Replacement of Mycophenolate Acid With Everolimus in Patients Who Became Neutropenic After Renal Transplant

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Abstract

Objectives: Neutropenia after kidney transplant is an adverse event usually treated with a dosage reduction of mycophenolic acid. We evaluated the efficacy and safety of substituting mycophenolic acid with everolimus in patients with persistent neutropenia after kidney transplant.

Materials and Methods: This study was a retrospective analysis. A total of 17 patients who were initially treated with mycophenolic acid (1912 ± 196 mg/d), calcineurin inhibitors, and methylprednisolone for kidney transplant were included.

Results: In 15 patients, neutropenia occurred within the first 3 months (during valganciclovir administration), and in 2 patients between the fourth and sixth month after transplant. One hundred eighteen episodes of neutropenia were recorded, originally treated by reducing the dosage of mycophenolic acid (765 ± 390 mg/d) and administering granulocyte colony-stimulating factor. Three patients experienced acute rejection 5 to 10 days after reducing the dosage of mycophenolic acid, and they were successfully treated with pulse steroids. Five patients developed cytomegalovirus infection 108 ± 65 days after the onset of neutropenia. After replacing mycophenolic acid with everolimus, episodes of neutropenia were observed in 6 patients. In 1 patient, discontinuing everolimus was necessary after 1.5 months of treatment. In 5 patients with cytomegalovirus infection, neutropenia subsided after termination of valganciclovir treatment. In the remaining 11 patients, no episodes of neutropenia were observed. No episodes of acute rejection occurred, and renal function remained stable during a follow-up of 47 ± 30 months (estimated glomerular filtration rate [eGFR MDRD]: 45 ± 14 mL/min/1.73 m² → 47 ± 22 mL/min/1.73 m²).

Conclusions: Replacing mycophenolic acid with everolimus appears to be a safe and effective alternative treatment in neutropenic renal transplant recipients.

Key words: Cytomegalovirus, Everolimus, Kidney transplant, Mycophenolic acid, Neutropenia

Introduction

Neutropenia is a frequent adverse event after a kidney transplant. Approximately 28% of renal transplant recipients will experience at least 1 episode of neutropenia in the first year after transplant. The incidence of leukopenia and neutropenia in kidney transplant recipients has been reported to be from 10% to 55.5% and from 4.9% to 37.5%. Its cause is usually multifactorial, but the main culprits are medications, systemic infections, and posttransplant lymphoproliferative disorders.1-3

Medications that commonly lead to neutropenia include antiproliferative agents (mycophenolic acid [MPA] and azathioprine) and antiviral agents (valacyclovir and valganciclovir). Less commonly, antithymocyte globulin, alemtuzumab, rituximab, tacrolimus (TAC), cyclosporine, sirolimus, cotrimoxazole, omeprazole, and angiotensin-converting enzyme inhibitors have been reported to
cause neutropenia.\textsuperscript{4-11} The contribution of these agents is difficult to assess because they are frequently used in combinations. Remission of neutropenia after drug elimination or discontinuation can be used as indirect evidence.

In clinical practice, in the absence of infection, management of neutropenia includes reduction of the dosage or discontinuation of MPA and/or valganciclovir, temporarily or permanently, and use of granulocyte colony-stimulating factor (G-CSF). These patients face an increased risk for acute rejection, graft loss, infections (bacterial or \textit{cytomegalovirus} [CMV]), and show worse survival rates.\textsuperscript{1,3,13-14}

Although neutropenia is a frequent complication after a kidney transplant, published data are scarce. Risk factors, natural history, therapeutic management, and outcomes have not been well defined. Substitution of MPA by m-TOR inhibitors in patients with neutropenia after a kidney transplant might be an alternative to maintain sufficient immunosuppression. In this study, efficacy and safety of MPA replacement with everolimus (EVE) in renal transplant recipients experiencing drug-related neutropenia was assessed.

\textbf{Materials and Methods}

\textbf{Patients’ clinical characteristics}

Data from patients who underwent a kidney transplant at the University Hospital of Patras, Greece, from 2003 to 2011, and who had mycophenolic acid replaced with EVE because of resistant neutropenia was retrospectively analyzed.

