Comparison of universal prophylaxis and preemptive treatment with valganciclovir in management of cytomegalovirus infection in heart transplant recipients

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Abstract

Background: Cytomegalovirus (CMV) is a major cause of infection in the early period after heart transplantation (HTx). There are limited data comparing universal prophylaxis with preemptive treatment of CMV infection in HTx recipients. Therefore, the goal of this study was to evaluate efficacy and safety of both strategies.

Methods: A total of 17 HTx recipients were prospectively enrolled in the universal prophylaxis group. This study cohort was matched with 18 HTx recipients who had the same immunosuppressive regimen and received preemptive therapy for CMV infection. All patients were CMV-seropositive. The study group received oral valganciclovir in a dose of 900 mg daily for 100 days. The second group was treated in case of CMV viraemia higher than 500 copies/ml. The incidence of CMV infection, other opportunistic infections and acute graft rejection and adverse events were evaluated at 3th, 6th and 12th months post-transplant.

Results: Universal prophylaxis was tolerated in 87.5% of the patients for a period of 100 days. Leukopenia was the most frequent side-effect that appeared in 25% of this group. This strategy decreased the rate of asymptomatic CMV infection during the first 3 months after HTx (11.7% vs. 55.6%, \( p = 0.006 \)) compared with preemptive therapy. This positive effect was associated with lower incidence of acute graft rejection at 12 months of follow up (6.3% vs. 41.2%, \( p = 0.015 \)).

Conclusion: Universal prophylaxis, with valganciclovir in CMV-seropositive HTx recipients, was acceptably safe and compared with preemptive therapy of CMV infection reduced the incidence of asymptomatic CMV infection and of acute graft rejection.

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1. Introduction

Cytomegalovirus (CMV) is a major cause of infection in the first months after heart transplantation (HTx). The reported incidence of CMV disease ranges between 10% and 60% depending on donor–recipient mismatch in CMV serology and on intensity of immunosuppression [1–3]. Besides to direct sequelae of infection, CMV viral load has been associated with indirect effects like an increased risk of opportunistic infections [1–3], high incidence of acute graft rejection and/or cardiac allograft vasculopathy [4–6]. Intravenous ganciclovir has been shown to prevent CMV disease both in CMV-seronegative [7] and CMV-seropositive [8] HTx recipients. The invention of valganciclovir a valine ester prodrug of

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ganciclovir with improved bioavailability has facilitated easier and more widespread use of CMV prophylaxis in these patients. As a universal prophylaxis in CMV-seronegative recipients of organs from seropositive donors, valganciclovir at dosage of 500 mg daily is equivalent to oral ganciclovir administered at a dose of 1000 mg three times daily [9]. Valganciclovir has also been studied in the setting of preemptive therapy in HTx recipients [10]. In such case the HTx recipients are monitored for early evidence of CMV replication and treated with antiviral therapy in case of documented viraemia.

Universal prophylaxis might be more effective way preventing both direct and indirect effects of CMV infection than preemptive therapy. On the other hand, preemptive therapy could reduce drug costs and toxicity. However, there are limited data about efficacy of universal prophylaxis with valganciclovir in CMV-seropositive HTx recipients. Similarly, direct comparison of universal prophylaxis and preemptive therapy is not available in this population. Therefore, we conducted a prospective cohort study comparing the efficacy and safety of the universal CMV prophylaxis with the preemptive treatment in HTx recipients at risk of CMV infection.

2. Methods and materials

2.1. Study protocol

This was a prospective single-center, case–control study. The inclusion criteria were as follows: de novo HTx, age of recipient above 18 years and an increased risk of CMV infection. The following combinations of CMV serology in a donor (D) and a recipient (R) were included: R+/D−, R+/D+ and R−/D+. We excluded individuals who deceased before the 10th postoperative day. The other exclusion criteria comprised acute renal or liver failure, severe leukopenia or trombocytopenia and known hypersensitivity to ganciclovir or valganciclovir.

