Efficacy and Safety of a Quadruple Regimen Compared with Triple Regimens in Patients with Mycophenolic Acid-Related Gastrointestinal Complications After Renal Transplantation: A Short-Term Single-Center Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BDE Zhiguo Peng
C Wanhua Xian
F Huaibin Sun
C Engang Li
D Lina Geng
AG Jun Tian

Department of Organ Transplantation, Qilu Hospital of Shan Dong University, Jinan, Shandong, P.R. China

Corresponding Author: Jun Tian, e-mail: tianjunql@163.com
Source of support: Departmental sources

Background: At present, there is no ideal conventional triple regimen that can effectively treat gastrointestinal (GI) complications in patients after kidney transplantation. We aimed to investigate the efficacy and safety of a quadruple regimen including standard-dose tacrolimus, low-dose enteric-coated mycophenolate sodium (EC-MPS), low-dose mizoribine (MZR), and corticosteroids, compared with regimens containing standard-dose tacrolimus, corticosteroids, plus either low-dose EC-MPS or standard-dose MZR in patients with mycophenolic acid (MPA)-related GI complications after renal transplantation.

Material/Methods: Between August 2016 and October 2018 in Qilu Hospital of Shandong University, 115 living donor kidney transplant recipients with MPA-related GI complications were enlisted in a single-center, prospective, randomized, control study. Thirty-six recipients were assigned to the low-dose EC-MPS plus low-dose MZR group, 37 recipients were assigned to the low-dose EC-MPS group, and 39 recipients were assigned to the standard-dose MZR group. We analyzed the Gastrointestinal Symptom Rating Scale (GSRS), estimated glomerular filtration rate (eGFR), graft rejection, serum creatinine, human leukocyte antigen (HLA) antibody, and the occurrence of adverse events among the 3 groups.

Results: Compared with baseline, gastrointestinal symptoms improved significantly in all 3 groups. The reduction in mean subscale scores from baseline to month 3 was more significant in the standard-dose MZR group compared with the other 2 groups. The low-dose EC-MPS plus low-dose MZR group had better renal function. The incidence of graft rejection and cytomegalovirus (CMV) and polyomavirus BK (BKV) infection, as well as the incidence of hyperuricemia, in the low-dose EC-MPS plus low-dose MZR group were all significantly reduced.

Conclusions: This quadruple regimen may be equivalent to regimens containing standard-dose tacrolimus, corticosteroids plus either low-dose EC-MPS or standard-dose MZR in improving GI symptoms after kidney transplant, and is also advantageous for kidney function, graft rejection, and the rates of adverse events.

MeSH Keywords: Gastrointestinal Diseases • Immunosuppression • Kidney Transplantation

Full-text PDF: https://www.annalsoftransplantation.com/abstract/index/idArt/919875
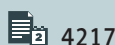
📄 4217   ⊞ 4   📊 5   📖 34

Background

Renal transplantation is the best way to treat patients with uremia. Advancements in immunosuppressive therapy and surgical techniques provide excellent graft survival [1]. However, gastrointestinal (GI) complications are common after kidney transplantation [2]. Severe GI complications can lead to weight loss and malabsorption syndrome and may even affect the long-term outcome of allograft survival [3,4].[3]

Immunosuppressive regimens, especially mycophenolic acid (MPA), were thought of as the main cause of GI complications after kidney transplantation. MPA has 2 formulations – mycophenolate mofetil (MMF) and enteric sodium mycophenolate mofetil (EC-MPS) – both providing MPA as the principle active ingredient.

According to conventional wisdom, MMF dose reduction should be the first intervention undertaken to relieve GI complications. However, studies have shown that reducing the MMF dose can increase the occurrence of acute rejection and kidney transplant allograft failure [5,6]. Although some patients were already receiving a reduced MMF dose, the majority of them were still suffering from GI symptoms, and their GSRS scores did not differ from those patients receiving a full MMF dose [7]. Consequently, conversion from MMF to an equivalent dose of EC-MPS has been used to manage patients with GI symptoms [8]. Recent data also suggest that switching from MMF to an equivalent dose of EC-MPS could be beneficial and improve GI symptoms in recipients experiencing GI disorders [9,10]. However, no significant difference in long-term improvement was found between patients taking MMF and those taking standard-dose EC-MPS [11].

Mizoribine (MZR) is a nucleoside analog that has been shown to selectively inhibit lymphocyte proliferation by inhibiting purine biosynthesis, thereby inhibiting humoral and cellular immunity [12,13]. MZR works similarly to MMF and can inhibit acute rejection of renal transplantation. Moreover, MZR is increasingly used in kidney transplantation as a substitute for MMF because of its superiority in reducing cytomegalovirus (CMV) infection [14] and its limited adverse effects, including infections or diarrhea. Therefore, conversion from MMF or EC-MPS to MZR seems to be an optimal management for MPA-associated GI complications after renal transplantation. Nevertheless, the optimal dose of MZR has not yet been established. A low dose of MZR was shown to be less potent in immunosuppression, whereas hyperuricemia developed more frequently among patients who received high-dose MZR after kidney transplantation [15,16]. At present, there is no immunosuppressive protocol that can manage GI complications in transplant recipients. The Guidelines for Immunosuppressive Therapy in Chinese Kidney Transplant Patients (2016 edition) recommend that when a patient develops MPA-related gastrointestinal complications, MPA should be reduced or stopped, or MPA should be converted to MZR (2B). Therefore, our study aimed to assess a quadruple regimen, including standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids in renal transplant patients with GI disorders. We examined whether the new regimen can reduce the adverse effects of gastrointestinal complications while maintaining efficacy and better tolerability.

Material and Methods

Study population

Between August 2016 and October 2018 in Qilu Hospital of Shandong University, 115 living donor kidney transplantation patients with MPA-related GI complications were enrolled in a single-center, prospective, randomized control study. None of these patients had a history of gastrointestinal disease before transplantation. Patient inclusion criteria were: (1) GI complications were not attributable to infectious diseases such as bacteria or fungi; (2) kidney transplantation performed at least 1 month to 8 years before inclusion; (3) stable renal function; (4) immunosuppression before inclusion: tacrolimus+MMF+corticosteroid. The dosage of tacrolimus was 0.1 to 0.15 mg/kg/day twice daily and corticosteroids 5–10 mg/day once daily. The dosage of MMF was 1.5 g/day twice daily. Patients were randomly assigned prior to initial enrollment by computer-generated selection to 3 groups: the low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids; the low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids; and the standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids.

They were observed for 1 year after initial enrollment. Patients gave their informed consent to be in the study, and the research protocol was approved by the Ethics Committee of Qilu Hospital of Shandong University, China (2016 Qilu Hospital of Shandong University IRB Approval No. 2016041).