Seventeen patients (12 men, 5 women; aged 40 ± 12 years old; range, 15-48 y) with MPA replacement by EVE were included in the study. Five patients had panel reactive antibodies titers > 30%. For 4 of these patients, this was their second kidney transplant, and for 1, it was the third. Most grafts (70%) came from donors with expanded criteria. Human leukocyte antigen compatibility was low (3-5 mismatches). The majority of these allograft recipients (n=16) received TAC as calcineurin inhibitor. Trough levels for TAC ranged between 7 to 11 ng/mL, and for cyclosporine, it ranged between 150 and 175 ng/mL. Delayed graft function was seen in 9 of 17 patients (53%). Clinical and demographic data of donors and recipients are summarized in Table 1. In all patients, white blood cell counts as well as absolute neutrophil count were within normal limits before the transplant.

\begin{table}
\centering
\caption{Patients’ Clinical and Demographic Data}
\begin{tabular}{|l|l|}
\hline
\textbf{Data} & \textbf{\((n=17)\)} \\
\hline
Sex, male/female (n) & 12/5 \\
Age, mean (y) & 40 ± 12 \\
CKD cause, (n) & \\
Chronic glomerulonephritis & 7 \\
Unknown & 3 \\
Vesicouretal reflex & 2 \\
Polyuric kidney disease & 2 \\
Alport syndrome & 1 \\
Nephronophthisis & 1 \\
Cystinuria & 1 \\
Previous kidney transplants (n) & \\
1 & 12 \\
2 & 4 \\
3 & 1 \\
Donors’ data & \\
Living/deceased (n) & 1/16 \\
Age, mean (y) & 55 ± 12 \\
Marginal (n) & 12 \\
Transplant data & \\
Mismatches, mean (n) & 4 ± 1 (3-5) \\
Delayed graft function (n) & 9 \\
CMV IgG donor/recipient, (n) & \\
+/+ & 11 \\
+/- & 5 \\
/- & 1 \\
PRA before transplant, (n) & \\
> 30% & 3 \\
> 80% & 2 \\
Immunosuppression, (n) & \\
TAC/MPA/steroids & 16 \\
CSA/MPA/steroids & 1 \\
\hline
\end{tabular}
\end{table}

Abbreviations: CKD, chronic kidney disease; CSA, cyclosporine; MPA, mycophenolate acid; PRA, panel reactive antibodies; TAC, tacrolimus

Neutropenia

Neutropenia is defined as \textit{absolute neutrophil count} < 2000/\mu{L}. Grades of neutropenia defined according to WHO criteria are: grade 1, absolute neutrophil count < 2000/\mu{L}; grade 2, absolute neutrophil count < 1500/\mu{L}; grade 3, absolute neutrophil count < 1000/\mu{L}; and grade 4, absolute neutrophil count < 500/\mu{L}.

\textbf{Immunosuppression}

All patients received an immunosuppressive regimen with induction therapy by anti-IL2 receptor monoclonal antibody (basiliximab) and maintenance therapy with calcineurin inhibitors (TAC or cyclosporine), MPA (mycophenolate mofetil or enteric-coated mycophenolate sodium), and methylprednisolone. Mycophenolic acid blood levels were not measured, because this was not a routine clinical practice at our center during the study.

\textbf{Concomitant medications}

According to our practice, all patients received 3 months’ prophylactic treatment with valganciclovir for CMV, and 6 months’ cotrimoxazole (800 + 160 mg every second day against \textit{Pneumocystis jirovecii}). Valganciclovir dosing was adjusted for kidney
function (900 mg for estimated glomerular filtration rate [eGFR] > 60 mL/min, 450 mg for eGFR = 40-59 mL/min, 450 mg every second day for eGFR = 25-39 mL/min, and 450 mg twice weekly for eGFR = 0-24 mL/min). Patients with known G6PD deficiency or previous allergic reaction to cotrimoxazole received inhaled pentamidine instead. The antihypertensive treatment used in patients with neutropenia included beta blockers and calcium channel blockers. None of the patients with neutropenia received angiotensin-converting enzyme inhibitors that could contribute to neutropenia. In addition, all patients received a protein pump inhibitor for gastric protection.

Cytomegalovirus

Cytomegalovirus was defined as either CMV clinical syndrome or tissue invasive CMV, with PCR detection of CMV in blood (more than 50 copies/mL).

