Prospetcive follow-up of primary CMV infections after 6 months of valganciclovir prophylaxis in renal transplant recipients

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Abstract

Background. The occurrence and clinical course of late primary CMV infections developing after valganciclovir prophylaxis in high-risk renal transplant recipients are poorly described.

Methods. Helsinki University Hospital district kidney allograft recipients between January 2004 and March 2007 (N = 175) were prospectively investigated. Patients with D+/R– CMV serostatus and 1-year follow-up were included (N = 25). After 6 months of oral valganciclovir prophylaxis, the patients were monitored for CMV-DNAemia with real-time quantitative plasma PCR at 2–6 weeks interval and if CMV infection was suspected. Infections were treated with i.v. ganciclovir or high-dose valganciclovir, followed by 1–3 months of secondary valganciclovir prophylaxis.

Results. CMV infection developed in 12/25 patients a mean of 107 days (range 26–330 days) after prophylaxis ended. Two were asymptomatic. Ten patients symptoms included fever (N = 7), gastrointestinal (N = 5), upper respiratory tract (N = 3) and hepatopathy (N = 2). One patient with infection had prophylaxis terminated after 5 months (leukopenia). The mean viral load at diagnosis was 49 517 (range 490–325 300), and peak viral load was 84 654 (range 1250–527 400) copies/ml. Five infections were treated with valganciclovir and six with i.v. ganciclovir resulting with negative PCR results. One mild infection with low viral load was treated successfully with minimization of immunosuppression. Infection relapse developed in three patients a mean of 31 (range 15–61) days after the end of the therapy. Relapses were treated with valganciclovir.

Conclusions. CMV primary infections were common after 6 months of valganciclovir prophylaxis and mostly symptomatic. Relapses commonly occurred. Primary infections seem to be delayed, but were not efficiently prevented by 6 months of prophylaxis.

Keywords: cytomegalovirus (CMV); kidney transplantation; valganciclovir

Introduction

Cytomegalovirus (CMV) remains a significant cause of morbidity and costs after organ transplantation also in the current era. In addition to the direct effects of viral infection, CMV has been associated with acute and chronic rejection after solid organ transplantation and decreased graft survival [1–3]. CMV seronegative patients receiving organs from seropositive donors (D+/R−) are at greatest risk of CMV infection; without prophylaxis, more than 50% of high-risk kidney allograft recipients develop symptomatic infection during the first 3 months after transplantation [4]. Especially with the introduction of orally bioavailable valine ester of ganciclovir, valganciclovir, many transplant centres nowadays use prophylaxis against CMV after organ transplantation [5]. Prophylaxis with either valganciclovir or oral ganciclovir is effective in preventing CMV infection [6,7], but after the cessation of prophylaxis, many patients still develop late-onset CMV infection.

The incidence of late-onset CMV disease after 3 months CMV prophylaxis in high-risk kidney transplant recipients is reported as being 18–31% [8–10], but prolonging prophylaxis up to 6 months may decrease the incidence of late-onset CMV disease [9]. There is no consensus of the optimal duration of prophylaxis after renal transplantation although Canadian guidelines for the diagnosis and treatment of CMV recommend 12–14 weeks of prophylaxis in high-risk patients [5]. Prophylaxis with valganciclovir is not without side effects, the most common being leukopenia [6], and valganciclovir prophylaxis is also very costly. In addition, the risk of drug resistance raises concerns [11].

The clinical course of late-onset CMV disease after prophylaxis is still poorly described although CMV disease after prophylaxis may include more tissue-invasive infections, especially gastrointestinal CMV disease, compared to infections occurring without prophylaxis [6,10]. The policy of our clinic was changed in 2004 to use 6 months...
of valganciclovir prophylaxis after renal transplantation in high-risk recipients to reduce the risk of late-onset CMV infections. The purpose of this study was to describe the occurrence and clinical course of late-onset CMV infection in the Finnish kidney transplant population after valganciclovir prophylaxis.

Subjects and methods

All Helsinki University Hospital district adult kidney transplant recipients who received a graft between January 2004 and March 2007 (N = 175) were prospectively investigated. All CMV seronegative recipients receiving a kidney from a seropositive donor (D+/R−) with a completed 1-year follow-up and functioning graft at 6 months were investigated (N = 25). Baseline immunosuppression was usually a triple-drug regimen with cyclosporine A, mycophenolate mofetil and steroids. In immunologically high-risk patients (long waiting time, poor match, re-transplantation), cyclosporine was replaced by tacrolimus, and/or induction therapy with basiliximab was administered. In patients with stable graft function and especially in patients with problems in glycaemic control or osteoporosis, steroids were withdrawn slowly during the second post-transplant year.

