Pre-emptive therapy of CMV pp65 antigen positive renal transplant recipients with oral ganciclovir: a randomized, comparative study

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Abstract

Background. About one-quarter of renal transplant patients will suffer from symptomatic cytomegalovirus (CMV) disease if no preventive therapeutic measures are taken. In this prospective, randomized single-centre study pre-emptive therapy with oral ganciclovir is compared with conventional deferred treatment.

Methods. Renal transplant recipients (n = 455) over 18 years of age were screened weekly for CMV pp65 antigenaemia during the first 12 weeks post-transplantation. If CMV pp65 antigen in leukocytes appeared within 8 weeks post-transplantation patients were randomized and included in the study. Five patients developed CMV disease before positive CMV pp65, and 14 patients with a positive antigen test developed CMV disease before randomization could take place, all these representing a limitation of the applicability of the results in the overall renal transplant population. Altogether 179 patients were not randomized for various reasons. Eighty patients completed the study, 42 were randomized to receive pre-emptive oral ganciclovir therapy and 38 to conventional deferred treatment (control group).

Results. Time from transplantation to start of ganciclovir capsules was 36 (12–60) days and duration of oral ganciclovir therapy was 49 (27–70) days, median (range). No patient in the pre-emptive treatment group, but nine of 38 patients (23.7%) in the control group, developed CMV disease during the first 12 weeks post-transplantation (P = 0.0009). In the period from 3 months to 1 year post-transplantation, two patients in each group developed CMV disease. There were no significant differences in acute rejection or renal function between treatment groups during the first post-transplant year.

Conclusions. Pre-emptive oral ganciclovir therapy in renal transplant recipients during the first 12 weeks post-transplantation effectively prevents CMV disease during this time period. The incidence of late CMV disease (3 months to 1 year after transplantation) was similar in the two groups, indicating that pre-emptive therapy does not result in late onset of CMV disease.

Keywords: cytomegalovirus disease; cytomegalovirus infection; ganciclovir capsules; kidney transplantation; pre-emptive therapy

Introduction

Cytomegalovirus (CMV) infection is a major cause of morbidity in the early phase following organ transplantation [1–3]. More than 60% of renal transplant recipients have diagnosed CMV infection and over 20% suffer from CMV disease [2,4,5]. CMV disease occurs in ~40–60% of seronegative recipients of seropositive donors (D+R−) (primary infection) and in about one-quarter of seropositive recipients (R+) (reactivated infection) [2–4]. Moreover, a high CMV antigen load (CMV pp65 >50/105 leukocytes) is associated with an increased risk of CMV disease in seropositive recipients [2]. CMV disease may be more prevalent with potent immunosuppressive drug regimens, especially when anti-lymphocyte preparations are used [1,4].

Several studies show that acute rejection episodes may induce CMV infection and disease [1,2,6]. On the other hand, recent studies have shown that CMV infection and disease also are independent risk factors of acute rejection episodes [7–9]. This emphasizes the importance of a successful treatment strategy to prevent CMV disease, since acute rejection episodes may affect long-term graft survival [10–13]. There are several strategies to deal with CMV infection and
disease. As CMV infections not always lead to clinical disease the traditional approach has been to restrict therapy to symptomatic treatment of CMV disease (deferred treatment). On the other hand, good results have been shown in preventing CMV infection and hence CMV disease by using prophylactic treatment with ganciclovir, especially in high-risk recipients [14]. However, with frequent screening of blood samples for CMV pp65 antigenaemia it is possible to monitor CMV infection and start treatment before CMV disease develops (pre-emptive treatment). With such an approach treatment of many patients with low risk of CMV disease is avoided.

Oral ganciclovir has a low and variable bioavailability, < 10%, and the systemic exposure is therefore most probably subtherapeutic for treatment of CMV disease, where ganciclovir i.v. is used, but may still be appropriate for prophylactic and pre-emptive use [15].

The present study was designed to investigate the efficacy of pre-emptive oral ganciclovir treatment with appearance of CMV pp65 antigenaemia compared with no treatment to prevent CMV disease during the first year following transplantation in renal transplant recipients.

The primary endpoint was appearance of CMV disease during the first 12 weeks after transplantation. Our hypothesis was that the incidence of CMV disease would be reduced by at least 50% in the pre-emptive treatment group, compared with the control group, during the first 12 weeks after transplantation.

Secondary endpoints were CMV disease between 12 weeks and 1 year after transplantation and renal function assessed by s-creatinine and glomerular filtration rate (GFR) in the two treatment groups after 12 weeks, 6 and 12 months. Other secondary endpoints were acute rejection episodes, graft survival and patient survival 6 and 12 months after transplantation.