2.2. Study groups

In total, 44 individuals who underwent de novo HTx between November 2007 and December 2008 were screened. Out of this cohort, 41 patients were at risk of CMV infection (85%). Three individuals died early after HTx and another three refused to participate in the study. A total of 35 patients participated in the study. Seventeen HTx recipients at risk of CMV infection were prospectively enrolled in the universal prophylaxis group. The remaining 18 individuals received preemptive treatment of CMV infection. The whole study group had the same induction therapy with polyclonal anti-human thymocyte immunoglobulin (Thymoglobuline, Genezyme Polyclonals S.A.S, Marcy L’ Etoile, France) 1.25 mg/kg day administered at the time of surgery and in the following 3–7 days until target through levels of tacrolimus were reached. Standard immunosuppressive regimen consisted of tacrolimus with a target through level of 10–15 ng/ml, mycophenolate mophetil 2000 mg daily, and prednisone at an initial dose of 1 mg/kg day with subsequent tapering to less than 0.3 mg/kg day at 1 month and 0.1 mg/kg day at 12 months after HTx. Both groups were followed using the same schedule of clinical and laboratory controls, as well as the institutional protocol of endomyocardial biopsy (EMB). Acute allograft rejection episodes ≥ grade Banff 3A were treated with intravenous methylprednisolone 1000 mg for 3 consecutive days.

2.3. Study treatment

The universal prophylaxis group was treated with 900 mg of oral valganciclovir once daily for 100 days starting within the first 10 days after HTx. The group of preemptive therapy was closely monitored to detect CMV viremia and received valganciclovir only in case of CMV viremia higher than 500 copies/ml. The therapeutic dosage of valganciclovir was 900 mg twice daily for 2–3 weeks until clearance of CMV viremia followed by a prophylactic dose for next 3 months. Individuals with tissue invasive CMV disease were treated with intravenous ganciclovir 5 mg/kg twice daily for 3 weeks followed by a prophylactic dose of valganciclovir for next 3 months. In cases of impaired renal function, dosages of valganciclovir and ganciclovir were adjusted appropriately.

2.4. Follow-up

Presence of CMV disease, CMV viremia, other infections and acute graft rejection, as well as adverse effects were analyzed at 3th, 6th and 12th months post-transplant. EMB were planned and performed according to the institutional protocol. In brief, patients underwent EMB every week until 30 days post-transplant, every 2 weeks until 3 months, every 1 month until 6 months, followed by EMB at 9th and 12th months after HTx. Biopsies were graded according to 1990 ISHLT classification (Banff classification) using the following scale: 0, 1A, 1B, 2, 3A, 3B, 4 [11]. Each EMB was accompanied with a clinical and laboratory control. Laboratory analysis included measurements of CMV-viremia, blood count, serum creatinine and liver function tests (aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase and alkaline phosphatase).

The pre-transplant CMV serology status of recipients and donors was assessed using a commercial enzyme-linked immunoassay detecting specific IgG and IgM antibodies. CMV viremia was measured in peripheral venous blood samples obtained into tubes containing ethylenediaminetraacetic acid. The measurement of CMV DNA concentration was performed using a commercially available real-time polymerase chain reaction (Artus™ CMV RG PCR kit, Qiagen, Hilden, Germany).

2.5. Definitions

CMV infection was defined as presence of CMV viremia > 500 copies/ml regardless of symptoms. For the purpose of statistical analysis, we divided CMV infection into asymptomatic CMV viremia (a positive CMV PCR without signs or symptoms) and CMV disease (detectable CMV PCR with attributable symptoms). Leukopenia referred to white blood
cell count of less than $4.0 \times 10^9 \text{l}^{-1}$ and trombocytopenia to platelet count of less than $150 \times 10^9 \text{l}^{-1}$.

2.6. Statistical methods

Categorical data were expressed as percentages and compared using $\chi^2$ analysis. Continuous variables were expressed as a mean and standard deviation. They were compared using the Student $t$-test for paired and unpaired data or by the non-parametric Mann–Whitney test when appropriate. A $p$ value $<0.05$ was considered statistically significant. Analysis was performed using the statistical software SPSS (Chicago, Illinois, USA) for Windows, version 17.0.

