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Cytomegalovirus Infection Associated Neutropenia and Acute Kidney Graft Rejection

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Abstract

Background: Cytomegalovirus (CMV) infection, neutropenia occurrence and mycophenolate (MMF) dose reduction are associated with an increased risk of acute kidney graft rejection. The aim of our retrospective clinical study was to evaluate the association of CMV associated neutropenia with a consequent MMF dose reduction and acute kidney graft rejection.

Method: 161 patients transplanted from January 2005 till December 2010, who received anti-CD25 antibodies induction, MMF, calcineurin inhibitor and steroids, were retrospectively analyzed for the incidence of neutropenia (leucocyte count <4.0 x 10^6/mL with reduced rate of neutrophils to <1.6 x 10^6/mL in differential white blood cell count, CMV viremia (>150 virus copies/mL detected by polymerase chain reaction), MMF dose modification, granulocyte colony-stimulating factor (G-CSF) therapy and rejection episodes.

Results: Neutropenia was detected in 41 (25.5%) patients. It was associated with CMV viremia (p<0.001) but not with CMV prophylactic therapy. MMF dose was reduced due to neutropenia in 29 patients (70.7%) and acute rejection occurred in 6 (14.6%) of them. The average reduction of MMF dose in these patients was 31% of the initial dose. All neutropenic patients with rejection had concomitant CMV infection. There was a trend to positive correlation between MMF reduction and CMV infection or rejection (p=0.06). G-CSF was used in 16 (39.02%) neutropenic patients. No significant correlation was found between G-CSF use and occurrence of acute rejection.

Conclusion: CMV infection was important cause of neutropenia that resulted in MMF dose reduction and increased rate of acute graft rejection. G-CSF therapy is an alternative therapeutic approach in neutropenic patients that enables the maintenance of optimal therapeutic dose of MMF and without significant influence on acute rejection occurrence.

Keywords: Cytomegalovirus; Neutropenia; Mycophenolate; Kidney transplantation; Granulocyte colony-stimulating factor; Acute rejection

List of Abbreviations

D: Donor; R: Recipient; MMF: Mycophenolate mofetil; CMV: Cytomegalovirus; G-CSF: Granulocyte colony-stimulating factor; PCR: Polymerase chain reaction

Introduction

Neutropenia is known to occur frequently in kidney transplant recipients and is often multifactorial with contributory effects from bone marrow toxicity arising from medications, systemic infection or posttransplant lymphoproliferative disease [1,2]. Evidence supports association of neutropenia with increased risk of allograft loss and death [3].

Cytomegalovirus (CMV) is the most common viral infection after solid organ transplantation. Although immediate effects of CMV, including leucopenic episodes are well described [4], long term effects of CMV, specifically its role in the pathogenesis of acute rejection and on graft loss are not as clearly understood [5-9]. Valgancyclovir is established for prophylaxis and treatment of CMV infection. Its use has been associated with an increased risk of neutropenia in kidney transplant recipients and according to the manufacturer, appears in 10-13% of patients. Valgancyclovir induced neutropenia shows dose dependency: in one of the meta-analyses the risk of developing leucopenia was significantly higher in patients receiving valgancyclovir 900 mg daily for CMV prophylaxis versus 450 mg, with similar efficiency in preventing CMV disease [10]. International survey of CMV management revealed use of the lower valgancyclovir prophylaxis dosing (sometimes called "mini dosing") to be quite common among clinicians with yet no firm evidence of increasing the CMV infection rate [11]. However, so far there are insufficient data to support the routine use of such dosing, and standard recommended dosing algorithms and adjustment to renal function are lately suggested by the Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation [12].

Mycophenolate mofetil (MMF) became a standard part of a immunosuppressive regimen with great impact on lowering the rate of kidney rejection, but with increasing risk for bone marrow suppression

and immunodeficiency-related complications [13,14]. Increased incidence of dose dependent leucopenia and neutropenia was noted in simultaneous valgancyclovir and MMF treatment, leading to reduction of immunosuppression which may precipitate acute rejection [2,15,16].