**Subjects and methods**

**Patients and study design**

The present open, randomized, single-centre, parallel group trial investigated the effect on CMV disease of the use of pre-emptive oral ganciclovir treatment at the appearance of CMV pp65 antigenaemia vs conventional deferred treatment in renal transplant recipients. All renal transplant patients at our centre were screened once weekly for CMV pp65 antigenaemia during the first 12 weeks. Patients included in the study were also screened at 6 and 12 months after transplantation. At the first sign of CMV infection (CMV pp65 antigen test ≥1/100 000 leukocytes) within 8 weeks post-transplantation patients were randomized to either oral ganciclovir or no treatment within a week after the first positive sample. The inclusion period of 8 weeks was chosen to ensure a minimum treatment period of 4 weeks. Traditionally, the patients leave the transplantation centre at 12 weeks after transplantation to be followed up by their local nephrologist. Thus, for practical reasons the treatment was stopped at 12 weeks after transplantation while the patients still were under supervision by the study investigator.

Patients over 18 years of age receiving a primary renal graft were eligible for inclusion. CMV negative recipients receiving CMV seronegative organs were not eligible for inclusion. No putative anti-CMV therapy was allowed during the study other than the investigational drug (ganciclovir).

Exclusion criteria were a neutrophil count of < 1.0 × 10⁹/l or a platelet count < 25 × 10⁹/l at randomization. Multiple organ recipients were excluded. Patients of child-bearing potential who were not on an effective form of contraception were also excluded. Other exclusion criteria were: HIV-positive patients, drug or alcohol abuse, the need of chemotherapy for malignant disease, severe gastrointestinal circumstances such as diarrhoea or vomiting, pre-transplant PRA positivity (> 20%), history of hypersensitivity to aciclovir, ganciclovir or similar drugs and CMV disease before randomization. Positive hepatitis C virus (HCV) antibody test was not an exclusion criterion. Only one of the randomized patients was HCV positive, and this patient was in the control group and did not develop CMV disease.

In the period from August 1998 to April 2001, a total of 455 patients, 18 years of age or more, received their first kidney transplant at our centre. Forty-nine of these were seronegative recipients of seronegative donors (none of these developed CMV pp65 antigenaemia during the course of the trial). Of the remaining 406 patients 262 had a positive test, but 179 of these were not randomized for various reasons shown in Figure 1.

Of the 33 patients who were not randomized due to ‘other inclusion criteria not fulfilled’ (Figure 1), six did not understand the Norwegian language, five suffered from gastrointestinal disease possibly interfering with drug absorption, 12 were not considered to be compliant and 10 had multiple or serious complications after transplantation and many of these were also in need of haemodialysis.

Altogether eight of the 455 observed patients (1.8%) developed CMV disease before CMV pp65 antigenaemia. Although CMV disease was verified in eight patients only five of these fulfilled the study inclusion criteria. In addition, 14 patients who had no CMV disease at the time of first positive CMV pp65 test developed CMV disease before randomization could take place (Table 1). Eleven of these patients were D+R− risk group, 10 were D+R+ and one was D−R+. Furthermore, 10 of the 22 patients experienced an acute rejection episode prior to CMV disease of which six were steroid resistant. Five of the 22 patients neither belonged to the D+R− high-risk group nor experienced a rejection episode. The randomization procedure was stratified for the following ‘risk groups’: D+R+, D+R− and D−R+. A total of 83 patients had been enrolled in the study when the predefined statistical stopping criterion was met at the second interim analysis. Three patients were substituted before the second interim analysis, one due to withdrawal of his consent and two due to violation of one or more exclusion criteria.

The patients who were randomized to active treatment received 1000 mg oral ganciclovir three times daily (adjusted for renal function). Ganciclovir was temporary discontinued with an absolute white cell count < 2.5 × 10⁹/l or a platelet count < 25 × 10⁹/l.

Information regarding infection, rejection, concomitant medication and adverse events as well as blood sampled for CMV pp65 and safety blood chemistry analyses were collected weekly following inclusion up to 12 weeks post-transplantation for all patients and, for safety reasons, for another 2 weeks in patients receiving pre-emptive treatment, i.e. 2 weeks after stop of therapy.
At 6 and 12 months after transplantation information regarding s-creatinine, CMV pp65, medication and adverse events was obtained from the local nephrologist.

Informed consent was obtained from all patients before inclusion in accordance with the Declaration of Helsinki. The patients were included in the screening phase without giving consent as the screening for CMV pp65 antigen in leukocytes is part of the standard procedure at this transplantation centre. The study was approved by the Regional Ethics Committee of Health Region II and by the Norwegian Medicines Control Authority, Oslo, Norway.