Acute rejection

Acute rejection episodes were diagnosed by biopsy and classified according to Banff criteria.

Kidney function

Kidney function was assessed by serum creatinine concentrations and e-GFR using MDRD6 formula.

Statistical analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 17.0, IBM Corporation, Armonk, NY, USA). Paired t and Mann-Whitney U tests were used to compare means. P values < .05 were considered as statistically significant.

Results

In total, 118 episodes of neutropenia (7 ± 4 per patient) were recorded (grade 1, 44%; grade 2, 34%; grade 3, 19%; and grade 4, 3%) before replacement of MPA by EVE (Figure 1). In 15 patients (88%), the first episode of neutropenia occurred within 3 months, while in 2 patients (12%), it occurred 4 and 5 months after transplant (mean, 59 ± 38 d). Patients who experienced the first episode of neutropenia within the first 3 months after transplant also were receiving valganciclovir.

At the initial phase of neutropenia, a careful clinical and laboratory investigation (urine culture, C-reactive protein concentrations, and detection of serum CMV) were performed in each patient to exclude any underlying infection. Also, during the whole maintenance phase of neutropenia, all patients were screened every 7 to 14 days for CMV and 3 times weekly with urine cultures.

Initial management of neutropenia

Initially, the MPA dose was gradually reduced from 2000 mg/day (n=14) or 1500 mg/day (n=3) to 500 to 1000 mg/day (1912 ± 196 → 765 ± 390 mg/d). Despite the dosage reduction, MPA was discontinued in 3 patients because of persistent neutropenia. At the same time, cotrimoxazole was substituted by inhaled pentamidine.

Patients who had grade 2, 3, and 4 episodes of neutropenia received G-CSF (7 ± 5 doses per patient) at a dosage ranging from 1 dose every second day up to 2 doses every 2 weeks. Calcineurin inhibitor (CNI) levels and valganciclovir dosing were kept unchanged.

After MPA dosage reduction or discontinuation, 3 episodes of acute rejection (Banff 1A) were confirmed. In 1 patient, acute rejection occurred 5 days after MPA was discontinued; and in 2 patients, acute rejection occurred 10 and 12 days after MPA dosage reduction (1000 mg/d). All 3 episodes were successfully managed with intravenous pulses of methylprednisolone. Despite these maneuvers, neutropenia persisted in all patients.

Replacement of mycophenolic acid by everolimus

Replacement of MPA with EVE was performed after a mean of 4 months (range, 5 d to 48 mo) after the first episode of neutropenia, and 6 months (range, 1-23 mo) after transplant (4 patients, 0-3 mo; 7 patients, 4-6 mo; 3 patients, 7-12 mo; and 3 patients > 12 mo after transplant).

After replacing MPA with EVE, no episodes of neutropenia were recorded in 11 of 17 patients (65%). From the remaining 6 patients who continued experiencing neutropenia, 5 patients (83%) developed symptomatic CMV disease, ranging from 48 to 200 days (median, 108 d) after the first episode of neutropenia and 4 to 8 months (mean, 5.5 mo) after receiving the transplant. All these patients were successfully treated with valacyclovir/valganciclovir, and 3 of them also received combined treatment with foscarnet. In these 5 patients replacement of MPA with EVE was performed after a median of 8 days (range, 1-50 d) after receiving a diagnosis of CMV disease. The
episodes of neutropenia were milder (grade 1, 53%; grade 2, 38%; grade 3, 9%) and with less frequent use of G-CSF (0-4 doses per patient) (Figure 1).

After discontinuing antiviral drugs, no more episodes of neutropenia were seen. Only 1 of 17 patients (6%) continued to experience episodes of neutropenia, necessitating frequent use of G-CSF. In this patient, EVE was discontinued 1.5 months later, and the patient received double immunosuppressive regimen with TAC plus corticosteroids.

No episode of acute rejection was recorded, and renal function remained stable 6 months after replacing MPA, and for the entire follow-up of 52 months (range, 6-84 mo) (Table 2). No major adverse reactions related to EVE were observed. The amount of urinary protein excretion remained unchanged (190 ± 140 mg/day → 290 ± 200 mg/day; P = NS) for the entire study.