Immunosuppression

All patients received oral tacrolimus at a dosage of 0.1 to 0.15 mg/kg/day twice daily and corticosteroids 5–10 mg/day once daily after initial enrollment. In the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group, EC-MPS was administered simultaneously with tacrolimus at a maintenance dosage of 0.36–0.72g/day, divided into 2 doses. In the standard-dose MZR group, MZR was administered at 200 mg/day twice daily. In the low-dose EC-MPS plus low-dose MZR group, MZR was administered at 100 mg/day twice daily.

Assessment of the severity of GI complications

The severity of GI complications was assessed using the GSRS [17]. This questionnaire is a 15-item instrument devised to evaluate symptoms related to usual GI complications. Questions are grouped into 5 main categories of symptoms (reflux, diarrhea, constipation, indigestion, and abdominal pain), each containing 3 questions. Answers are rated from 1 to 7 on a scale of increasing severity. The mean rating of all 15 questions represents the total score of the questionnaire, and the mean rating of the 3 questions of each group represents each group's subscales. Higher scores represent higher symptom burden, which means more discomfort. GI symptoms scores (reflux, diarrhea, constipation, indigestion, and abdominal pain) were evaluated at the time of enrollment (baseline) and after 3 months in all groups. To ensure unbiased responses to the questionnaires, participants completed the self-administered paper questionnaires prior to any clinical evaluation or procedure.

Study parameters

Routine laboratory tests

At each follow-up visit (1 month, 2 months, 3 months, 6 months, 9 months, and 12 months after enrollment), we analyzed serum creatinine, trough levels of tacrolimus and MZR, the MPA area under the plasma concentration-time curve (AUC), graft rejection, uric acid concentration, proteinuria, HLA antibodies, and CMV and BKV infection; a positive result was defined when quantitative BKV DNA load was greater than 1.0×10^4 copies per milliliter, and CMV DNA load was greater than 1.0×10^3 copies per milliliter in plasma samples. We also assessed patient and graft survival. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [18] at each visit.

Determination of MPA-AUC$_{0-12\,h}$

We used a limited number of timed samples (suitable for a typical clinic visit) and a multiple regression linear equation for predicting the actual MPA exposure. Blood samples (3 mL each) were taken from a forearm vein into heparin-containing tubes at 0.5, 2, 4, and 8 h after the morning dose of EC-MPS, and the samples were transferred directly to the analytical laboratory for measurement of MPA levels within 24 h.

Graft biopsy

When serum creatinine levels were elevated, or if proteinuria was identified during the study, renal biopsies were occasionally performed. The pathological criteria for graft rejection were based on the Banff classification 2015.

Donor-specific alloantibody

We assessed the presence of donor-specific alloantibody (DSA) when rejection or proteinuria occurred. Detection of HLA antibodies was performed using HLA antibody testing using the Luminex method with LAB-Screen assays (One Lambda, Inc., Canoga Park, CA) in accordance with the manufacturer's instructions. A graft kidney biopsy to diagnose chronic antibody-mediated rejection was conducted in recipients with *de novo* DSA after informed consent was obtained.

This was a prospective, single-center, short-term study that met the ethics standards of the 2000 Declaration of Helsinki. We obtained informed consent from all patients before their inclusion in the research.

Statistical analyses

The main goal of our research was to evaluate the changes in GI symptom severity before and after intervention with different 3 regimens (measured by GSRS score). The minimal clinically relevant difference (MCRD) of the GSRS total score was presumed to be 0.36 score points. When combined with other study parameters such as graft rejection or eGFR, a sample size of 115 evaluable patients was calculated, assuming a standard deviation of 1.3 for the *t* test, an alpha level of 0.05, and a power of 90%.

Changes in the severity of GI disorders from baseline to month 3 within the 3 groups were tested using paired *t* tests. Differences in the severity of GI disorders among the 3 groups were tested using one-way analysis of variance (ANOVA). The Mann-Whitney U test was used for other continuous data. The categorical variables were analyzed by χ^2 or Fisher's exact test. The results are expressed as the mean±standard deviation (SD) or percentages. All statistical analyses were performed using the SPSS program (version 17.0; SPSS, Inc, Chicago, IL, USA). *P*<0.05 was considered significant.

Results

We prospectively included 115 patients between August 2016 and October 2018, and 112 fulfilled the inclusion criteria. Baseline patient characteristics of age, sex, causes of end-stage renal disease, time since transplant, and antigen mismatches were similar among the 3 groups and are summarized in Table 1.

Table 1. Demographics and Characteristics of the patients.

	Low-dose EC-MPS* (N=37)	Standard-dose MZR** (N=39)	Low-dose EC-MPS plus Low-dose MZR*** (N=36)	P value
Variable				
Recipient age (y)	32.8±7.3	30.5±6.1	31.2±6.8	0.62
Sex (Male/Female), n	21/16	20/19	19/17	0.46
Systolic pressure (mmHg)	138.2±12.8	136.7±11.7	136.7±13.9	0.81
Diastolic pressure (mmHg)	85.9±7.7	86.1±6.3	86.1±5.9	0.36
Pre-transplant eGFR (mL/min)	8.5±1.3	9.7±1.1	7.7±1.5	0.24
Duration of dialysis before transplantation, (mo)	9.5±3.7	9.8±2.6	10.6±2.1	0.28
Cause of end-stage renal disease (%)				
Chronic glomerulonephritis	51.5	51.8	50.6	0.55
Diabetic nephropathy	8.2	6.5	6.9	0.47
Hypertensive nephropathy	5.6	5.2	4.3	0.39
Polycystic kidney disease	1.5	0.8	1.6	0.31
Uncertain	33.2	35.7	36.6	0.57
Donor age (y)	50.7±3.5	48.9±7.1	49.1±6.3	0.50
Time since transplant (mo)				
Mean±SD	16.2±15.1	13.8±12.5	15.6±13.9	0.39
Median	8.3	6.6	7.5	0.40
Range	1.2–98.6	1.6–96.7	1.3–95.9	0.08
Warm ischemia time (min)	2.68±0.89	3.16±1.39	2.27±1.21	0.08
Antigen mismatches – A, B, and DR (no.)	2.9±1.2	3.1±1.6	2.8±1.3	0.59
Baseline GSRS scores	2.87±1.12	3.04±0.92	2.83±0.93	0.43
Baseline eGFR (mL/min)	75±15.8	77±17.1	70±16.6	
Baseline 24-h proteinuria (g/day)	0.17±0.07	0.21±0.05	0.19±0.08	

* The low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids;** the standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids; *** the low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids. EC-MPS – enteric-coated mycophenolate sodium; MZR – mizoribine.