**CMV serologic features**

The ABBOTT AxSym system was used for detection of CMV IgG antibodies (ABBOTT, Chicago IL).

**CMV pp65 assay**

CMV infection was monitored by detection of CMV pp65 lower matrix protein in leukocytes from EDTA blood samples. The assay for CMV pp65 antigen was performed, as described previously, as a modification of a reported

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**Fig. 1.** Flow-chart of the 455 patients who received a renal graft in the study period. 179 patients were not randomized due to various reasons listed in the flow-chart. Forty-three patients were randomized to pre-emptive ganciclovir treatment, of whom 42 completed the study. Forty patients were randomized to the control group, of whom 38 completed the study.

**Table 1.** Patients not randomized due to CMV disease

<table>
<thead>
<tr>
<th>CMV donor and recipient</th>
<th>CMV disease before CMV pp65 antigenaemia n = 5</th>
<th>CMV disease with CMV pp65 antigenaemia before randomization n = 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV IgG serostatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+R−</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>D+R+</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>D−R+</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rejection²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+R−</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>D+R+</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>D−R+</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

²Rejection prior to CMV disease.
procedure [16]. Screening was performed every week, and the results were given as the number of CMV pp65 antigen-positive cells per 100,000 leukocytes. CMV infection was defined as a positive CMV pp65 antigen test (>1/100,000 leukocytes).

CMV disease

Diagnosis of CMV disease was made by two physicians following the preset criteria: positive CMV pp65 test accompanied either with CMV syndrome with fever, muscle pain or leucopenia (<4 x 10^9 cells/l) and/or thrombocytopenia (<100 x 10^9 cells/l) or fall in leucocytes and/or thrombocytes of >50% over the last week or organ involvement. Hepatitis: at least twice the baseline value of two liver enzyme tests (ASAT, ALAT) without any other known or detectable cause. Pneumonia: absence of other pathogens (e.g. Pneumocystis carinii) in addition to two of the following signs: interstitial infiltrates on chest X-ray, dyspnoea or requirement for supplementary oxygen and/or ventilatory assistance or decreased pO2 (<80 mmHg) with an increased A-a gradient (i.e. >20). Oesophagitis and gastroenteritis: confirmed by endoscopy and biopsy showing following; CMV inclusions or positive immunofluorescence staining or in situ hybridization for CMV or isolation of CMV virus from biopsy, presence of inflammation and/or necrosis, absence of other pathogens. Polyradiculopathy: confirmed by positive CMV PCR, CMV pp65 or culture of cerebrospinal fluid in addition to both progressive flaccid paraparesis and CSF analysis showing polymorphonuclear pleocytosis, decreased glucose and elevated protein. Retinitis: diagnosed as CMV disease without any other known or detectable cause. Other CMV disease: encephalitis, other CNS affections, etc., without any other known cause. In the event of clinical CMV disease, i.e. ganciclovir was substituted for oral treatment for at least 14 days. Compliance was evaluated by the counting of capsules and patients were excluded if they missed >7 days of oral ganciclovir treatment or took <80% of the allocated ganciclovir treatment.

Immunosuppressive treatment

In recipients of living donor grafts, immunosuppression was instituted 2 days before transplantation while recipients of cadaveric donor grafts had the first dose immediately prior to the transplantation. The immunosuppressive therapy was based on prednisolone and cyclosporine A (CsA) during the whole study. All patients received prednisolone, four patients received tacrolimus instead of CsA. Other components of the immunosuppressive regimen changed during the study. Azathioprine (AZA) was used before 1 April 1999, basiliximab between 1 April 1999 and 1 January 2001, and thereafter mycophenolate mofetil (MMF) was given as baseline immunosuppression in addition to CsA and steroids.

Doses of immunosuppressive drugs

Methylprednisolone i.v.: 40 mg preoperatively, 500 mg during surgery, 40 mg post-operatively and another 80 mg day one. Prednisolone p.o.: 30 mg/day preoperatively, the post-operative dose of 80 mg/day was tapered to 20 mg/day during the first week post-operatively, further tapered to 15 mg after 1 month and subsequently to 10 mg after 2 months. CsA p.o.: 15 mg/kg/day for 2 days before transplantation, 5 mg/kg 2 h preoperatively followed by 5 mg/kg after surgery, 10 mg/kg on day 1. The CsA dose was then adjusted to whole blood trough CsA concentrations of 300–400 μg/l the first 14 days, 250–350 μg/l the next 2 weeks, 150–250 μg/l the second month followed by 125–200 μg/l. AZA p.o.: 3 mg/kg preoperatively, first week 2 mg/kg/day and thereafter 1 mg/kg/day. Basiliximab i.v.: 20 mg preoperatively on day 0 and 20 mg on day 4. MMF p.o.: 2 g/day from the first day after transplantation (1.5 g/day if body weight <60 kg).