All the patients included in this study received oral valganciclovir prophylaxis for 6 months after transplantation (900 mg once daily if normal renal function). After the cessation of prophylaxis, patients were monitored for CMV-DNAemia with TaqMan-based real-time quantitative plasma PCR [12] at 2–6 weeks interval for the first 3–6 months and also if CMV disease was suspected (fever, leucopenia, gastrointestinal symptoms, hepatopathy, upper respiratory tract symptoms). The quantitative real-time method used in this study correlates well with the most used commercial CMV-PCR method Cobas Amplicor Monitor and the CMVpp65 antigenemia test [12]. CMV infections were treated with i.v. ganciclovir or high-dose valganciclovir (900 mg twice daily if normal renal function), followed by 1–6 months of secondary oral valganciclovir prophylaxis.

Baseline clinical data at the time of transplantation and clinical follow-up data at 1, 3, 6, 12, 18 and 24 months after transplantation and at last follow-up were collected from patient charts and laboratory database. Baseline data included recipient and donor age and sex, cold ischaemia time, delayed graft function as defined by the need of dialysis during the first post-transplant week and HLA A-, B- and DR-mismatch. Follow-up data included kidney function as measured by plasma creatinine and estimated creatinine clearance using the four-variable Modification of Diet in Renal Disease (MDRD) equation [13], doses and trough levels of cyclosporine and tacrolimus, doses of mycophenolate mofetil and steroids, and CMV-PCR results.

All data are expressed as mean ± 1 standard deviation, unless otherwise indicated. Statistical significances between the groups were measured by the nonparametric Mann–Whitney’s U-test and Fisher’s exact test, using SPSS statistical software (version 12.0.1, SPSS Inc., Chicago, IL, USA).

Results

One patient lost a graft during the study period due to chronic allograft nephropathy 37 months after transplantation and returned to haemodialysis. Other grafts were functioning at the end of follow-up. The mean time of follow-up was 31 months (range 16–47). The mean estimated GFR at the end of follow-up was 58 ± 16 ml/min. Tacrolimus was used as an immunosuppressive agent in six patients, while others received cyclosporine. Induction treatment with basiliximab was administered to three patients. Three patients showed acute rejection in biopsy histology, of which two were treated with high-dose intravenous steroids and conversion of cyclosporine to tacrolimus, and one rejection resolved spontaneously and was controlled with repeated biopsies.

No CMV infections were detected during the 6 months of valganciclovir prophylaxis. Primary CMV infection, as detected by positive CMV-PCR results, developed in 12 patients (48%) a mean of 101 days after the cessation of prophylaxis (range 26–330 days). Only two of these late-onset infections were asymptomatic; the occurrence of CMV disease was 40% (10/25). In the 10 symptomatic patients, symptoms included fever (N = 7), gastrointestinal (nausea, vomiting, diarrhoea, abdominal pain) (N = 5), upper respiratory tract (N = 3) and elevated liver enzymes (N = 2). Lymphopaenia (blood lymphocyte count <1.30 × 10⁹/l) was detected in five patients at the time of CMV infection diagnosis. Of the 10 symptomatic patients, 2 presented only with fever. Valganciclovir prophylaxis was terminated prematurely in three patients due to leucopaenia at 4, 5 and 5.5 months after transplantation, respectively. One of these patients developed CMV infection 60 days after the end of prophylaxis. The frequency of late-onset CMV infection did not differ between patients on cyclosporine or tacrolimus. Of the three patients who received basiliximab induction, two developed late-onset CMV infection.

The mean viral load at diagnosis in symptomatic patients was 56 934 copies/ml (range 490–325 300), and peak viral load was 91 235 copies/ml (range 2100–527 400). The viral loads of the two asymptomatic patients at diagnosis were 4300 and 45700, respectively. The patients with CMV infection and no CMV infection are compared in Table 1. Patients with or without CMV infection did not differ at 1 year or at the end of follow-up in renal function, HLA mismatch, cold ischaemia time, age, sex or calcineurin inhibitor used or any other parameter studied (data not shown).

All except one patient with CMV infection received treatment with antiviral drugs. One patient with abdominal pain, vomiting and slightly elevated liver enzymes but no fever and relatively low viral load of 2500 copies/ml was treated successfully with mycophenolate mofetil dose reduction and followed until plasma CMV-PCR was negative repeatedly. Of the other 11 patients...
with CMV infection, 5 were initially treated with oral valganciclovir and 6 with i.v. ganciclovir. One patient with an increasing viral load after 7 days of valganciclovir was thereafter treated with i.v. ganciclovir for 25 days (Figure 1). All treatments were successful resulting with negative PCR results. No clinically suspected cases of ganciclovir resistant virus were detected, and no laboratory testing for resistance was performed. The mean duration of intravenous ganciclovir treatment was 16 days (range 7–25 days). One patient received only 7 days of intravenous ganciclovir but received 2-week course of treatment dose valganciclovir.