Efficacy measurements

Changes in the GSRS score after patient enrollment

All the GI symptoms in our study presented after transplantation. The most frequently reported GI symptoms before enrollment were diarrhea (80%), abdominal pain (75%), indigestion (50%), constipation (42%), and reflux (40%). The results of changes in the GSRS score from baseline to month 3 in every group of patients are shown in Figure 1. The GSRS total

score in all of the patients at the beginning of the study (baseline) was 2.88±0.99, and at month 3 the total GSRS score was 1.98±0.81 ($P<0.001$). The mean scores improved significantly for diarrhea ($P<0.001$), abdominal pain ($P<0.001$), indigestion ($P<0.001$), and reflux ($P<0.001$) in the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group between baseline and month 3 due to an improvement in the GSRS total score. We also observed that the mean scores improved significantly for diarrhea ($P<0.0001$), abdominal pain ($P<0.0001$), indigestion ($P<0.0001$), and reflux ($P<0.0001$) in
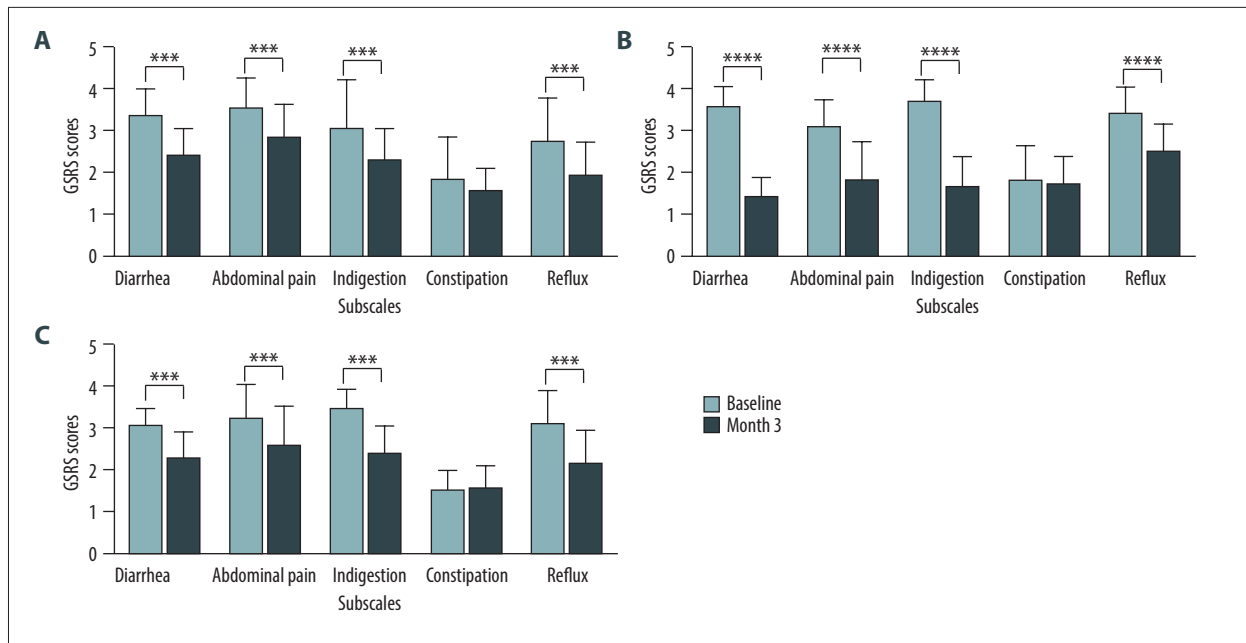
Figure 1. Changes in mean subscale GSRS scores from baseline to 3 months in every group. Higher scores indicate the more significant burden of symptoms (*** *P*<0.001). (**A**) Changes in the mean GSRS scores of patients in the low-dose EC-MPS group from baseline to 3 months. (**B**) Changes in the mean GSRS scores of patients in the standard-dose MZR group from baseline to 3 months (**** *P*<0.0001). (**C**) Changes in the mean GSRS scores of patients in the low-dose EC-MPS plus low-dose MZR group from baseline to 3 months (*** *P*<0.001).
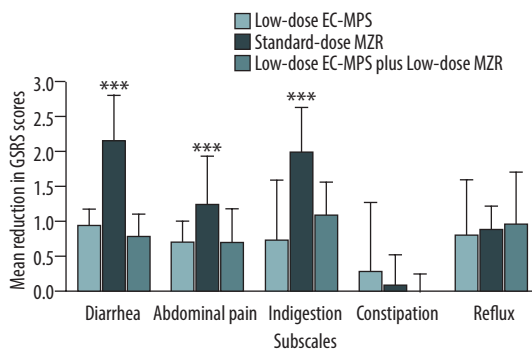

Figure 2. Changes in mean subscale GSRS scores from baseline to 3 months among the 3 groups. Significant reductions in mean subscale scores from baseline to 3 months was found for diarrhea (*** *P*<0.001), abdominal pain (*** *P*<0.001), and indigestion (*** *P*<0.001) in the standard-dose MZR group compared with the other 2 groups. Values shown are mean reductions of GSRS scores from baseline to month 3 of each group.

the standard-dose MZR group between baseline and month 3. Analysis of changes in GSRS subscale scores from baseline to month 3 among the 3 groups is shown in Figure 2. Mean subscale scores from baseline to month 3 were significantly reduced for diarrhea (*P*<0.001), abdominal pain (*P*<0.001), and

indigestion (*P*<0.001) in the standard-dose MZR group compared with the other 2 groups.

Graft rejection

All patients with clinically suspected graft rejection underwent a renal graft biopsy. The biopsy-proven graft rejection rates and Banff classification in each group after enrollment are shown in Table 2. There was a higher graft rejection rate in the low-dose EC-MPS group and the standard-dose MZR group than in the low-dose EC-MPS plus low-dose MZR group (18.9% *vs.* 8.3% and 20.5% *vs.* 8.3%; *P*<0.01). There were 5 Type 1b and 5 Type 2a T cell-mediated acute rejections, 6 T cell-mediated chronic rejections, and 2 antibody-mediated rejections. For the occurrence time of graft rejection, there were 5 cases in the low-dose EC-MPS group and 6 cases in the standard-dose MZR group who experienced rejection within the first 3 months after enrollment.

Renal function evolution

The serum creatinine level and mean eGFR remained relatively stable from baseline to 12 months after enrollment in the low-dose EC-MPS plus low-dose MZR group. Conversely, the serum creatinine level significantly increased in the low-dose EC-MPS group and the standard-dose MZR group from month 1. A higher level was found in the 2 groups at 1, 2, 3,

Table 2. Biopsy-proven graft rejection of patients during the study.