Some patients were switched from the baseline treatment to MMF or tacrolimus during the study due to rejection episodes or side effects.

All patients received prophylactic treatment with sulfamethoxazole (400 mg) trimethoprim (80 mg) against P. carinii infection the first 6 months of the study.

Rejection

Acute rejection episodes were diagnosed clinically by a >20% rise in s-creatinine in the absence of urinary tract obstruction and renal graft artery stenosis (excluded by an ultrasonography and duplex Doppler examination of the renal graft). Dehydration, infection and nephrotoxic medication (including CsA) were also excluded. The first rejection episode was usually confirmed by an ultrasound guided core biopsy.

First rejection episodes were treated with a total of 1000–1500 mg of methylprednisolone i.v. during 4–8 days. Later rejection episodes were treated by methylprednisolone boluses of 1/3 of the initial total dose. Oral prednisolone was increased to 30 mg/day, then tapered by 5 mg every 14 days to a maintenance dose of 10 mg.

Steroid-resistant rejections were diagnosed as biopsy verified rejections with no fall in maximum s-creatinine on the fifth day after institution of standard methylprednisolone treatment and were treated by antithymocyte globulin or OKT 3.

Glomerular filtration rate

Renal function was assessed 10 weeks post-transplantation as clearance of [125I]iothalamate. Twenty-five to 50 mCi [125I]iothalamate was injected i.v. and GFR was calculated from plasma samples up to 360 min following administration according to the Brøchner-Mortensen formula \( \frac{\text{GFR}}{[Q_0 b]^{0.99078} - ([Q_0 b]^2 \times 0.001218]) \), where \( Q_0 \) is the amount at time 0 and \( b \) is the terminal elimination constant [17].

Drugs and chemicals

Ganciclovir 250 mg capsules the first 2 years and thereafter 500 mg capsules were supplied by F. Hoffmann La-Roche AG (Basel, Switzerland).

Statistics

The study was designed as a group-sequential study where two interim analyses of the incidence of CMV disease were planned to be performed; the first when 40 patients had been followed for 3 months post-transplantation and the second
analysis after 80 patients had been followed for the same time. Fisher’s exact test was applied to evaluate the hypotheses of equal risks of CMV disease in the two treatment groups. The significance level was 0.005 and 0.014 according to the O’Brien and Fleming procedure in the first and second interim analysis, respectively. Statistical significance was not reached at the first interim analysis, while the second interim analysis of the main variable is presented in this report.

\( \chi^2 \) and Fisher’s exact test were applied to evaluate the hypotheses of equal risks of categorical data between different treatment groups. An unpaired \( t \)-test (adjusted for unequal variances if necessary) was used to compare normally distributed data between different groups. For non-normally distributed data Wilcoxon or Mann–Whitney tests were applied. The statistical software SPSS was used to perform the calculations (SPSS 10.0, Inc., Chicago, IL).

**Results**

**Patients**

Of the 83 patients enrolled into the study, 43 were randomized to receive pre-emptive oral ganciclovir therapy and 40 to no treatment (control group). One patient in the treatment group was substituted before the first interim analysis due to withdrawal of his consent. Two patients in the control group were substituted before the second interim analysis, one due to genital herpes simplex demanding treatment with aciclovir tablets, and the other developed herpes zoster and received valaciclovir. Baseline characteristics of the patients who completed the study are shown in Table 2. There were no demographic differences between the two treatment groups. Donor and recipient CMV IgG serogroups, level of CMV pp65 antigen at inclusion and the number of patients receiving CsA, AZA, tacrolimus, MMF and prednisolone at inclusion were also similar in the two treatment groups.

There was no significant difference in the incidence rates of CMV disease between the group of patients receiving MMF at the time of inclusion (\( n = 13 \)) and the rest of the patients (\( n = 67 \)) (Fisher’s exact test, \( P = 0.053 \)).

The patient and graft survival at 3 months was 100%. One patient in the control group died suddenly and unexpectedly at home 8 months after transplantation with a functioning graft. This patient had not suffered from CMV disease. One patient in the treatment group experienced relapse of Wegeners granulomatosis 11 months after transplantation. He died from septicæmia 14 months after transplantation and received haemodialysis in the terminal phase.