According to guidelines on management of CMV infection in solid organ transplantation [12], dose reduction or discontinuation of valgancyclovir/gancyclovir due to side effects such as leucopenia should be avoided due to risk of resistance. Instead, other potential causes of leucopenia should be addressed, with dose reductions and modifications made where possible to any myelosuppressive therapies including MMF. The addition of granulocyte colony- stimulating factor (G-CSF) should also be considered before dose reduction or cessation of antiviral therapy. It was demonstrated that a cumulative number of days without MMF (after reduction or withdrawal due to side effects) was a strong predictor of acute rejection [15]. Risks and benefits of discontinuing MMF must therefore be weighed carefully.

G-CSF has been used to treat posttransplant neutropenia and was found to be safe and effective in neutropenic kidney graft recipients [17]. However, experience in using G-CSF after solid organ transplantation is still limited and their possible role in acute rejection remains controversial [18].

The aim of this retrospective study was to evaluate the association of acute rejection episodes with MMF dose reduction succeeding neutropenia caused by CMV infection and/or its treatment (gancyclovir or valgancyclovir) or anti-CMV prophylaxis (valgancyclovir).

Patients and Methods

Patients

A total of 161 kidney transplant recipients who were transplanted at University Medical Centre Ljubljana between January 2005 and December 2010 were included in the study, 90 man and 71 women. Three of them had combined transplantations: one liver after kidney graft transplantation and 2 simultaneous kidney and pancreas transplantation. The average age of patients at the time of transplantation was 50 ± 13.1 years.

All patients were treated with our standard centre immunosuppressive protocol with anti-CD25 antibodies induction, methylprednisolone, MMF and calcineurin inhibitor. Six patients were on tacrolimus regimen, all other on cyclosporine. Valgancyclovir or gancyclovir were used as a prophylaxis in 28 (17.3%) seronegative recipients (R-) of seropositive donor (D+) graft. Patients treated with lymphocyte depleting antibodies as induction therapy or as therapy for rejection treatment were excluded from the analysis. They were followed up for at least 6 months (range 0.5 to 5.9 years). All patients were retrospectively analyzed for the incidence of leucopenia, neutropenia, MMF dose modification, CMV viremia, rejection episodes and G-CSF therapy.

Blood samples were obtained daily in the first week after surgery, twice weekly in the first month, once weekly during the second month, every second week during the third month, once monthly from 3rd month to 6th month, and once every 3 months thereafter. All drug modifications undertaken due to neutropenia were recorded.

The study is in compliance with the Helsinki Declaration and was approved by the Republic of Slovenia National Medical Ethics Committee, ref. no. 28/04/15.

Immunosuppression

All recipients received quadruple sequential immunosuppression, including anti-CD25 antibodies induction (basiliximab), MMF, calcineurin inhibitor (either cyclosporine or tacrolimus) and steroids.

Prophylaxis

In the observed period of time CMV prophylaxis with valgancyclovir or gancyclovir was given for a time period of 3 months only to seronegative recipients (R-) who received seropositive graft (D +). Most of the patients had prophylaxis with valgancyclovir except those with delayed graft function with creatinine clearance less than 15 ml/min, who were temporary treated with ganciclovir. The dose of the drug was adjusted according to the kidney graft function.

Definitions of CMV infection and disease

CMV infection was defined by CMV viremia, which was detected with polymerase chain reaction (PCR) and expressed as the number of viral DNA copies/mL blood. Viremia was defined as detection of >100 copies of CMV DNA/mL blood. Quantitative PCRCMV DNA monitoring was performed in patients in case of symptoms or signs suggesting viral infection and in patients experiencing one or more episodes of leucopenia.

Leucopenia and neutropenia definitions

Leucopenia was defined as leucocyte count less than 4.0×10^6/mL. All leucopenic episodes during the observational period were analyzed and neutropenic episodes extracted for further analysis. Neutropenia was defined as an absolute neutrophil count less than 1.6×10^6/mL. Only patients with two consecutive measurements of leucopenia or neutropenia were included.