The duration of valganciclovir treatment in four patients who received only valganciclovir was a mean of 19 days (range 14–26 days). All patients with primary CMV infection received secondary prophylaxis with valganciclovir for a mean of 80 days (range 30–180 days). Three patients developed recurrent CMV infection a mean of 32 days after the end of secondary prophylaxis (range 15–60 days). The duration of secondary prophylaxis in these patients was 30, 45 and 90 days, respectively. Two of these were asymptomatic and one infection relapse presented with fever and upper respiratory tract symptoms. All infection relapses were treated with valganciclovir for total 30–45 days, starting in two patients with treatment dose for 2 weeks.

### Discussion

Despite prolonged valganciclovir prophylaxis of 6 months in high-risk kidney transplant recipients, 48% of these patients developed late-onset CMV infection and 40% CMV-associated clinical symptoms in our material. Infection relapse developed in three patients after secondary prophylaxis. Most of the infections were symptomatic, with the most common symptoms being fever and gastrointestinal symptoms. All infections were successfully treated with either i.v. ganciclovir or oral valganciclovir and in one patient with reduction of immunosuppression.

Previous studies have reported the incidence of late-onset CMV infection in high-risk renal allograft recipients after 3 months of prophylaxis being 14–31% [7–10]. In a study comparing 3 and 6 months of prophylaxis in high-risk renal transplant recipients, prolonged prophylaxis of 6 months reduced the incidence of CMV infection from 31% to 7% [9]. Our results do not support these findings, as the incidence of late-onset primary CMV infections was still relatively high despite prolonged prophylaxis. Some evidence suggests that prophylaxis with a potential antiviral agent such as ganciclovir may impair the development of virus-specific T-cell responses after bone marrow transplantation [14] or the maturation of antibodies after kidney transplantation [15] and may by these mechanisms increase the risk of infection after prophylaxis. As an alternative to universal prophylaxis some authors recommend pre-emptive therapy, which may reduce this problem [16]. A recent study comparing CMV prophylaxis and pre-emptive treatment, however, showed improvement in graft survival with prophylaxis [8]. In a study by Kliem et al., 50% of D+/R− kidney transplant recipients in the prophylaxis group suffered from CMV infections, of which most developed after the end of oral ganciclovir prophylaxis. Current guidelines recommend CMV prophylaxis in high-risk recipients for 12–14 weeks after transplantation [5].

The intensity of immunosuppression did not seem to be associated with the risk of CMV infection in our study, as the frequency of tacrolimus or basiliximab induction therapy or steroid dose was not different in patients with or without CMV infections. However, our analysis of the association of immunosuppression with the risk of CMV infections is limited by the low number of patients using tacrolimus or induction therapy. A majority of patients included in the other studies of CMV prophylaxis in high-risk recipients received quadruple immunosuppression including induction therapy with either ATG or IL-2-receptor antagonists [7,9,10]. A possible explanation for our higher incidence of late-onset CMV infection could also be that the impact of CMV prophylaxis may not be equal in patients receiving relatively conservative immunosuppression. The Finnish kidney transplant population also differs from other transplant populations in that most of the kidneys are from well-matched cadaveric donors in our genetically isolated population. In addition, the incidence of delayed graft function is

### Table 1. Comparison of patients with late-onset primary CMV infection and no CMV infection after 6 months of valganciclovir prophylaxis; no patients were lost during follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Late-onset CMV infection (N = 12)</th>
<th>No CMV infection (N = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age</td>
<td>46 ± 9</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Donor age</td>
<td>49 ± 10</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>HLA A-, B- and DR-mismatch</td>
<td>2.2 ± 0.9</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Delayed graft function</td>
<td>5/7</td>
<td>5/8</td>
</tr>
<tr>
<td>(yes/no)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rejection (yes/no)</td>
<td>1/11</td>
<td>2/11</td>
</tr>
<tr>
<td>C0-level at 3 months (µg/l)</td>
<td>142 ± 29</td>
<td>140 ± 46</td>
</tr>
<tr>
<td>C0-level at 6 months (µg/l)</td>
<td>124 ± 24</td>
<td>119 ± 27</td>
</tr>
<tr>
<td>C0-level at 12 months (µg/l)</td>
<td>90 ± 16</td>
<td>104 ± 33</td>
</tr>
<tr>
<td>Number of patients on</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>methylprednisolone dose at 6 months (mg)</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>eGFRc at 3 months (ml/min)</td>
<td>68 ± 22</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>eGFRc at 6 months (ml/min)</td>
<td>71 ± 21</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>eGFRc at 12 months (ml/min)</td>
<td>68 ± 18</td>
<td>66 ± 16</td>
</tr>
<tr>
<td>eGFRc at the end of follow-up (ml/min)</td>
<td>59 ± 20</td>
<td>57 ± 12</td>
</tr>
<tr>
<td>Plasma creatinine at 3 months (µmol/l)</td>
<td>108 ± 32</td>
<td>115 ± 17</td>
</tr>
<tr>
<td>Plasma creatinine at 6 months (µmol/l)</td>
<td>102 ± 24</td>
<td>105 ± 11</td>
</tr>
<tr>
<td>Plasma creatinine at 12 months (µmol/l)</td>
<td>106 ± 26</td>
<td>110 ± 23</td>
</tr>
<tr>
<td>Plasma creatinine at the end of follow-up (µmol/l)</td>
<td>123 ± 39</td>
<td>125 ± 30</td>
</tr>
<tr>
<td>Number of grafts lost during follow-up</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