Median (range) compliance assessed by capsule count was 100% (83.2–100%). Time from transplantation to start of ganciclovir capsules was 36 (12–60) days and duration of pre-emptive oral ganciclovir treatment was 49 (27–70) days, median (range). Two patients in the pre-emptive treatment group developed leukopenia and had ganciclovir discontinued for 6 and 7 days, respectively. Both of these patients had received previously OKT 3 due to steroid-resistant rejection, and they continued

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Treatment group ( n = 42 )</th>
<th>Control group ( n = 38 )</th>
<th>( P )-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recipient age, year mean (range)</strong></td>
<td>55 (22–79)</td>
<td>56 (21–78)</td>
<td>( P = 0.7^a )</td>
</tr>
<tr>
<td><strong>Donor age, year mean (range)</strong></td>
<td>46 (2–73)</td>
<td>48 (3–81)</td>
<td>( P = 0.8^a )</td>
</tr>
<tr>
<td><strong>Male recipient gender</strong></td>
<td>28 (67%)</td>
<td>29 (76%)</td>
<td>( P = 0.3^b )</td>
</tr>
<tr>
<td><strong>Recipient weight at inclusion, kg (mean ± SD)</strong></td>
<td>70.5 ± 11.6</td>
<td>75.2 ± 13.9</td>
<td>( P = 0.1^c )</td>
</tr>
<tr>
<td><strong>Dialysis before Tx, no. (%)</strong></td>
<td>31 (74)</td>
<td>34 (89)</td>
<td>( P = 0.09^d )</td>
</tr>
<tr>
<td><strong>Dialysis time pre-transplantation, months</strong></td>
<td>12.0</td>
<td>15.0</td>
<td>( P = 0.3^e )</td>
</tr>
<tr>
<td><strong>Living donor, no. (%)</strong></td>
<td>14 (33)</td>
<td>11 (29)</td>
<td>( P = 0.7^f )</td>
</tr>
<tr>
<td><strong>HLA-AB mismatches, mean ± SD</strong></td>
<td>2.2 ± 1.2</td>
<td>2.3 ± 1.3</td>
<td>( P = 0.6^g )</td>
</tr>
<tr>
<td><strong>HLA-DR mismatches, mean ± SD</strong></td>
<td>0.6 ± 0.7</td>
<td>0.5 ± 0.7</td>
<td>( P = 0.7^h )</td>
</tr>
<tr>
<td><strong>Recipient CMV IgG serogroup, no. (%)</strong></td>
<td>5 (12)</td>
<td>3 (8)</td>
<td>( P = 0.7^i )</td>
</tr>
<tr>
<td><strong>Diabetes mellitus before Tx, no. (%)</strong></td>
<td>24 (57)</td>
<td>24 (63)</td>
<td>( P = 0.6^j )</td>
</tr>
<tr>
<td><strong>Cold ischaemia time, hour, mean (range)</strong></td>
<td>10.4 (0.9–22.8)</td>
<td>12.2 (1–27.7)</td>
<td>( P = 0.4^k )</td>
</tr>
<tr>
<td><strong>CMVpp65 antigen at inclusion.(^{m})Median (range)</strong></td>
<td>2 (1–121)</td>
<td>3 (1–28)</td>
<td>( P = 0.9^l )</td>
</tr>
<tr>
<td><strong>CsA treated patients at inclusion, no. (%)</strong></td>
<td>38 (90)</td>
<td>38 (100)</td>
<td>( P = 0.1^m )</td>
</tr>
<tr>
<td><strong>AZA treated patients at inclusion, no. (%)</strong></td>
<td>14 (37)</td>
<td>15 (36)</td>
<td>( P = 0.6^n )</td>
</tr>
<tr>
<td><strong>MMF treated patients at inclusion, no. (%)</strong></td>
<td>6 (14)</td>
<td>7 (18)</td>
<td>( P = 0.6)</td>
</tr>
<tr>
<td><strong>Prednisolone treated patients at inclusion, no. (%)</strong></td>
<td>42 (100)</td>
<td>38 (100)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( ^a \)Versus group with pre-emptive ganciclovir (Mann–Whitney test).

\( ^b \)Versus group with pre-emptive ganciclovir \( (\chi^2 \text{ test}) \).

\( ^c \)Versus group with pre-emptive ganciclovir \( (\chi^2 \text{ test}) \).

\( ^d \)Versus group with pre-emptive ganciclovir \( (\chi^2 \text{ test}) \).

\( ^e \)CMV pp65 positive cells/10\(^5\) leukocytes.
pre-emptive ganciclovir treatment when the white blood cell count had recovered.