Table 2. Renal Function Before and After Everolimus Initiation (n=17)

<table>
<thead>
<tr>
<th>Renal Function</th>
<th>Before Everolimus</th>
<th>6 Months After Everolimus</th>
<th>52 Months After Everolimus</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mmol/L)</td>
<td>167.96 ± 34</td>
<td>150.28 ± 72</td>
<td>176.80 ± 39</td>
<td>NS</td>
</tr>
<tr>
<td>e-GFR (mL/min/1.72m²)</td>
<td>45 ± 14</td>
<td>51 ± 16</td>
<td>47 ± 22</td>
<td>NS</td>
</tr>
</tbody>
</table>

Discussion

Although neutropenia is a common adverse event after renal transplant, its management is controversial and complicated by variable practice patterns. Dosage reduction, or discontinuation of immunosuppressive and/or anti-infective drugs during the early posttransplant period, increases the risk either for rejection or infection.

Mycophenolic acid seems to cause dose-dependent neutropenia,18-20 while its dosage reduction or discontinuation increases the risk of acute rejection.12-15 Use of MPA in combination with TAC has been associated with neutropenia more often than the combination of MPA with cyclosporine.3 This effect seems to be related with increased patient exposure to MPA because of increased bioavailability.21 However, it remains unclear whether MPA dose or blood levels can predict the risk of developing neutropenia.18,22,23 Calcineurin inhibitor trough levels ranged between the expected limits for the time after transplant. In our study, the vast majority of patients (16 of 17) who received TAC in combination with MPA developed resistant neutropenia. Also, 17% of patients with MPA dosage reduction or discontinuation because of neutropenia had an acute rejection episode. However, these episodes were mild (Banff 1A) and were effectively treated with steroids.

Bone marrow toxicity of valganciclovir seems to be dose-dependent, especially if dosing is not adjusted to kidney function (e-GFR).15-17 In 88% of our patients, neutropenia appeared within the first 3 months after the transplant, during which all patients received prophylactic treatment with valganciclovir. Neutropenia persisted despite valganciclovir discontinuation after 3 months. However, after MPA substitution by EVE, only 5 of 17 patients (29%) diagnosed with a CMV infection who received antiviral treatment, experienced episodes of neutropenia. Whether infection itself or valganciclovir was responsible for persistence of neutropenia cannot be definitely determined.

Administration of G-CSF for managing neutropenia is considered with skepticism, given the paucity of evidence in the literature.24,25 Additionally, it remains unclear which grade of neutropenia should be regarded as a threshold for using G-CSF. We used G-CSF at an absolute neutrophil count less than 1500/mL (grade 2). Using G-CSF was followed by an increase in the white blood cell count, as well as in absolute neutrophil count, but this effect was temporary and necessitated frequent re-administration. There were no adverse events recorded after the use of G-CSF, except for mild bone pain in few patients.

Introduction of EVE in the immunosuppressive regimen, in combination with MPA or low-dose CNI aim at maintaining kidney function and improving graft survival. Moreover, with its possible antineoplastic and antiviral properties, along with cardiovascular protection properties, it aims at improving overall patient survival.26-30
In an attempt to maintain a sufficient triple immunosuppressive regimen, EVE was introduced to replace MPA, as MPA was considered responsible for resistant neutropenia. Patients in our study were relative young (range, 15-58 y), 5 of them had received 1 or 2 kidney transplants in the past and had an increased cytotoxic antibody titers, while 70% of the donors were donors with expanded criteria. In addition, MPA dosage reduction led to acute rejection episodes in 3 patients.

Variations in the time EVE was introduced to manage resistant neutropenia (range, 5 d-48 mo) was related to the limited availability of the drug and the small experience with this maneuver.

Although EVE has been reported as a cause of neutropenia, only 1 patient (6%) continued experiencing neutropenia and had the drug discontinued. The remaining 5 patients who experienced neutropenia were patients who had developed CMV infection and were receiving treatment. Finally, the absence of acute rejection episodes in these patients, maintenance of stable kidney function, and the absence of serious adverse events, established this practice in our center for managing resistant neutropenia especially in high-immunologic risk patients.

In conclusion, replacing MPA with EVE in renal transplant recipients who receive triple maintenance immunosuppressive regimen and experience resistant neutropenia seems to be an efficient and safe alternative option. However, prospective randomized trials are needed to confirm these findings.

References


