (79%)	4 (6%)	
Tacrolimus	15 (21%)	66 (94%)	
Sirolimus	9 (12%)	0 (0%)	
Mycophenolate	41 (57%)	70 (100%)	
Azathioprine	5 (7%)	0 (0%)	

CMV: cytomegalovirus, D: donor, HSV: herpes simplex virus, PRA: panel-reactive antibody, R: recipient

for a mean duration of 44 ± 32 days. Only two cases of asymptomatic CMV viraemia relapse were observed after the end of valganciclovir therapy and resolved without antiviral therapy. It was noteworthy that 2 patients developed adverse events during valganciclovir treatment: one case of thrombocytopenia and one case of pancytopenia which resolved after discontinuation of valganciclovir.

Agranulocytosis during universal valganciclovir prophylaxis

Four cases (5.7%) of agranulocytosis (absolute neutrophil count <500 cells/mm^3) occurring during the first three months after transplantation were observed in period 2. The agranulocytosis was delayed and transient, i.e. it occurred abruptly 74 ± 20 days after transplantation, was asymptomatic and recovered within 3–7 days after discontinuation (or interruption) of MMF and valganciclovir. G-CSF was safely administered to 2 out of 4 patients to help reverse the neutropenia. No patient was found to have CMV infection or any other viral or bacterial infection (which might have played an aetiological role) at the time of the agranulocytosis episode and, importantly, there were no cases of agranulocytosis-associated sepsis. No significant association with prior thymoglobulin administration (during induction immunosuppression) was found. In 2/4 patients, MMF could be successfully resumed (but at a lower dosage) without any subsequent problem or neutropenia relapse. Valganciclovir was discontinued prematurely in 3/4 patients, and was resumed in 1/4 patient to complete the drug prophylaxis course. A point to note was that none of the 3 patients with premature dicontinuation of valganciclovir had CMV reactivation in the ensuing weeks or months.

BK virus nephropathy, PTLD and fungal infection

There were no differences between the two periods in terms of incidence of BK virus nephropathy (4% vs. 4%) or post-transplant lymphoproliferative disease (0% vs. 1%). As mentioned above and in Table 2, a single case of probable invasive aspergillosis was diagnosed.

Table 2

Patient outcome after 12-month follow-up.

	Period 1 Jan 00-Mar 03	Period 2 Apr 03-Jul 05	p value
Number of patients	73	70	
Patients with acute rejection	31 (42%)	5 (7%)	<0.001
Total episodes of acute rejection	38	5	
Median creatinine (mmol/dl)	156	137	0.10
Agranulocytosis episodes*	0 (0%)	4 (5.7%)	0.055
BK virus nephropathy**	3 (4%)	3 (4%)	1.0
PTLD**	0 (0%)	1 (1%)	1.0
Invasive fungal infection	1 (1%)	0 (0%)	1.
Graft loss	8 (11%)	2 (3%)	0.09
Death	3 (4%)	0 (0%)	0.24

* Agranulocytosis: <500 neutrophils per ml
** All cases of BK virus nephropathy and PTLD were biopsy-proven
PTLD: post-transplant lymphoproliferative disorder

Table 3

Incidence of CMV infection at 12 months after organ transplantation.

	Period 1 Jan 00-Mar 03	Period 2 Apr 03-Jul 05	p value
Number of patients	73	70	
CMV active infection or disease requiring therapy*	22 (30%)	8 (11%)	0.007
D+/R-	9/22 (41%)	6/8 (75%)	0.2
CMV asymptomatic infection	16 (22%)	0 (0%)	<0.001
Time from transplantation to first CMV viraemia detection, days	44 (27-128)	–	
CMV disease	6 (8%)	8 (11%)	0.66
Syndrome	5 (7%)	7 (10%)	
Tissue-invasive disease	1 (1%)	1 (1%)	
Time from transplantation to CMV disease diagnosis, days	118 (33-213)	139 (108-171)	0.3
Duration of antiviral therapy	26 ± 12	44 ± 32	0.015
Relapse episodes after stopping therapy	12	2	0.22

* Significant CMV active infection/disease requiring therapy (CMV disease and/or CMV PCR positive >10.000 copies/10^6 cells or ≥10 infectious units /10^6 WBC by blood culture)

Discussion

In the present study we analysed the effect of introducing universal anti-CMV valganciclovir prophylaxis in conjunction with a tacrolimus/MMF-based maintenance immunosuppressive regimen in kidney transplant recipients. Despite the higher net state of immunosuppresion in the second period, the incidence of CMV infection requiring treatment was significantly reduced from 30 to 11%. This reduction was due to a significant decrease in the incidence of asymptomatic CMV infection associated with valganciclovir use in period 2, while the incidence of CMV disease was not significantly reduced.

There are some advantages of the universal prophylaxis approach with respect to the preemptive approach. First, it is simpler. During the first three months after transplantation, valganciclovir very effectively inhibits CMV replication and prevents the appearance of CMV infection during the time of most intense immunosuppression. In contrast, the preemptive approach is relatively complex and in our experience more difficult to implement. We have previously shown that morbidity due to CMV infection depends on compliance with preemptive approach guidelines [15]. However, it has been argued that by exposing recipients to some low-level viral replication, the preemptive approach may be somewhat beneficial and lead to an earlier anti-CMV immune response (compared to the prophylactic approach), i.e. it may reduce the risk of late CMV disease [18]. Here it should be emphasised that there are as yet no data regarding late CMV disease in large, prospective, comparative studies of these two (prophylactic vs. preemptive) approaches [19, 20]. At our centre we previously identified a pattern of deleterious protracted infection with recurrent episodes of high level CMV viraemia by using the preemptive approach [13]. The major predictor of protracted CMV infection was the same as for late disease, namely the high-risk (D+/R-) CMV serostatus pattern.