**Incidence of CMV disease during the first 12 weeks after transplantation**

There was a highly significant difference in the incidence of clinical CMV disease between the two treatment groups ($P = 0.0009$) (Figure 2). None of the 42 patients randomized to oral ganciclovir treatment had clinical CMV disease diagnosed during the first 12 weeks post-transplantation, whereas nine of the 38 patients (23.7%) in the control group developed clinical CMV disease during this time period.

Within the control group, all three patients in the $D+R-$ risk group, six of 24 patients in the $D+R+$ risk group whereas none in the $D-R+$ risk group developed clinical CMV disease (Table 3).

Two of the nine patients with CMV disease had duodenal ulcer, and tissue biopsies demonstrated shedding of CMV (culture positivity). One patient showed multiple duodenal ulcer, and tissue biopsy showed both CMV shedding and presence of CMV antigen (positive immunofluorescence staining). Five of the patients suffered from CMV hepatitis. The ninth patient had diagnosed CMV syndrome with flares of fever during 8 days up to 39 °C with chills, headache and muscle pain. All three patients with gastrointestinal ulcer, two of the patients with hepatitis and the patient with CMV syndrome with flares of fever were hospitalized. The other three patients with CMV hepatitis were treated with ganciclovir i.v. on an outpatient basis.

**Incidence of late CMV infection and CMV disease**

Twenty-one patients, who all had been negative for CMV pp65 antigenaemia for >1 month, developed a new CMV infection in the 9 month follow-up period. The number of CMV pp65 positive leukocytes ranged from 1 to 2400. Seven of them were in the control group, and 14 were in the treatment group, the difference was not statistically significant, $P = 0.13$ (Table 4).

![Fig. 2.](image)

**Table 3.** Incidence of clinical CMV disease overall and in subgroups

<table>
<thead>
<tr>
<th>CMV IgG serostatus groups</th>
<th>CMV disease</th>
<th>Treatment group</th>
<th>Control group</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first 12 weeks after transplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0/42</td>
<td>9/38</td>
<td>0.0009\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>$D+R-$</td>
<td>0/5</td>
<td>3/3</td>
<td>0.02\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>$D+R+$</td>
<td>0/24</td>
<td>6/24</td>
<td>0.02\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>$D-R+$</td>
<td>0/13</td>
<td>0/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between 12 weeks and 1 year after transplantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>2/42</td>
<td>2/38</td>
<td>NS\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>$D+R-$</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D+R+$</td>
<td>1/24</td>
<td>1/24</td>
<td>NS\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>$D-R+$</td>
<td>1/13</td>
<td>1/11</td>
<td>NS\textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Versus CMV disease in the treatment group (Fisher’s exact test).
Four patients developed late CMV disease, two in each treatment group. Both patients in the control group developed CMV hepatitis and were treated with ganciclovir i.v., and both were treated with MMF. One of these was hospitalized for late CMV hepatitis after a late acute rejection episode (treated with steroids and ATG). The other patient suffered from an acute rejection episode during the first 3 months after transplantation.

One patient in the treatment group had 22 CMV pp65 positive leukocytes and developed fever and leukopenia while being treated with MMF. He was hospitalized and received ganciclovir i.v. as CMV disease was suspected. His leukopenia worsened, and 2 weeks later MMF was withdrawn with subsequent normalization of white cell count. This patient suffered from a steroid-resistant rejection during the first 3 months post-transplantation.

Finally one patient had recurrence of Wegener’s granulomatosis 11 months after transplantation. Over next 3 months he was hospitalized several times with fluctuating fever, flu-like symptoms and pneumonia responding to antibiotics. He did not receive ganciclovir. Fourteen months after transplantation an open lung biopsy was performed which revealed a positive CMV culture and histology compatible with Wegener’s granulomatosis. He died a few days later from bacterial septicaemia. This patient never experienced any rejection episode, but he received MMF from the time of transplantation. CMV disease was suspected but not diagnosed in another four patients with late CMV infection. Three were untreated. The fourth (treatment group) received ganciclovir capsules pre-emptively again for 14 days as he had 30 CMV pp65 positive leukocytes and simultaneously was also treated for a rejection. CMV affection of the renal graft was suspected, but this was not confirmed in the renal biopsy.

Renal function

There was no difference in s-creatinine between the groups at any time during 1 year post-transplantation. There was, however, a significant decrease in s-creatinine at 6 and 12 months in the control group compared with the baseline value (Table 5). GFR (iothalamate clearance) measured at 10 weeks post-transplantation did not differ between the two treatment groups ($P = 0.09$).