2.7. Ethics

The investigation conformed to the principles outlined in the Declaration of Helsinki. It was approved by the local human ethics committee. All subjects gave their written informed consent prior to the participation in the study.

3. Results

Table 1 shows the study population characteristics. The universal prophylaxis group and the preemptive therapy group were well matched. All recipients were CMV-seropositive. There was no case of D+/R-CMV mismatch, which indicated an intermediate risk of post-transplant CMV infection in our study group. Two patients died during follow-up. In the universal prophylaxis group, one patient died of intracerebral hemorrhage on the 16th postoperative day which was not related to treatment with valganciclovir. In the preemptive therapy group, one patient died of sepsis of unknown origin in the 6th postoperative week. The remaining 33 patients completed 12 months of follow-up.

4. Efficacy of treatment

Compared with the preemptive therapy, universal prophylaxis with valganciclovir resulted in significant reduction of asymptomatic CMV viraemia during the first 3 months of follow-up: 2 pts (11.7%) vs. 10 pts (55.6%), $p = 0.006$ (Table 2). The relative risk reduction reached 80%. In addition, four individuals (22%) from the preemptive therapy group experienced a tissue invasive form of CMV disease, which was not observed in the universal prophylaxis group. These four cases included histologically proven CMV gastritis and CMV myocarditis (0–3 months of follow-up) and histologically proven CMV colitis and interstitial pneumonia with detection of CMV and pneumocystis jiroveci in bronchoalveolar lavage specimens (4–6 months of follow-up). Importantly, there was no increase in late-onset CMV infection after completion of valganciclovir prophylaxis. Three months after HTx, asymptomatic CMV infection affected about 12% of patients in both groups in each time period (Table 2).

During the follow up period, four cases of opportunistic infection were observed. Two of them occurred in the universal prophylaxis group. The first case was pneumocystis jiroveci pneumonia diagnosed at 7 weeks after HTx that resolved after cotrimoxazole treatment. The second case was pneumocystis jiroveci pneumonia complicated by invasive pulmonary aspergillosis at 8 weeks after HTx. It was successfully treated with cotrimoxazole and voriconazol. The remaining two cases of opportunistic infection appeared in the preemptive therapy group. One case comprised mixed CMV and pneumocystis jiroveci interstitial pneumonia at 4 months of follow-up, treated again with a combination of cotrimoxazol and voriconazol. The second case was invasive pulmonary aspergillosis diagnosed at 4 months of follow-up, successfully treated with itraconazol.

Interestingly, during the first 3 months of follow up, the universal prophylaxis group presented with lower incidence of acute cellular rejection grade Banff 2 compared with the preemptive therapy group [0 pts vs. 5 pts (27.8%), $p = 0.019$]. Within 12 months of follow-up, only one patient (6.3%) from the universal prophylaxis group experienced one episode of acute cellular rejection, grade Banff 2. On the contrary, 7 pts (41.2%) from the preemptive therapy group had within the same time period a total of 10 episodes of acute cellular rejection grade Banff 2 ($p = 0.015$).

5. Safety

Universal prophylaxis with valganciclovir was well tolerated by 14 individuals (87.5%) for the entire treatment period of 100 days. Valganciclovir had to be discontinued in 2 pts (12.5%) due to significant leukopenia and neutropenia on the 57th and 85th day of prophylaxis. In these two patients, the count