aExpressed as mean ± SD unless otherwise indicated; all differences are nonsignificant.
bTrough level of cyclosporine A.
cEstimated glomerular filtration rate using the four-variable Modification of Diet in Renal Disease (MDRD) equation, ml/min.
relatively high, partly explained by increased cold ischaemia times due to the long geographic distances in our country (mean cold ischaemia time in the study population was 21 h and 23 min). These factors may also contribute to the risk of CMV infections.

Risk factors for late-onset CMV infection in high-risk recipients are not well described. A large study comparing the efficacy of oral ganciclovir and valganciclovir prophylaxis in CMV D+/R− solid organ transplant recipients (PV 16000) reported reduced renal function and female sex as the only significant risk factors for late-onset CMV infection [17]. Other studies have identified bacterial and fungal infections and delayed graft function as risk factors for late-onset CMV infection after prophylaxis [9,10]. Seroconversion occurring in some patients during or after prophylaxis, on the other hand, was a poor predictor of CMV infection [18]. In our study, we failed to identify any risk factors for late-onset CMV infection, and late-onset CMV infection in our study was not associated with poorer graft function or graft loss at the end of follow-up. The small number of patients with CMV infection may limit our analyses. A recent study associated delayed-onset CMV infection with allograft loss and mortality [10].

Most of the late-onset CMV infections in our study were symptomatic. A relatively high frequency of symptomatic infections after 100 days of CMV prophylaxis was similarly seen in a study by Khoury et al. [19], which included also CMV antibody-positive kidney transplant recipients. Of the D+/R− patients developing late-onset CMV infection after prophylaxis, 3/6 had symptoms related to the infection, mostly fever and fatigue [19]. In addition to fever, the most common symptoms of CMV infection in our study were of gastrointestinal origin. Similar findings of changed clinical picture towards gastrointestinal disease of delayed-onset primary CMV infection have been reported by previous studies, including studies of also solid-organ transplantations [10,20,21]. It has been suggested that primary infections after CMV prophylaxis may include more tissue-invasive disease than without prophylaxis [10,16]. In our study, five patients suffered from gastrointestinal symptoms, suggestive of gastrointestinal CMV disease. Our study is however limited by the lack of confirmation of
tissue-invasive CMV disease by endoscopy or biopsy findings; the description of CMV disease was limited to the description of symptoms.

Due to a 2 to 6-week interval in the late monitoring of CMV infection, some asymptomatic reactivations of short duration or asymptomatic viraemia developing before the onset of symptoms may have been missed, partly explaining the high percentage of symptomatic infections. Considerable variation was seen in the peak viral loads of patients with CMV infection, with also very high viral loads in some patients (up to 527 400 copies/ml). The method used for CMV-DNA detection from plasma is comparable to other common methods to detect DNAemia and pp65 antigenemia [12]. Although using whole blood specimens for the detection of CMV-DNAemia in transplant recipients may be more sensitive, the use of plasma samples is also considered adequate for the detection of CMV infection [22]. This study is also limited by the relatively low number of D+/R− recipients in our material although being a prospective study. We continue to collect more material to confirm these findings. However, because of the previously unreported high incidence of infections in our well-matched material with relatively conservative immunosuppression, we feel these results need to be reported.

In conclusion, the incidence of late-onset primary CMV infections after 6 months of valganciclovir prophylaxis in the Finnish kidney transplant recipient population was considerably higher than previously reported, and relapses were common. CMV infections seem to be delayed but not prevented by prolonged prophylaxis. These preliminary findings warrant further studies, and because of the high costs associated with valganciclovir, the prevention strategies for CMV need to be reconsidered.

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Conflict of interest statement. None declared.

References

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