Valganciclovir-Induced Leucopenia in Renal Transplant Recipients treated with Mycophenolate Mofetil

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ABSTRACT

Objective
Cytomegalovirus (CMV) is a viral infection commonly affecting renal transplant recipients. Current guidelines recommend the prophylactic treatment of patients at risk from CMV with oral Valganciclovir (VGC), however, myelotoxic side effects have been reported. The severity of leucopenia is reported to be increased when used in conjunction with Mycophenolate Mofetil (MMF), although some studies have shown conflicting evidence.

Method
Retrospective analysis of patient clinical data, post-renal-transplant, was performed. Patients included were treated with; MMF and VGC [MMF(+)VGC(+)], MMF but not VGC [MMF(+)VGC(-)], no MMF but with VGC [MMF(-)VGC(+)] and, neither MMF or VGC [MMF(-)VGC(-)]. Blood results and other relevant data were collected from clinical databases.

Results
In total, data from 61 patients were analysed. 13 patients were MMF(+)VGC(+), 48 patients were MMF(+)VGC(-), 5 patients were MMF(-)VGC(+) and 12 patients were MMF(-)VGC(-). Of these, 6 MMF(+)VGC(+) patients and 3 MMF(+)VGC(-) patients were leucopenic within the first 3 months post-renal-transplant (p=0.001). This difference was not apparent in patients that were not treated with a MMF regime.

Conclusion
Patients treated with MMF and VGC are at a significantly higher risk of leucopenia when compared to patients not treated with MMF and VGC in the first 3 months post-renal-transplant.

Key Words: Leucopenia, Mycophenolate, Renal, Transplant, Valganciclovir.

Introduction
Human cytomegalovirus (CMV) is the most important viral infection in renal transplant recipients, as it is a major cause of morbidity and mortality. [1] Approximately 30-97% of the general population are thought to be seropositive for CMV, and the prevalence has been shown to increase with age. [2] Solid organ transplant (SOT) is one of the many ways in which CMV may be transmitted to the seronegative patients. Primary infection with the virus is often asymptomatic in an immunocompetent host. However, in an immunocompromised host, primary infection or reactivation of a latent infection can cause CMV disease. This is of...
particular concern in the first 3 to 6 months post-SOT when recipients are aggressively immunosuppressed to avoid acute graft rejection. \[2\]

Several recent studies have shown oral Valganciclovir (VGC) prophylaxis to be superior to pre-emptive therapy, when CMV infection and disease is a risk following SOT. \[3, 4\] In prophylactic therapy all patients “at risk” are given Valganciclovir whereas in pre-emptive therapy patients are monitored for any indications of disease prior to starting therapy. Current guidelines recommend the prophylactic treatment of renal transplant patients at risk from CMV with oral Valganciclovir, 900mg once daily dose, started within 10 days and continued for at least 100 days. \[5\] Unfortunately, Valganciclovir is known to have myelotoxic effects and studies have reported leucopenia in patients treated with Valganciclovir alone as high as 10-28%. \[6, 7\]

Mycophenolate Mofetil (MMF) is a commonly used immunosuppressive agent which is often used in combination calcineurin inhibitors as standard practice. However, MMF too can cause leucopenia. The severity of leucopenia has been reported to increase when used in conjunction with VGC. \[8\] Some studies have reported the use of VGC and MMF not to be associated with a greater incidence of leukopenia. \[9\] It is important to clarify these conflicting results as it could help prevent the establishment and complications of severe neutropenia in this patient group. Consequently, the aim of this study was to investigate whether the use of VGC causes leucopenia in patients treated with a MMF regime post-renal-transplant or not.

**Methods**
We retrospectively analysed clinical follow-up data of renal transplant patients from Glasgow, Scotland, United Kingdom. Ethical approval for the study was deemed unnecessary as patient follow-up data was being analysed in order to evaluate existing patient care. Caldicott guardian approval was not sort as no patient sensitive data was collected.

**Study Population**
Men and women of all ages were included in this study if they had a renal transplant. Patients who were not treated with a MMF regime and those with insufficient data available were excluded from this study. Prophylactic therapy with VGC was indicated in patients if the serum CMV status in the donor was positive and recipient was negative (D+R-). On occasions VGC therapy was given to patients where the donor was negative and recipient was positive (D-R+) or both the donor and recipient were positive (D+R+). Data from consecutive patients who had a renal transplant in Glasgow between August 2008 and May 2009 were analysed. This time frame was selected due to limitations in time available to collect data.