The advantages of universal prophylaxis for all at-risk patients may be the reduction of both the "direct" and "indirect effects" of CMV infection [4], in particular a decrease in acute rejection and a reduction in other infectious complications. In our study the reduction in the incidence of acute allograft rejection in period 2 was probably mainly due to the switch toward a more potent and efficacious immunosuppressive regimen. Whether valganciclovir prophylaxis, as an independent parameter, may also have helped to reduce the incidence of acute rejection (e.g. by suppressing asymptomatic CMV viraemia) cannot be demonstrated in the present study due to its retrospective, non-randomised nature.

However, there are some drawbacks to universal prophylaxis, such as late-onset CMV disease or increased exposure to an anti-CMV drug which has potential side-effects. Late-onset CMV disease in D+/R- recipients remains a significant problem, and CMV disease appearing after discontinuation of antiviral prophylaxis is indeed increasingly described in the literature [10, 18, 21]. Thus, monitoring for CMV infection after discontinuing prophylaxis is mandatory, particularly in D+/R- recipients. Late-onset CMV disease is often characterised by tissue-invasive CMV disease, especially colitis or hepatitis. Although it has been reported that the clinical manifestations of late-onset CMV disease are more severe [22], in our series this was not so since all cases responded well to anti-CMV therapy without sequelae.

Second, even if valganciclovir is generally well tolerated, toxicity may still be a concern. In this regard it seems important to emphasise the description in this series of 4 cases (5.7%) of abrupt agranulocytosis in period 2, i.e. when they were receiving both valganciclovir and MMF. All cases were reversible without complications after drug discontinuation (or interruption), but this adverse event does raise the question of establishing the minimally effective valganciclovir prophylactic dose for each individual recipient. We suggest that this unique clinical finding of "delayed asymptomatic agranulocytosis" in 5.7% of kidney transplant recipients receiving two potentially myelotoxic drugs should be monitored closely (and reported) as an additional endpoint in future clinical studies using valganciclovir prophylaxis. The precise explanation for the delayed nature of the severe neutropenia was not determined. A possible mechanism may be the previously documented progressive increase in the bioavailability of MMF during the first three months after transplantation, interacting with concomitantly administered valganciclovir and resulting in myelotoxicity [23]. With the exception of this haematological adverse event, valganciclovir was well tolerated and very user-friendly.

In period 2 a fixed dose of 450 mg per day of valganciclovir was administered regardless of allograft function. On average, kidney allograft function was good or excellent (median serum creatinine at one month of 130 mol/l, estimated glomerular filtration rates [GFRs] ranging from 50 to 70 ml/min). We did not identify breakthrough CMV disease during prophylaxis but we cannot exclude some low-level asymptomatic CMV viraemia since CMV surveillance by PCR was performed only after discontinuation of prophylaxis. It is noteworthy that all patients who developed CMV disease after discontinuation of antiviral prophylaxis received oral valganciclovir again and responded well to the second course of antiviral therapy. Thus we encountered no clinical suspicion of the emergence of ganciclovir-resistant CMV strains. Prospective randomised trials are also needed to determine the optimal dose (ef-

ficacious and safe) and duration of valganciclovir for CMV prophylaxis after kidney transplantation [24, 25]. In particular, valganciclovir pharmacokinetic analysis should be performed for GFRs of around 60 ml/min or higher, in order to validate (or not) the dose of 450 mg/day we now routinely use in our centre.

There are some limitations to the current analysis. Because of the retrospective nature of the study, tissue-invasive CMV disease may be underdiagnosed due to incomplete clinical information. For example, some patients with CMV syndrome and diarrhoea were not diagnosed as having "tissue-invasive disease" because colonoscopy with biopsies was not performed in all cases. In addition, two different methods of detecting CMV were used during the 5.5-year study period, quantitative PCR showing higher sensitivity than blood culture. Because CMV infection may carry a risk of being over-diagnosed in period 2 (because of the more sensitive PCR technique), we decided to define and use a comparable endpoint, i.e. significant CMV active infection or disease requiring treatment.

In conclusion, our analysis showed that valganciclovir prophylaxis during period 2 was very effective in reducing the incidence of CMV infection requiring therapy in a cohort of patients receiving a maintenance immunosuppressive regimen of tacrolimus/mycophenolate. The protocol used in period 2 led to a significantly improved outcome both in terms of acute rejection and of CMV infection/disease, thus achieving the goal of an integrated immunosuppressive and antimicrobial approach which as been referred as "the therapeutic prescription" by R. Rubin [26]. However, we did not observe a decrease in late-onset CMV disease after discontinuation of valganciclovir prophylaxis in D+/R- patients. Occurrence of late-onset CMV disease was not associated with severe morbidity or mortality. Surveillance with PCR for CMV and a low clinical diagnostic threshold are probably necessary after discontinuation of valganciclovir prophylaxis in D+/R- patients. Finally, our findings emphasise that special attention to new and unexpected clinical events, such as "delayed transient agranulocytosis", is mandatory in the current era of the more effective but also more powerful drugs that are used in transplant recipients.

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