Rejection episodes

Rejection was suspected when acute deterioration in allograft function was detected with more than 20% increase in serum creatinine concentration. Each rejection episode was biopsy proven and classified by the Banff classification for the histologic diagnosis of rejection [19].

Statistical analysis

Statistical analysis was performed using the Statgraphics Centurion XVI Version 16.1.11 for Windows. Results for descriptive statistics were expressed as median or mean ± standard deviation. Chi-square test was performed on categorical variables. Correlation between two continuous variables was established using linear regression; multivariate logistic regression was used to identify variables independently associated with the occurrence of leucopenia or graft rejection. P-value <0.05 was considered statistically significant.

Results

In a cohort of 161 kidney transplant recipient's leucopenia was detected in 47 (29.1%) patients after at least 6 months follow up duration. Among them 41 (25.5%) were neutropenic. Neutropenia occurred at a mean of 115 days post-transplant (range 4 - 360 days) and was not associated with CMV prophylaxis (p=0.13). It lasted more than 7 days in 15 (36.6%) patients.

In 12/41 (29.3%) recipients with neutropenia the dose of MMF was not modified. In 29/41 (70.7%) recipients with neutropenia MMF dose was on average reduced to 31% of the starting dose or even temporarily discontinued in 13 patients (31.7%).

Neutropenia was associated with CMV infection in 22 (53.6%) recipients ($p<0.001$). CMV viremia was detected at a median time of 89 days (range 10-270 days) post-transplant, 17 (41.5%) patients with CMV viremia had signs of CMV disease (infectious syndrome or tissue invasive disease).

Acute rejection occurred in 7/41 (17.1%) neutropenic recipients at a median time of 64 days posttransplant (range 11-308 days). Rejections were antibody mediated (2 recipients), borderline (2 recipients), 4/IA (3 recipients), 4/IB (1 recipient), 4/IIA (1 recipient). Two patients had combined cellular and antibody mediated rejection. The relationships among the investigated parameters are shown in Table 1.

Neutropenia (n=41)													
MMF reduction Yes (n=29)								**MMF reduction No (n=12)**					
CMV viremia Yes (n=18)				CMV viremia No (n=11)				CMV viremia Yes (n=4)		CMV viremia No (n=8)			
G-CSF Yes (n=7)		G-CSF No (n=11)		G-CSF Yes (n=7)		G-CSF No (n=11)		G-CSF Yes (n=7)		Yes (n=1)	G-CSF No (n=11)		
AR Yes (n=2)	AR No (n=5)	AR Yes (n=2)	AR No (n=5)	AR No (n=4)		AR Yes (n=1)	AR No (n=6)	AR Yes (n=1)	AR No (n=3)	AR No (n=8)			
n: Number of patients. AR: Acute rejection, G-CSF: Granulocyte colony stimulating factor, CMV: Cytomegalovirus, MMF: Mycophenolate mophetil													

Table 1: Acute rejection episodes in neutropenic kidney graft recipients. The number of patients in each subgroup is represented by the width of the columns.

MMF dose was previously decreased in 6 out of 7 neutropenic patients with rejection: median time period of MMF dose reduction before rejection was 18 days (range 6 -210 days). All neutropenic patients with rejection had concomitant CMV infection in 3 of them it was detected before rejection occurred and in 3 recipients afterwards.

There was a positive and statistically significant correlation between MMF dose reduction and rejection ($p=0.04$). There was also a trend to positive correlation between CMV infection and rejection ($p=0.05$). The treatment of rejection was successful in all seven patients with improvement of graft function to the initial value of serum creatinine concentration. Graft function remained stable one year after rejection treatment.

Overall, G-CSF therapy was used in 16 neutropenic patients, in 11 of them the dose of MMF was modified (Table 1). There was no significant correlation between G-CSF use and occurrence of acute rejection ($p=0.2$).