	Low-dose EC-MPS* (N=37)	Standard-dose MZR** (N=39)	Low-dose EC-MPS plus Low-dose MZR*** (N=36)
Rates of graft rejection	7 (18.9%)	8 (20.5%)	3 (8.3%)
Rejection classification			
T cell-mediated acute rejection			
Type 1b	2	2	1
Type 2a	3	2	
T cell-mediated chronic rejection	1	3	2
Antibody-mediated rejection	1	1	
Time of graft rejection episodes			
Day 0–90	5	6	
Day 90–180	1	1	2
Day 180–365	1	1	1

* The low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids; ** The standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids; *** The low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids. EC-MPS – enteric-coated mycophenolate sodium; MZR – mizoribine.

months after enrollment compared with the low-dose EC-MPS plus low-dose MZR group (*P*<0.01; Figure 3A). The eGFR did not change significantly in the low-dose EC-MPS plus low-dose MZR group, while it significantly decreased in the other 2 groups. At 1, 2, 3, months, there was a significant difference in the eGFR between the low-dose EC-MPS plus low-dose MZR group and the other 2 groups (*P*<0.01; Figure 3B). There was a slightly higher serum creatinine level and lower eGFR in the low-dose EC-MPS group and standard-dose MZR group at 6, 9, and 12 months compared to the low-dose EC-MPS plus low-dose MZR group, although this difference was not statistically significant.

We also assessed the quantity of urine protein at month 12 to further evaluate the renal function of patients, showing that 24-h proteinuria increased significantly in the low-dose EC-MPS group and standard-dose MZR group compared to the low-dose EC-MPS plus low-dose MZR group (0.68±0.76 g/day *vs.* 0.18±0.39 g/day; 0.73±0.69 g/day *vs.* 0.18±0.39 g/day; *P*<0.01; Figure 3C). Consistent with this, the percentage of patients with proteinuria was higher in the low-dose EC-MPS group and standard-dose MZR group than in the low-dose EC-MPS plus low-dose MZR group (16% *vs.* 5%; 19% *vs.* 5%; *P*<0.01; Figure 3D). We also analyzed graft biopsy findings among 13 transplant recipients with proteinuria >150 mg/day. Transplant glomerulopathy was the most common finding (6 cases), followed by IgA nephropathy (3 cases), chronic rejection (2 cases), and

acute rejection (2 cases). Figure 4 lists 2 examples of graft biopsy in patients with proteinuria or graft rejection.

Survival

At 12 months after initial enrollment, the survival rate of patients or grafts in each group was 100%, and no difference was found among the 3 groups.

Safety evaluation

Adverse effects in patients

Table 3 lists the most common adverse events during the study. CMV infection was significantly higher in the low-dose EC-MPS group (7 cases; 18.9%), whereas it was less frequent in the standard-dose MZR group and low-dose EC-MPS plus low-dose MZR group (18.9% *vs.* 7.6%; 18.9% *vs.* 5.5%, respectively, *P*<0.01). The frequency of BKV infection in the low-dose EC-MPS group was also higher than in the standard-dose MZR group and low-dose EC-MPS plus low-dose MZR group (16.2% *vs.* 5.1% and 16.2% *vs.* 5.5%; *P*<0.01). The incidence of hyperuricemia was significantly different between the standard-dose MZR group and the other 2 groups. Eleven patients had hyperuricemia in the standard-dose MZR group (28.2%), and 3 cases occurred in the low-dose EC-MPS group (8.1%). Four cases occurred in the low-dose EC-MPS plus low-dose
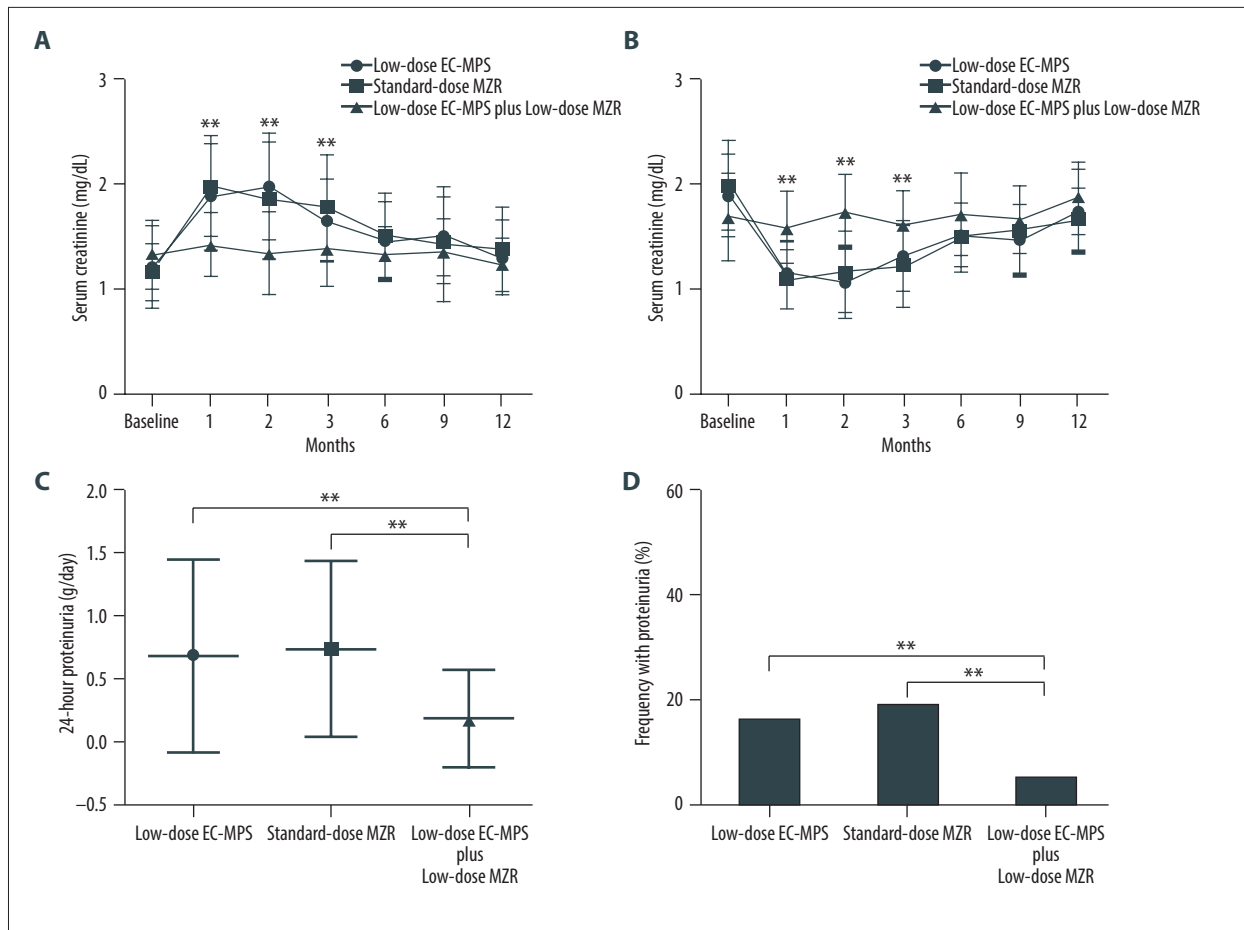
Figure 3. Serial follow-up of renal function in the 3 groups. (**A**) The serum creatinine level greatly increased in the low-dose EC-MPS group and the standard-dose MZR group from month 1. A higher level was found in the 2 groups at 1, 2, and 3 months compared with the low-dose EC-MPS plus low-dose MZR group (F [1.143, 6.861]=5.039, * *P*<0.05). (**B**) The eGFR did not change greatly in the low-dose EC-MPS plus low-dose MZR group, while it gradually decreased in the other 2 groups. At 1, 2, and 3 months, there was also a significant difference in the eGFR between the low-dose EC-MPS plus low-dose MZR group and the other 2 groups (F [1.207, 7.45]=5.429, * *P*<0.05). (**C**) 24-h proteinuria was higher in the low-dose EC-MPS group and standard-dose MZR group compared to the low-dose EC-MPS plus low-dose MZR group (0.68±0.76 g/day *vs.* 0.18±0.39 g/day; 0.73±0.69 g/day *vs.* 0.18±0.39 g/day; ** *P*<0.01). (**D**) Protein excretion rate in month 12 was higher in the low-dose EC-MPS group and standard-dose MZR group than in the low-dose EC-MPS plus low-dose MZR group (16% *vs.* 5%; 19% *vs.* 5%; ** *P*<0.01).