<table>
<thead>
<tr>
<th>Table 1 – The study group characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Universal prophylaxis</strong>, $n = 17$ pts</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Aethiology of heart failure</strong></td>
</tr>
<tr>
<td>CAD 4 pts (23%)</td>
</tr>
<tr>
<td>DCM 7 pts (42%)</td>
</tr>
<tr>
<td>Other 6 pts (35%)</td>
</tr>
<tr>
<td><strong>Immunosuppression</strong></td>
</tr>
<tr>
<td>Tacrolimus 17 pts (100%)</td>
</tr>
<tr>
<td>MMF 17 pts (100%)</td>
</tr>
<tr>
<td>Prednisone 17 pts (100%)</td>
</tr>
<tr>
<td><strong>CMV serology</strong></td>
</tr>
<tr>
<td>D+/R−0</td>
</tr>
<tr>
<td>D+/R−5 pts (29%)</td>
</tr>
<tr>
<td>D+/R−12 pts (71%)</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; CMV, cytomegalovirus; DCM, dilated cardiomyopathy; D, donor; R, recipient; MMF, mycophenolate mophetil; NS, not significant; pts, patients.
of leukocytes and neutrophils reached $2.2 \times 10^9 \text{l}^{-1}$ and $3.3 \times 10^9 \text{l}^{-1}$ and $1.52 \times 10^9 \text{l}^{-1}$ and $1.83 \times 10^9 \text{l}^{-1}$, respectively. Another two individuals experienced leukopenia that resolved after dose reduction of valganciclovir to 450 mg daily (Table 3). The first case appeared on the 79th day (leukocytes $109 \text{l}^{-1}$, and 1.83 $\times 10^9 \text{l}^{-1}$), while the second case was diagnosed on the 83rd day (leukocytes $3.9 \times 10^9 \text{l}^{-1}$, neutrophiles $2.11 \times 10^9 \text{l}^{-1}$). In the first 3 months, the prevalence of leukopenia in the universal prophylaxis group was higher (25%) than in the preemptive therapy group (0%). However, this difference did not reach statistical significance ($p = 0.089$).

Table 3 shows the other parameters of safety. We observed a mild elevation of aspartate amino-transferase in the universal prophylaxis group at 3 months. However, this elevation was only mild and did not exceed the upper limit of normal values in any patient. No other drug-related side-effects were observed.

6. Discussion

The main findings of our study can be summarized as follows. First, universal prophylaxis was significantly more effective than preemptive treatment in reduction of subclinical CMV infection. Second, universal prophylaxis with valganciclovir was safe and tolerated by 83% of the study group for the entire treatment period of 100 days. Third, universal prophylaxis reduced incidence of acute graft rejection Banff 2 during the first 3 months post-transplant.

7. Comparison with previous studies

The first experience with preemptive treatment of CMV infection with valganciclovir was reported by Devyatko et al. [10]. Subsequently, Potena el al. [12] demonstrated in a cohort study that universal prophylaxis with valganciclovir compared with preemptive intravenous ganciclovir reduces CMV viral burden and prevents progression of cardiac allograft vasculopathy. In another study by the same author, an aggressive CMV prophylaxis in CMV R+/D+HTx patients decreased risk of CMV infection, acute graft rejection and progression of cardiac allograft vasculopathy below levels seen in CMV-seropositive HTx patients receiving standard prophylaxis [13]. The aggressive prophylaxis protocol consisted of CMV hyperimmune immunoglobulin plus 4 weeks of treatment with intravenous ganciclovir followed by 2 months of valganciclovir. Standard prophylaxis consisted of intravenous ganciclovir administered for 4 weeks. A direct comparison of universal prophylaxis and preemptive therapy with valganciclovir was performed by Khoury et al. [14] in a randomized study in renal transplant recipients. The study demonstrated greater efficacy of prophylactic valganciclovir given for 100 days to suppress subclinical CMV infection for 12 months than preemptive therapy. However, 22% of patients in the universal prophylaxis group experienced late-onset CMV viraemia. This occurred more frequently in individuals with pre-transplant serology CMV D+/R− (in 38%). A randomized trial in 364 CMV D+/R− solid organ transplant recipients compared valganciclovir 900 mg once daily with oral ganciclovir 1000 mg three times a day for 100 days [9]. Valganciclovir provided greater reduction of CMV viraemia associated with reduced occurrence of acute graft rejection. Nevertheless, this study demonstrated high incidence of late-onset CMV infection, which appeared approximately in 50% of these high risk patients after completion of prophylaxis.

Our study extends the available evidence by direct comparison of universal prophylaxis and preemptive therapy with valganciclovir in CMV-seropositive HTx recipients. It confirms results of previous studies in terms of reduced incidence of CMV infection and acute allograft rejection achieved through universal prophylaxis. However, its results may not be applicable to individuals with pre-transplant CMV serology.
In comparison with preemptive anti CMV therapy, universal prophylaxis with valganciclovir in CMV-seropositive HTx recipients have a high risk of CMV infection and may need a combination of prophylactic approaches or prolonged prophylaxis with valganciclovir. Further studies are needed to elucidate the best management in these individuals.