Rejection episodes

There was no difference between the two treatment groups regarding the incidence of clinically suspected or biopsy verified acute rejection episodes before inclusion or during the study period up to 12 months post-transplantation (Table 6). In the control group, the incidence of clinically suspected or biopsy verified rejection episodes did not seem to differ between the

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**Table 4. Number of patients with late CMV infection and late CMV disease**

<table>
<thead>
<tr>
<th>CMV IgG serostatus groups</th>
<th>Late CMV infection</th>
<th>Late CMV disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment group</td>
<td>Control group</td>
</tr>
<tr>
<td>All</td>
<td>14/42 (33%)</td>
<td>7/38 (18%)</td>
</tr>
<tr>
<td>D+/R+</td>
<td>10/24 (42%)</td>
<td>5/24 (21%)</td>
</tr>
<tr>
<td>D−/R−</td>
<td>2/13 (15%)</td>
<td>1/11 (9%)</td>
</tr>
<tr>
<td>D+/R−</td>
<td>2/5 (40%)</td>
<td>1/3 (33%)</td>
</tr>
</tbody>
</table>

*Late CMV infection is defined as positive CMV pp65 test (CMV pp65 > 1 positive cell per 100 000 leukocytes) at any time between 12 weeks and 1 year after transplantation in patients who have been CMV pp65 negative for at least 1 month prior to the positive test.

*NS vs CMV infection in the treatment group ($\chi^2$ test).

*NS vs CMV infection in the treatment group (Fisher’s exact test).

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**Table 5. Renal function in the treatment group and the control group**

<table>
<thead>
<tr>
<th></th>
<th>Treatment group ($n=42$)</th>
<th>Control group ($n=38$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-creatinine at inclusion, mmol/l (mean ± SD)</td>
<td>154 ± 80</td>
<td>158 ± 68</td>
</tr>
<tr>
<td>s-creatinine at 12 weeks, mmol/l (mean ± SD)</td>
<td>133 ± 43</td>
<td>147 ± 40</td>
</tr>
<tr>
<td>s-creatinine at 6 months, mmol/l (mean ± SD)</td>
<td>138 ± 53</td>
<td>146 ± 67</td>
</tr>
<tr>
<td>s-creatinine at 12 months, mmol/l (mean ± SD)</td>
<td>138 ± 53</td>
<td>136 ± 42</td>
</tr>
<tr>
<td>GFR (10 weeks post-Tx, ml/min/1.73 m&lt;sup&gt;2&lt;/sup&gt; (mean ± SD)</td>
<td>56 ± 17</td>
<td>50 ± 14</td>
</tr>
</tbody>
</table>

*Versus treatment group (Mann–Whitney test).

*Versus s-creatinine at inclusion in treatment group (Wilcoxon signed ranks test).

*Versus treatment group (Mann–Whitney test).

*Versus s-creatinine at inclusion in the treatment group (Wilcoxon signed ranks test).

*GFR determined as $[^{135}]$iothalamate clearance.

*Versus treatment group (Unpaired $t$-test).
groups with and without CMV disease (data not shown). Furthermore, 10 patients in the study population experienced a steroid-resistant rejection during the first 12 weeks after transplantation and were treated with ATG or OKT3. Nine of these occurred before inclusion in the study. Of these nine patients, six were randomized into the treatment group and three to the control group (not significant). None of these nine patients experienced CMV disease. One patient (treatment group) had a steroid-resistant rejection after inclusion. This patient did not experience CMV disease. Between 3 months and 1 year after transplantation only one patient suffered from steroid-resistant rejection. This patient was in the control group and developed CMV disease 1 week after she received antithymocyte globulin.

General outcome
The incidence of bacterial and viral (other than CMV) infections did not differ between the two treatment groups during the first 12 weeks of the study (not assessed after 12 weeks).

Discussion
The main finding of the present study is that preemptive therapy with oral ganciclovir is highly effective in preventing CMV disease the first 12 weeks after transplantation in renal transplant recipients. None of the ganciclovir-treated patients developed CMV disease during the first 12 weeks post-transplantation while almost one out of four patients in the control group developed disease.

The incidence of late CMV disease (3 months to 1 year post-transplantation) was only four of 80 (5%), two in each group. Thus, it appears that late occurrence of CMV disease is unaffected by the pre-emptive anti-CMV treatment during the first 12 weeks after transplantation. One patient (treatment group) developed late CMV disease simultaneously with relapse of Wegener's granulomatosis, which may have contributed to reactivation of CMV.

To our knowledge this is the first prospective, randomized study to show effect of pre-emptive oral ganciclovir treatment in renal transplant recipients. Intravenous ganciclovir for pre-emptive treatment has been found previously to result in relatively low CMV-associated morbidity, although the effect was not so striking as in the present study [18]. Oral ganciclovir treatment is more convenient than i.v. treatment but has an absolute bioavailability <10% [15]. Oral valganciclovir was not available at the time of the study.