MZR group (11.1%). No significant differences were found in the incidence of other adverse events, such as fungal infection, pneumonia, anemia, hyperglycemia, and hypertension.

Mean trough levels of immunosuppression

Table 4 shows the trough levels of the immunosuppressant during the study. The tacrolimus trough levels of each group were all maintained at 5–10 ng/ml during the study. No significant difference in MPA AUC was found between the low-dose EC-MPS plus low-dose MZR group and the low-dose EC-MPS group. The trough level of MZR in the standard-dose MZR group was significantly higher than that in the low-dose EC-MPS plus low-dose MZR group (*P*<0.01).

Incidence of HLA antibodies

We assessed the presence of HLA antibodies when proteinuria or rejection occurred. Nine cases showed HLA antibodies. Among them, 2 patients showed DSA for the HLA antigen. Table 5 lists the incidence of HLA antibodies in each group.

Discussion

To the best of our knowledge, it is the first research to demonstrate that MPA-related GI symptoms in kidney transplant patients can be relieved after switching to the quadruple regimen, including standard-dose tacrolimus, low-dose EC-MPS,
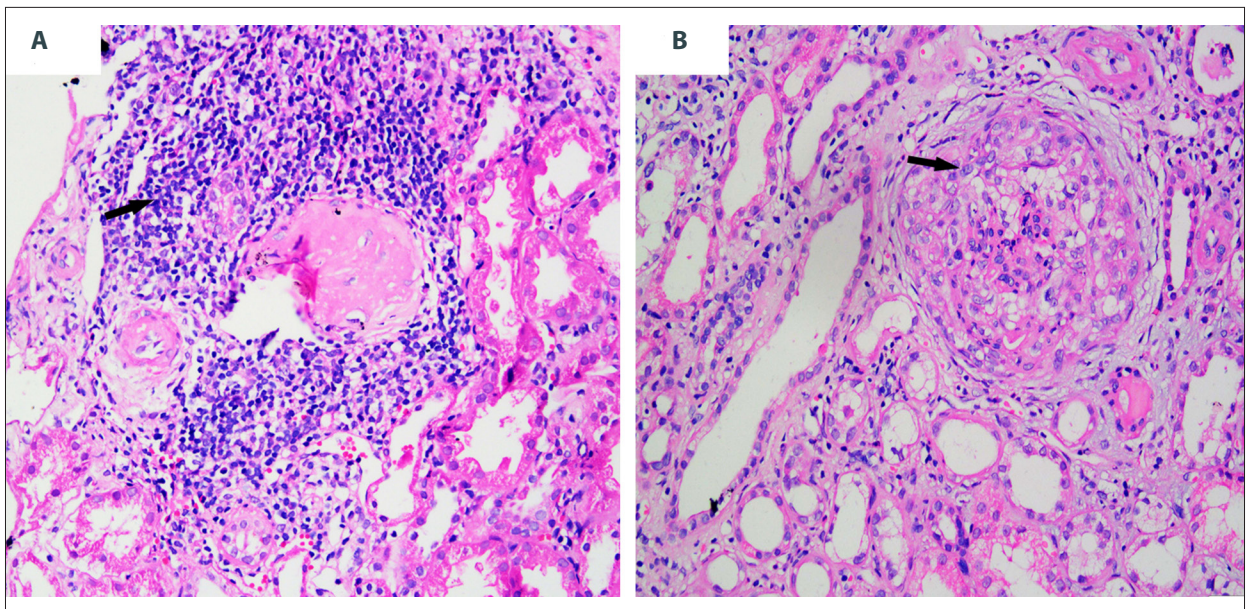
Figure 4. Two examples of graft biopsy in patients with proteinuria or graft rejection. (**A**) T cell-mediated acute rejection (Banff Ia) in a 27-year-old female who suffered from increased serum creatinine. In addition to moderate tubulitis and tubule atrophy, significant interstitial inflammation was observed (arrow). An interstitial arterial wall thickened with hyaline degeneration was also present. (**B**) Recurrent IgA nephropathy in a 36-year-old male who suffered from proteinuria. Mild glomerular mesangium diffuse proliferation with moderate mesangial matrix increase. The formation of the crescent was found in a glomerulus of fibrosis (arrow). Moderate grade (2+) mesangial IgA deposition was observed (data not shown), confirming the diagnosis of recurrent IgA nephropathy.

low-dose MZR, and corticosteroids. In addition, the quadruple regimen was associated with a satisfactory immunosuppressive effect and with better kidney function and a lower incidence of adverse effects compared with the 2 conventional triple regimens in kidney transplant recipients with GI disorders.

Tacrolimus, MMF, and corticosteroids are the initial, classical maintenance immunosuppressive medications after kidney transplantation [19]. However, there was a higher frequency of GI symptoms in patients treated with this protocol [3]. MMF plays a major role in the occurrence of GI symptoms. Therefore, strategies, including MMF dose reduction and the conversion of MMF to EC-MPS or MZR, have been advised to alleviate the MPA-related GI symptoms. Although these measures may help alleviate GI symptoms, new problems can arise, such as inadequate immunosuppression or serious adverse effects [6,20]. In our research, we considered that reducing the doses of EC-MPS would alleviate gastrointestinal symptoms caused by MPA. At the same time, we were concerned that a reduction in the doses of EC-MPS would lead to insufficient immunosuppression. Therefore, we added low-dose MZR to the quadruple regimen. We also wanted to assess whether MZR could replace EC-MPS in kidney transplant patients with MPA-related GI symptoms. Thus, we chose standard-dose MZR instead of EC-MPS in one triple regimen.