### Table 3 – Safety of the universal prophylaxis compared with the preemptive therapy. Leukopenia was defined as white blood cells count less than 4000 x 10^9/l.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Group</th>
<th>Bilirubin (µmol/l)</th>
<th>AST (µkat/l)</th>
<th>ALT (µkat/l)</th>
<th>S-Cr (µmol/l)</th>
<th>Leukopenia number of patients (%)</th>
<th>WBC (x 10^9 l^-1)</th>
<th>Hb (g/l^-1)</th>
<th>Platelets (x 10^9 l^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10th day</td>
<td>UP, n = 17</td>
<td>16.3±9.0</td>
<td>0.54±0.61</td>
<td>0.90±0.35</td>
<td>74.9±18.2</td>
<td>0</td>
<td>11.0±5.2</td>
<td>102.6±26.7</td>
<td>267.5±101.3</td>
</tr>
<tr>
<td></td>
<td>FT, n = 18</td>
<td>16.7±9.0</td>
<td>0.38±0.22</td>
<td>0.88±0.44</td>
<td>78.4±22.9</td>
<td>0</td>
<td>11.5±3.3</td>
<td>105.4±19.4</td>
<td>266.9±78.3</td>
</tr>
<tr>
<td>3 months</td>
<td>UP, n = 16</td>
<td>12.2±8.2</td>
<td>0.44±0.11*</td>
<td>0.77±0.29</td>
<td>93.3±128.0</td>
<td>4 (25%)</td>
<td>6.3±2.2</td>
<td>129.7±47.9</td>
<td>208.3±56.9</td>
</tr>
<tr>
<td></td>
<td>FT, n = 17</td>
<td>12.5±6.4</td>
<td>0.35±0.14</td>
<td>0.57±0.24</td>
<td>98.6±24.5</td>
<td>3 (17.6%)</td>
<td>7±1.0</td>
<td>121.1±3.8</td>
<td>196.1±51.5</td>
</tr>
<tr>
<td>6 months</td>
<td>UP, n = 16</td>
<td>13.2±8.9</td>
<td>0.39±0.10</td>
<td>0.61±0.24</td>
<td>100.6±38.3</td>
<td>0</td>
<td>6.7±1.4*</td>
<td>132.1±14.2</td>
<td>188.2±51.0</td>
</tr>
<tr>
<td></td>
<td>FT, n = 17</td>
<td>11.8±3.8</td>
<td>0.43±0.18</td>
<td>0.65±0.28</td>
<td>109.4±25.4</td>
<td>2 (11.1%)</td>
<td>6.0±1.9</td>
<td>126.2±14.4</td>
<td>185.1±51.1</td>
</tr>
<tr>
<td>12 months</td>
<td>UP, n = 16</td>
<td>13.4±6.3</td>
<td>0.42±0.14</td>
<td>0.56±0.16</td>
<td>107.2±47.4</td>
<td>0</td>
<td>7.7±2.6</td>
<td>132.0±16.3</td>
<td>199.3±58.0</td>
</tr>
<tr>
<td></td>
<td>FT, n = 17</td>
<td>13.2±7.1</td>
<td>0.41±0.12</td>
<td>0.58±0.18</td>
<td>121.4±30.0</td>
<td>1 (5.8%)</td>
<td>7.2±2.9</td>
<td>128.2±17.8</td>
<td>173.1±57.3</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALT, alanine amino-transferase; AST, aspartate amino-transferase; FT, preemptive therapy; S-Cr, serum creatinine; UP, universal prophylaxis; WBC, white blood cells count. P-value for comparison between the universal prophylaxis group and the preemptive therapy group at each period of follow-up was coded: p < 0.05, *p < 0.01.*
recipients reduced more effectively incidence of CMV infection and acute allograft rejection grade Banff 2 during the first 3 months after transplantation. Prophylactic treatment was well tolerated and safe.

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