Discussion

According to our data post-transplant neutropenia in kidney recipients is mostly associated with CMV infection; as a consequence frequent dose-reduction of MMF therapy leads to increased rate of acute graft rejection episodes. The use of G-CSF to avoid MMF dose reduction is successful in reversing severe neutropenia without any influence on kidney graft rejection.

In our study neutropenia was found in 25.5% of kidney transplant recipients during the observational period, which is comparable with the reported frequency of neutropenia in the French study group [1] and is almost twice as high as reported data on neutropenia from the United States Renal Data System [3].

In our series, CMV viremia with or without symptoms and signs of infectious syndrome or CMV disease was found to be the major risk factor associated with neutropenia ($p<0.001$) and was detected in almost one third of patients with a peak occurrence after 3 months post-transplant due to cessation of prophylactic therapy. Namely, in the period of 2004-2010 CMV prophylaxis was mainly devoted to seronegative recipients of seropositive kidney donors for the period of three months; in our study population the peak occurrence of CMV infection timely coincided with ceasing of the three months CMV prophylaxis. In this period of time CMV viremia was detected in almost two thirds of our kidney transplant recipients (unpublished data), and one third of patients with CMV viremia showed signs of infectious syndrome or tissue invasive disease. The higher rate of neutropenic episodes in our study could therefore be probably addressed to high incidence of CMV viremia among our patients.

It was already confirmed that benefits of antiviral CMV prophylaxis are limited to the time duration of prophylaxis. In the high risk group recipients 200 days prophylaxis regimen was shown to be superior compared with the standard 100 days prophylaxis [20]. Consecutively, the duration of the prophylactic therapy was extended to six months period in our transplantation center and all risk groups were included.

Acute rejection episodes were quite common in our neutropenic patients. CMV infection occurred in almost equal proportions in a six-month period pre- or post-rejection and is probably not directly involved in triggering an acute rejection episode. The cause of increased occurrence of acute rejection associated with neutropenia is unclear, but our study suggests that increased risk originate from reduction of immunosuppression. Reduction or discontinuation of MMF therapy was already shown to be associated with the higher incidence of acute graft rejection [1,15,21]. In our patients neutropenia was associated with reduction or discontinuation of MMF in 70.7% of patients, and MMF dose reduction was associated with acute graft rejection with the peak appearance of acute rejection 48 days after MMF dose reduction [22]. Our experience is not in concordance with the latest recommendations on CMV prophylaxis and therapy [12] where antiviral therapy reduction in this setting should be avoided and dose reduction of other myelosuppresive drugs like MMF, mammalian

target of rapamycin inhibitors or azathioprine is recommended. These steps should be considered with great caution, as according to our data, acute rejection could be induced. Fortunately, antirejection therapy was efficient in all our patients, and the kidney graft function resolved completely.

Finally, we observed that G-CSF therapy had a safe profile and was efficient in restituting normal leukocyte count without increasing the risk of acute rejection or graft loss. However, G-CSF was used late and as a second line therapy after MMF was already reduced or discontinued in severely neutropenic patients, exposing patients to rejection risk. Revisited guidelines on the management of CMV infection and neutropenia [12] now recommend addition of G-CSF before dose reduction or cessation of myelosuppresive therapy.

Conclusion

In the first post-transplant year of our study population with 3 months prophylactic anti-CMV therapy neutropenia was frequent and in around half of patients caused by CMV infection. The consequential reduction of MMF was associated with an increased rate of acute graft rejection. Strategies to avoid post-transplant neutropenia, including extended CMV prophylaxis regimen for high-risk population, and especially the early use of G-CSF to avoid MMF dose reduction could be successful in lowering the rate of acute graft rejections and graft loss.

Limitations of the Study

The main limitation of our study is small sample size for the observed parameters. In order to provide firm evidence, larger confirmatory studies are needed in the future.

Authors' Contributions

Z Veceric-Haler: acquisition of data, analysis of data, interpretation of data, manuscript concept; D Kovac: critical revision, contributions to concept and draft; J Tomazic: critical revision, contributions to concept and draft; J Lindic: help in data interpretation, participation in the concept and design, critical revision and coordination.

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