Our study has shown that both the total GSRS score and the mean GSRS score in the quadruple regimen and 2 conventional triple regimens were improved compared with the baseline. We think the main reason for this improvement in GI symptoms is associated with the type and dose of immunosuppressants in our protocols. Previous research has demonstrated that a reduction or discontinuation of MMF dosage is related to an increased risk of allograft failure [4,6,21]. Therefore, we chose EC-MPS or MZR as the antiproliferative agent in our immunosuppressant protocols. Our results have also shown that MMF conversion to low-dose EC-MPS, standard-dose MZR, or low-dose EC-MPS plus low-dose MZR are all beneficial in reducing MPA-associated GI complications. Further, we compared changes in GSRS subscale scores difference among the 3 groups. Our results showed that the reductions in mean subscale scores from baseline to month 3 were significant for diarrhea, abdominal pain, and indigestion in the standard-dose MZR group compared with the other 2 groups. These results demonstrated MZR can be more beneficial in reducing MPA-associated GI complications compared with EC-MPS.

In our study, the rates of graft rejection in the low-dose EC-MPS plus low-dose MZR group were lower than in the other 2 groups. Furthermore, in the low-dose EC-MPS group and standard-dose MZR group, graft rejection mainly occurred of within the first 3 months after enrollment. Our study demonstrated

Table 3. Other adverse effects in patients during the study.

	Low-dose EC-MPS* (N=37)	Standard-dose MZR** (N=39)	Low-dose EC-MPS plus Low-dose MZR*** (N=36)	P value
Opportunistic infection				
Cytomegalovirus	7 (18.9%)	3 (7.6%)	2 (5.5%)	<0.01
BK virus	6 (16.2%)	2 (5.1%)	2 (5.5%)	<0.01
Fungal infection	2 (5.4%)	1 (2.5%)	1 (2.7%)	0.19
Other infection				
Pneumonia	1 (2.7%)	2 (5.1%)	1 (2.7%)	0.18
Upper respiratory tract infection	3 (8.1%)	2 (5.1%)	2 (5.5%)	0.17
Blood system				
Anemia	1 (2.7%)	3 (7.6%)	1 (2.7%)	0.16
Metabolism system				
Hyperglycemia	3 (8.1%)	2 (5.1%)	2 (5.5%)	0.23
Hyperlipidemia	5 (13.5%)	5 (12.8%)	6 (16.6%)	0.38
Hypertension	6 (16.2%)	6 (15.3%)	7 (19.4%)	0.35
Hyperuricemia	3 (8.1%)	11 (28.2%)	4 (11.1%)	<0.01
Hypokalemia	2 (5.4%)	1 (2.5%)	1 (2.7%)	0.19

* The low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids; ** The standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids; *** The low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids. EC-MPS – enteric-coated mycophenolate sodium; MZR – mizoribine.

that low-dose EC-MPS and standard-dose MZR did not provide adequate immunosuppression in the conventional triple regimen groups. Therefore, the rates of graft rejection in the low-dose EC-MPS group and standard-dose MZR group were very high. However, the frequency of graft rejection in the low-dose EC-MPS plus low-dose MZR group was significantly lower than in the other 2 groups. As a result, the combination of low-dose EC-MPS and low-dose MZR can compensate for the relatively less potent immunosuppressive effect of each, and thereby adequately prevent graft rejection.

We compared the renal function among the 3 groups during the observation. There was an increase in creatinine level and a decreased of eGFR in the low-dose EC-MPS group and standard-dose MZR group from 1 month to 3 months after enrollment, with a significant difference observed compared with the low-dose EC-MPS plus low-dose MZR group. Protocol biopsies during this period were not performed. However, our occasional kidney graft biopsy findings indicate that the worsening of graft function that occurred in this period may have been related to the high rates and occurrence time of graft rejection in the 2 triple regimen groups. A slightly higher serum creatinine level and lower eGFR were be observed in the 2 triple regimen groups from 6 months to 12 months. This difference, although not statistically significant, might be clinically relevant in the long term.

Proteinuria is common in patients after kidney transplant. Early occurrence of proteinuria is a high-risk factor for patients receiving a calcineurin inhibitor-based immunosuppressive regimen [22]. Other studies have suggested that post-transplant proteinuria is related to poor graft survival [23–25]. To further evaluate renal function, we assessed urine protein quantity at month 12. The results show that proteinuria was present in nearly 20% of the patients in the low-dose EC-MPS and standard-dose MZR groups at 12 months but in only 5% of patients in the low-dose EC-MPS plus low-dose MZR group. The 24-h proteinuria was also higher in the low-dose EC-MPS group and standard-dose MZR group compared to the low-dose EC-MPS plus low-dose MZR group.

Table 4. Trough levels of tacrolimus and MZR, as well as MPA AUC, in the 3 groups.

		Tacrolimus (ng/ml)	MPA AUC$_{0-12 h}$ (mg·h/L)	MZR (ng/ml)
Low-dose EC-MPS* **(N=37)**	Baseline	7.73±1.87	52.56±13.71	
	Month 1	7.32±1.59	25.12±11.15	
	Month 2	8.22±2.06	23.37±10.38	
	Month 3	7.83±1.78	25.66±9.89	
	Month 6	7.37±2.27	21.86±10.17	
	Month 9	8.21±2.01	22.71±11.89	
	Month 12	7.67±1.98	21.98±9.95	
Standard-dose MZR** **(N=39)**	Baseline	7.12±1.76		
	Month 1	6.66±2.16		2.12±0.50[#]
	Month 2	7.13±1.80		2.75±0.37[#]
	Month 3	6.92±2.09		2.51±0.46[#]
	Month 6	8.28±1.98		2.35±0.43[#]
	Month 9	7.67±2.39		2.62±0.32[#]
	Month 12	8.36±1.59		2.59±0.40[#]
Low-dose EC-MPS plus Low-dose MZR* **(N=36)**	Baseline	6.86±1.98	55.79±12.87	
	Month 1	7.93±1.91	22.89±10.26	0.78±0.39
	Month 2	7.92±2.56	25.66±12.01	0.96±0.29
	Month 3	7.61±2.11	23.19±9.56	0.86±0.50
	Month 6	7.81±1.98	25.01±11.53	0.73±0.46
	Month 9	7.18±2.12	20.23±9.56	0.70±0.39
	Month 12	7.22±2.26	23.99±10.28	0.83±0.35

* The low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids; ** The standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids; *** The low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids. EC-MPS – enteric-coated mycophenolate sodium; MZR – mizoribine; MPA – mycophenolic acid. AUC – area under the concentration-time curve from time zero to 12 h. # $P<0.01$ between the standard-dose MZR and low-dose EC-MPS plus low-dose MZR groups.