**Collection of data**
Initially the transplant register was searched to identify suitable patients. Data was then retrieved from two separate databases, namely the “Western Infirmary General (WIG) renal proton” system and NHS greater Glasgow and Clyde Clinical Portal, to verify the search results and collect further data required. The WIG renal proton system contains up-to-date and past blood results along with a list of prescribed medications along with their respective doses. Relevant demographic and procedure related data were collected from the NHS greater Glasgow and Clyde Clinical Portal. \[10\] We collected patient demographic data (age, and gender), the white cell count (WCC) at 3 months, 6 months and 12 months, VGC dose and duration, MMF dose at 1 year, serum creatinine at 1 year, and rejection at 1 year.
Furthermore, the presence of leucopenia (i.e. WCC less than $4 \times 10^9$/L) with in the first 3 months post-transplant was also noted.

**Primary outcome**

The primary outcome was leucopenia during the first 3 months. To investigate whether the use of VGC affected the leucocyte count in patients on a MMF regime post-renal-transplant, we grouped patients into 4 groups. Patients that were treated with MMF and VGC [MMF(+)]VGC(+)], patients treated with MMF and no VGC [MMF(+)]VGC(-)], patients not treated with MMF but with VGC [MMF(-)]VGC(+)] and finally patients not treated with MMF and VGC [VCG(-)]MMF(-)]. Patients not treatment with MMF were treated with Azathioprine. The use other immunosuppressive agents (i.e. Tacrolimus and Sirolimus etc.) was not evaluated in this study.

**Secondary outcome**

We also investigated the mean white cell count (WCC) at 3, 6 and 12 months in the individual groups and renal function at 1 year by measuring the serum creatinine (Cr) levels at 1 year post-transplant. The relationship between leucopenia and number of infections experienced by patients was not evaluated in this study, as numerous previous studies have reported there to be no association in this patient group. [8]

**Statistical analysis**

All analyses were performed using the Statistical Software Minitab 15 (Minitab Inc). A chi-square test, fisher exact test or 2-sample t-test were performed, where possible, to identify any differences between groups. All p-values less than 0.05 were considered significant. Data which met the assumptions of the normal distribution were presented with the mean and 2-tailed 95% confidence interval.

**Results**

In total, data from 79 patients had a renal transplant. 1 patient was excluded due to insufficient data. Therefore data from 78 patients were analysed in this study.

**Baseline characteristics**

Of the 78 patients analysed, 42 patients were male (53.8%) and 36 patients were female (46.2%). The average age of patients in the study was 45.6 years (95% CI, 42.5 - 48.7 years). 30 patients were seronegative for CMV and 28 were seropositive. The serum CMV status of 20 patients was not commented on. 52 patients received a transplant from cadavers, 17 patients received a transplant from live related donors and 9 patients received transplants from live unrelated donor. The serum CMV status of donors was not obtained. In total 61 patients were treated with MMF and 18 patients treated with VGC (see figure 1).

**Primary outcome**

The WIG proton system showed 13 patients to be MMF(+)]VGC(+)], 48 patients to be MMF(+)]VGC(-)], 5 patients to be MMF(-)]VGC(+)] and 12 patients to be MMF(-)]VGC(-)] (see figure 1). 6 MMF(+)]VGC(+)], 3 MMF(+)]VGC(-)], 3 MMF(-)]VGC(+)] and 4 MMF(-)]VGC(-)] patients were leucopenic at some point within the first 3 months post-renal-transplant. The difference between MMF(+)]VGC(+) and MMF(+)]VGC(-) patients was statistically significant (p=0.001). The were no significant differences between MMF(-)]VGC(+) and MMF(-)]VGC(-)] groups.

The mean dose of MMF at 1 year in patients treated with VGC was significantly lower than patients not treated with VGC (0.87g; 95% CI: 0.53-1.22g versus 1.27g, 95% CI: 1.07-1.47g, p-
value=0.046). 15 patients received a single course and 3 patients received multiple courses. In total, 23 courses of Valganciclovir were administered, 5 at 900mg, 17 at 450mg and 1 at 125mg. 16 patients received VGC as prophylaxis. However only 10 patients had treatment started with 10 days of SOT. The mean duration of treatment for all patients treated with prophylactic VGC was 69.2 days (95% CI, 51.8 days to 86.7 days). 2 patients received a prophylactic dose of 900mg, 13 patients received a prophylactic dose of 450mg and 1 patient received a prophylactic dose of 125mg.