In a study of Rayes et al. [19] pre-emptive oral ganciclovir therapy was given to liver transplanted patients for 14 days in contrast to a median duration of 49 days in the present study. In that study, 10% of the patients in the treatment group developed CMV disease, and 7.5% of all observed patients developed CMV disease without previous CMV pp65 antigenemia in contrast to 1.8% (eight of 455) of the observed patients in the present study. Moreover, in the study of Rayes et al., a positive result was a count of at least one CMV pp65 positive cell per 10000 leukocytes vs one CMV positive cell per 100 000 leukocytes in the present study. Thus, a lower cut off used in the present study may explain why CMV antigenemia more often preceded CMV disease in the study of Rayes et al. However, the fact that eight of all observed patients (five of them available for randomization) developed CMV disease before antigenemia underlines the importance of intensive monitoring.

Another 14 patients developed CMV disease before randomization could take place underscoring the importance of prompt identification and treatment of positive patients. This may be feasible in a clinical setting. The procedure of informed consent and randomization may have caused the delay in these 14 patients. The fact that these 22 patients actually developed CMV disease represent a limitation for the applicability of the results in this study. Prophylaxis may have prevented CMV disease in these patients. However, it is obviously beyond the scope and design of the present study to discuss benefits vs costs of different treatment strategies.

Both a prophylactic and a pre-emptive regimen may delay the onset of CMV disease. Lowance et al. [5] found that treatment with valacyclovir for 90 days after transplantation significantly reduced the risk but delayed the onset of CMV disease. However, the
Pre-emptive ganciclovir treatment in renal transplant patients could not confirm a delayed onset of CMV disease in the treatment group during the follow-up period.

Although the present study only included a few patients belonging to the D+R− risk group, all three D+R− patients in the control group (100%) developed CMV disease. This is in accordance with previous reports of high incidence of CMV disease in this high-risk group and in particular when the patient has CMV pp65 antigenaemia [2]. The present study indicates that pre-emptive treatment with ganciclovir is a successful approach also for this high-risk group. None of the patients in the D+R− risk group who received pre-emptive ganciclovir therapy developed CMV disease during the first 12 months after transplantation.

The D+R+ patients, with somewhat lower risk of CMV disease, also showed great benefit of pre-emptive ganciclovir treatment in the present study. None of the 24 ganciclovir treated patients in this risk group developed CMV disease during the first 12 weeks in contrast to 25% of the 24 patients in the control group. In the D−R+ subgroup none of the 24 patients in either group developed CMV disease during the first 12 weeks. This is in conflict with previous reports indicating that the D+R+ and the D−R+ risk groups show the same risk of CMV infection and disease, but it is in agreement with a large population-based study showing that D−R+ had a significantly lower risk of hospitalized CMV disease than D+R+[2,20]. Although s-creatinine decreased somewhat in the control group and remained unchanged in the treatment group there was no evidence that renal function was influenced by the treatment.

Besides CMV disease, CMV also has been associated with rejection episodes [7–9]. In the study of Lowance et al. [5], prophylaxis with valacyclovir significantly reduced the incidence of CMV disease, and in seronegative recipients of seropositive donors acute allograft rejection was reduced by almost 50%. In the present study the incidence of clinically suspected and biopsy verified acute rejections were similar in the treatment and the control groups. Moreover, in the present study none of the 10 patients receiving ATG or OKT 3 during the first 12 weeks for treatment of steroid-resistant rejections developed CMV disease even though ATG/OKT 3 has been shown to be a significant risk factor for CMV disease in previous studies [1]. One explanation could be that in the present study only three of these patients were randomized to the control group while seven of the patients receiving ATG/OKT 3 were randomized to the treatment group.

In conclusion, pre-emptive peroral ganciclovir treatment up to 12 weeks post-transplantation effectively prevents CMV disease in renal transplant recipients. However, a pre-emptive therapy approach requires frequent monitoring of CMV pp65 antigenaemia and prompt start of treatment in patients with CMV antigenaemia.

Acknowledgements. The study was financially supported by grants from The Research council of Norway and Hofmann-La Roche, Norway. Thanks to Birgitte Bjerkeley for skilful patient data handling and to biomedical statistician Hans Fagertun for date assessment.

Conflict of interest statement. The co-author A. Aasberg was previously full-time employed at the Laboratory for Renal Physiology, Department of Internal Medicine, Rikshospitalet, University hospital, Oslo, Norway. From autumn 2000, he is only part-time employed at the Laboratory for Renal Physiology and works part-time as medical advisor for Roche Norway AS.

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Received for publication: 23.10.02
Accepted in revised form: 18.4.03