Table 5. Incidence of HLA antibodies in the patients with rejection or proteinuria.

	Low-dose EC-MPS*	Standard-dose MZR**	Low-dose EC-MPS plus Low-dose MZR***
HLA antibody	3	5	1
Non-donor-specific antibody	2	4	1
Donor-specific antibody	1	1	

* The low-dose EC-MPS group, which received standard-dose tacrolimus, low-dose EC-MPS, and corticosteroids; ** The standard-dose MZR group, which received standard-dose tacrolimus, standard-dose MZR, and corticosteroids; *** The low-dose EC-MPS plus low-dose MZR group, which received standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids. EC-MPS – enteric-coated mycophenolate sodium; MZR – mizoribine.

Furthermore, the most common graft biopsy findings of these patients with proteinuria were *de novo* glomerular or recurrent disease. Therefore, the occurrence of proteinuria may be associated with inadequate immunosuppression. Compared with the low-dose EC-MPS group and standard-dose MZR group, the low-dose EC-MPS plus low-dose MZR group had a lower incidence of proteinuria. We think this is also related to the adequate immunosuppression effect in the low-dose EC-MPS plus low-dose MZR group. Since proteinuria after renal transplantation is associated with poor graft survival, we think the immunosuppressant protocols in the low-dose EC-MPS plus low-dose MZR group had an advantage in protecting the renal function over the other 2 groups, and thus might help prolong graft survival.

Viral infection is a serious problem in kidney transplant recipients, and patients may suffer from severe manifestations of this infection along with the increased risk of death. MMF is thought to be related to CMV and BKV infection. However, many studies have shown that MZR has inhibitory effects on the replication of some DNA and RNA viruses, including CMV, respiratory syncytial virus, bovine viral diarrhea virus, and influenza virus [26–29]. Kuramoto et al. reported that the antiviral mechanism of MZR might be related to its chemical structure being similar to ribavirin, a well-known broad-spectrum antiviral agent [30]. In our study, the frequency of CMV and BKV infection was significantly lower in the standard-dose MZR group and low-dose EC-MPS plus low-dose MZR group compared with the low-dose EC-MPS group. This result suggests that standard-dose or low-dose MZR can inhibit CMV and BKV infection. Therefore, this is an important consideration factor in selecting an ideal immunosuppressive agent.

Hyperuricemia is common in kidney transplant patients receiving high-dose MZR (6 mg/kg), and it can lead to acute kidney failure [14,31,32]. Therefore, we did not use a high dosage of MZR in our study design. Our study still showed that hyperuricemia developed more frequently in the standard-dose MZR group compared with the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group. However, no significant difference was found between the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group. We used EC-MPS instead of MZR in the low-dose EC-MPS group, which can explain the low incidence of hyperuricemia in the low-dose EC-MPS group. We think the differences in the occurrence of hyperuricemia between the standard-dose MZR group and the low-dose EC-MPS plus low-dose MZR group is related to the dosage of MZR. Decreasing the dose of MZR may reduce the occurrence of hyperuricemia risk. Moreover, the combination of low-dose MZR and low-dose EC-MPS did not increase the incidence of hyperuricemia. No significant difference was found in the rate of other adverse events among the 3 groups.

In our study, the drug concentration of the immunosuppressive agents was assessed. The tacrolimus trough levels of each group were within the target range (5–10 ng/mL) during the entire study period. The MPA AUC of the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group maintained a relatively lower level of about 20 mg·h/L. Studies showed that the target range of MPA after renal transplantation is 30–60 mg·h/L [33]. It is obvious that the mean AUC of MPA in the low-dose EC-MPS group and low-dose EC-MPS plus low-dose MZR group did not achieve targeted therapeutic concentrations because of the low dose of EC-MPS in the protocol. However, the patients in the low-dose EC-MPS plus low-dose MZR group had lower graft rejection rates and better renal function compared to the low-dose EC-MPS group. These results indicate that the low-dose MZR can compensate for the reduced immunosuppressive effect of MPA in the low-dose EC-MPS.

Owing to the different dosages of MZR in the standard-dose MZR group and low-dose EC-MPS plus low-dose MZR group, the trough level of mizoribine in the standard-dose MZR group was higher than in the low-dose EC-MPS plus low-dose MZR group. Although the trough level of MZR in the low-dose EC-MPS plus low-dose MZR group was lower, the patients still had a lower graft rejection rate and better renal function compared to the standard-dose MZR group, probably because the combination of low-dose EC-MPS and low-dose MZR can compensate for the relatively less potent immunosuppressive effect of each, and is adequate for preventing graft rejection.

We also assessed the presence of HLA antibodies when rejection or proteinuria occurred. Nine cases exhibited the HLA antibody. Among them, 2 patients exhibited a DSA for the HLA antigen. Our data have shown that there were more HLA antibody-positive cases in the low-dose EC-MPS group and the standard-dose MZR group. The DSA was not found in the low-dose EC-MPS plus low-dose MZR group. Our previous study showed that the HLA antibody and proteinuria are both associated with poor 5-year graft survival [34]. Therefore, the quadruple regimen may be beneficial for inhibiting the production of antibodies and prolonging graft survival.

This study had some limitations. Firstly, this was a short-term study of patients at our hospital. Secondly, given that the research was conducted at a single hospital, the patient characteristics may be biased. However, the patients we studied had similar baseline characteristics. Therefore, these research findings might apply to larger populations. Due to these limitations, in future research, we plan to enlarge the sample size and extend the follow-up time to evaluate the long-term benefits of this quadruple regimen to kidney transplant patients with GI complications.

e919875-11

Indexed in: [Science Citation Index Expanded]   [Index Medicus/MEDLINE]
[Chemical Abstracts]   [Scopus]

Conclusions

In our research involving kidney transplant recipients experiencing MPA-induced GI symptoms, therapy for 12 months with a quadruple regimen including standard-dose tacrolimus, low-dose EC-MPS, low-dose MZR, and corticosteroids not only provided improved GI symptom burden, but also showed adequate immunosuppression, with better renal function and less adverse events as compared with 2 conventional triple regimens including standard-dose tacrolimus, corticosteroids plus either low-dose EC-MPS or standard-dose MZR. This new quadruple protocol appears to provide a safe treatment option when trying to relieve MPA-related GI symptoms in kidney transplant patients.