Secondary outcome
No significant differences were observed in MMF(+)VGC(+) and MMF(+)VGC(-) groups at 3, 6 and 12 months. The average serum creatinine at 1 year was; 123.5µmol/L (95% CI 100.8 – 146.3µmol/L) in the MMF(+)VGC(+) group, 122.5µmol/L (95% CI 84.2 – 160.8µmol/L) in the MMF(+)VGC(-) group, 413.0µmol/L (95% CI -269 – 1096µmol/L) in the MMF(-)VGC(+) group and 207.5µmol/L (95% CI 63.8 – 351.2µmol/L) in the MMF(-)VGC(-) group. The mean serum creatinine at 1 year in patients that were leucopenic in the first 3 months was 135.7µmol/L, 404.0µmol/L, 182.3µmol/L and 113.5µmol/L in MMF(+)VGC(+), MMF(+)VGC(-), MMF(-)VGC(+) and MMF(-)VGC(-) patients, respectively. No significant differences were observed.

Discussion
The use of VGC prophylaxis has a clear evidence based benefit in reducing the rates of CMV viraemia, which can lead to graft rejection, graft loss and death. To the best of our knowledge only two studies have evaluated VGC induced leucopenia in patients treated with a MMF immunosuppressive regime post-transplant. [8, 9] The study by Brum et al (2008) reported an increased frequency of leucopenia were VGC prophylaxis was given to patients in combination with MMF. [8] However, results from this study were contradicted by Perez et al (2009) who reported no differences in the incidence of leucopenia in patients treated with VGC alone when compared to VGC and MMF, although leucopenia was observed in both groups. Both these studies evaluated similar parameters to those used in our study, with the exception of the analysis of calcineurin inhibitor levels and patient BMI. Our definition of leucopenia also differed, as we defined it as a WCC less than 4x10^9/L in comparison to a WCC less than 3 x 10^9/L, which was used in both studies.

Outcome measures
In this retrospective study, investigating whether the use of VGC affected the leucocyte count in patients treated with a MMF regime post-rental-transplant, we found that significantly more patients in the patient group treated with MMF and VGC [MMF(+)VGC(+)] experienced leucopenia when compared patients treated with MMF alone [MMF(+)VGC(-)] in the first 3 months after renal transplant. However, this difference was not apparent in patients that were not treated with MMF. Consequently, leucopenia was associated with the combined use of MMF and VGC. However the absence of any significant differences in leucopenic episodes between [MMF(+)(VGC(+))] and [MMF(-)(VGC(-))] was surprising and questions the significance of our findings. Insufficient data was recorded to allow for the quantification of the magnitude of leucopenia observed during this period. No significant differences were observed in the different patient groups when analysing the WWC at 3, 6 and 12 months. This may have been due to the cessation of simultaneous VGC and MMF therapy by these time points.

As recommended by current guidelines, the most popular dose of MMF prescribed was 2g (twice daily dose of 1g). However, we observed patients treated with VGC to be given significantly lower doses of MMF when compared to patients that were not treated with VGC but on a MMF regime. Consequently the physician may have been guided by patients
WCC to adjust the doses of both drugs in an effort to avoid their cumulative side-effects. Current guidance surrounding the concomitant use of MMF and VGC is sparse and future research is required to improve current guidelines. The most commonly used dosage of VGC was 450mg, which is half the recommended dosage (900mg). Use of this lower dosage may have been justified by patients having a reduced renal function, physicians experience or literature suggesting 450mg VGC to be non-inferior to 900mg VGC. [11]

We did not observe any significant differences in the number of successful renal transplants at 1 year when comparing the difference groups. Furthermore, no difference in renal function was also observed at 1-year post-renal-transplant.

**Limitations**
The low number of patients in this study is an obvious limitation; consequently larger studies are required in order to validate these trends. Several potential confounding factors were not accounted for in this study. The omission of data on other immunosuppressive agents (i.e. sirolimus) used is important to bear in mind as they too can cause myelosuppression. Data relating to CMV viraemia was also not documented, which may be significant as this too can cause leucopenia in patients who did not receive prophylactic VGC. Finally the absence of the CMV status of 20 recipients and all donors in this study makes it impossible to comment on whether VGC was used based on current guidelines.

**Conclusion**
Patients treated with Mycophenolate Mofetil and Valganciclovir [MMF(+)/VGC(+)] are at a significantly higher risk of leucopenia when compared to patients treated with Mycophenolate Mofetil and not Valganciclovir [MMF(+)/VGC(-)] in the first 3 months post-renal-transplant. However, leucopenia whilst an adverse event is reversible and not associated with long-term adverse outcomes.

**References**