References:

1. Hariharan S, Johnson CP, Bresnahan BA et al: Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med, 2000; 342(9): 605–12

2. Helderman JH, Goral S: Gastrointestinal complications of transplant immunosuppression. J Am Soc Nephrol, 2002; 13(1): 277–87

3. Bunnapradist S, Neri L, Wong W et al: Incidence and risk factors for diarrhea following kidney transplantation and association with graft loss and mortality. Am J Kidney Dis, 2008; 51(3): 478–86

4. Machnicki G, Ricci JF, Brennan DC, Schnitzler MA: Economic impact and long-term graft outcomes of mycophenolate mofetil dosage modifications following gastrointestinal complications in renal transplant recipients. Pharmacoeconomics, 2008; 26(11): 951–67

5. Knoll GA, MacDonald I, Khan A, Van Walraven C: Mycophenolate mofetil dose reduction and the risk of acute rejection after renal transplantation. J Ame Soc Nephrol, 2003; 14(9): 2381–86

6. Bunnapradist S, Lentine KL, Burroughs TE et al: Mycophenolate mofetil dose reductions and discontinuations after gastrointestinal complications are associated with renal transplant graft failure. Transplantation, 2006; 82(1): 102–7

7. Savvidaki E, Papachristou E, Kazakopoulos P et al: Gastrointestinal disorders after renal transplantation. Transplant Proc, 2014; 46(9): 3183–86

8. Cooper M, Deering KL, Slakey DP et al: Comparing outcomes associated with dose manipulations of enteric-coated mycophenolate sodium versus mycophenolate mofetil in renal transplant recipients. Transplantation, 2009; 88(4): 514–20

9. Chan L, Mulgaonkar S, Walker R et al: Patient-reported gastrointestinal symptom burden and health-related quality of life following conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium. Transplantation, 2006; 81(9): 1290–97

10. Ortega F, Sanchez-Fructuoso A, Cruzado JM et al: Gastrointestinal quality of life improvement of renal transplant recipients converted from mycophenolate mofetil to enteric-coated mycophenolate sodium drugs or agents: Mycophenolate mofetil and enteric-coated mycophenolate sodium. Transplantation, 2011; 92(4): 426–32

11. Shehata M, Bhandari S, Venkat-Raman G et al: Effect of conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium on maximum tolerated dose and gastrointestinal symptoms following kidney transplantation. Transpl Int, 2009; 22(8): 821–30

12. Sonda K, Takahashi K, Tanabe K et al: Clinical pharmacokinetic study of mizoribine in renal transplantation patients. Transplant Proc, 1996; 28(6): 3643–48

13. Takahashi S, Wakui H, Gustafsson JA et al: Functional interaction of the immunosuppressant mizoribine with the 14-3-3 protein. Biochem Biophys Res Commun, 2000; 274(1): 87–92

14. Yoshimura N, Ushigome H, Akioka K et al: The beneficial effect of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroids on CMV infection in renal transplant recipients. Clin Exp Nephrol, 2013; 17(1): 127–33

15. Yoshimura N, Ushigome H, Nobori S et al: Excellent results of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroids in renal transplant recipients – 4-year results. Transplant Proc, 2013; 45(4): 1472–75

16. Ju MK, Huh KH, Park KT et al: Mizoribine versus mycophenolate mofetil in combination therapy with tacrolimus for de novo kidney transplantation: evaluation of efficacy and safety. Transplant Proc, 2013; 45(4): 1481–86

17. Revicki DA, Wood M, Wiklund I, Crawley J: Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease. Qual Life Res, 1998; 7(1): 75–83

18. Levey AS, Stevens LA, Schmid CH et al: A new equation to estimate glomerular filtration rate. Ann Intern Med, 2009; 150(9): 604–12

19. Kasiske BL, Zeier MG, Chapman JR et al: KDIGO clinical practice guideline for the care of kidney transplant recipients: A summary. Kidney Int, 2010; 77(4): 299–311

20. Su V, Greanya ED, Ensom MH: Impact of mycophenolate mofetil dose reduction on allograft outcomes in kidney transplant recipients on tacrolimus-based regimens: A systematic review. Ann Pharmacother, 2011; 45(2): 248–57

21. Tierce JC, Porterfield-Baxa J, Petrilla AA et al: Impact of mycophenolate mofetil (MMF)-related gastrointestinal complications and MMF dose alterations on transplant outcomes and healthcare costs in renal transplant recipients. Clin Transplant, 2005; 19(6): 779–84

22. Stephany BR, Augustine JJ, Krishnamurthi V et al: Differences in proteinuria and graft function in de novo sirolimus-based vs. calcineurin inhibitor-based immunosuppression in live donor kidney transplantation. Transplantation, 2006; 82(3): 368–74

23. Cantarovich F, Martinez F, Heguilen R et al: Proteinuria >0.5 g/d, a prevalent prognostic factor for patient and graft survival in kidney transplantation. Clin Transplant, 2010; 24(2): 175–80

24. Ibis A, Akgul A, Ozdemir N et al: Posttransplant proteinuria is associated with higher risk of cardiovascular disease and graft failure in renal transplant patients. Transplantat Proc, 2009; 41(5): 1604–8

25. Djamali A, Samaniego M, Torrealba J et al: Increase in proteinuria >200 mg/g after late rejection is associated with poor graft survival. Nephrol Dial Transplant, 2010; 25(4): 1300–6

26. Shiraki K, Ishibashi M, Okuno T et al: Effects of cyclosporine, azathioprine, mizoribine, and prednisolone on replication of human cytomegalovirus. Transplant Proc, 1990; 22(4): 1682–85

27. Shigeta S: Recent progress in antiviral chemotherapy for respiratory syncytial virus infections. Expert Opin Investig Drugs, 2000; 9(2): 221–35

28. Stuyver LJ, Lostia S, Patterson SE et al: Inhibitors of the IMPDH enzyme as potential anti-bovine viral diarrhoea virus agents. Antivir Chem Chemother, 2002; 13(6): 345–52

29. Hosoya M, Shigeta S, Ishii T et al: Comparative inhibitory effects of various nucleoside and nonnucleoside analogues on replication of influenza virus types A and B in vitro and in ovo. J Infect Dis, 1993; 168(3): 641–46

30. Kuramoto T, Daikoku T, Yoshida Y et al: Novel anticytomegalovirus activity of immunosuppressant mizoribine and its synergism with ganciclovir. J Pharmacol Exp Ther, 2010; 333(3): 816–21

31. Xing S, Yang J, Zhang X, Zhou P: Comparative efficacy and safety of mizoribine with mycophenolate mofetil for Asian renal transplantation – a meta-analysis. Clin Biochem, 2014; 47(7–8): 663–69

32. Akioka K, Ishikawa T, Osaka M et al: Hyperuricemia and acute renal failure in renal transplant recipients treated with high-dose mizoribine. Transplant Proc, 2017; 49(1): 73–77

33. Muller H, Solari S, Zuniga C et al: Therapeutic drug monitoring of mycophenolic acid in kidney transplant patients: An abbreviated sampling strategy. Transplant Proc, 2007; 39(3): 596–99

34. Zhang LW, Peng ZG, Xian WH et al: Association of de novo human leukocyte antigen and major histocompatibility complex class I chain-related gene-A antibodies and proteinuria with graft survival 5 years after renal transplantation. Transplant Proc, 2013; 45(9): 3249–53