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Improvement in long-term graft survival in cadaveric renal transplant recipients treated with mycophenolate mofetil

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Abstract Though mycophenolate mofetil has markedly reduced the incidence of acute rejection in renal transplantation, a significant improvement in graft survival has been more difficult to demonstrate. This retrospective study compares an historical control group of 210 consecutive renal transplant patients, who had received ATG induction associated with cyclosporin, prednisolone and azathioprine, with 187 patients receiving mycophenolate instead of azathioprine. The incidence of acute rejection was decreased with mycophenolate. In rejection-free patients, the 3-year graft survival rates were equivalent. In contrast, graft survival at 3 years improved significantly for patients who experienced a rejection crisis and remained under the initial triple drug regimen with mycophenolate compared to the patients of the historical group who were kept on azathioprine after a rejection episode. In conclusion, mycophenolate mofetil is not only able to reduce the incidence of acute rejection but could also improve the prognostic significance of acute rejection crises.

Keywords Renal transplantation · Chronic rejection · Mycophenolate mofetil · Azathioprine

Introduction

Large prospective clinical trials have established that the association of mycophenolate mofetil (MMF) and cyclosporin (CsA) dramatically reduces the incidence of acute rejection episodes in renal transplantation [1, 2, 3]. Further, it has been suggested that this low rate of acute rejection would lead to a low incidence of chronic rejection, which remains one of the main causes of late graft loss. However, it has been more difficult for those prospective studies to demonstrate that this new potent immunosuppressive drug resulted in a significant improvement of graft survival rates, when compared with azathioprine (AZA), after a 3 years follow-up [4, 5].

Nevertheless, in the 3-year data of the European placebo controlled trial [6], a reduction of 7.6% of graft loss was displayed in the MMF 2 g arm if death was excluded as a cause of failure.

In our centre, MMF replaced AZA in the prophylactic immunosuppressive protocol of kidney transplant patients in 1997. To assess this new regimen, we retrospectively compared a group of patients who had undergone transplantation from 1997 to 1999, with MMF ($n=187$), with an historical control group of patients who had received grafts between 1994 and 1996, under AZA ($n=210$). The two groups were compared in two consecutive equivalent periods of time of 3 years. All patients had received the same immunosuppressive regimen (anti-thymocyte globulin induction, delayed introduction of CsA and prednisolone) except for AZA or MMF. We here report on the incidence of graft rejection and the 3-year graft survival rate in these two groups.

Patients and method

Patients

This retrospective study includes 397 consecutive cadaveric kidney transplantations performed under a quadruple sequential immunosuppressive regimen between 1994 and 1999. All recipients completed their 3-year follow-up.

The patients were divided into two groups according to therapy with AZA or MMF. Thus, from January 1994 to December 1996, the first 210 recipients that received AZA were referred to as the AZA group, while the 187 remaining patients, who had received grafts between January 1997 and December 1999, corresponded to the MMF group. The classical pre-transplantation characteristics of the two groups are shown in Table 1. The only significant differences were: better HLA-B matching in the AZA group and a slightly shorter cold ischaemia time in the MMF group. The proportion of high immunological risk patients, defined as re-transplant and/or sensitized patients (PRA > 30%), was comparable in each group (14% vs 16% in the AZA and MMF group, respectively).

Prophylactic immunosuppressive protocol and drug monitoring

All patients received an initial course of anti-thymocyte globulins (rabbit ATG) during the first 5 to 10 days, depending on the occurrence of delayed graft function (DGF) requiring dialysis, associated with prednisolone (1 mg/kg per day for 15 days, then progressively reduced to 0.15 mg/kg per day after 3 months) and AZA (2 mg/kg per day) or MMF (2 g/day). CsA was started the day preceding the discontinuation of ATG, and doses were adjusted to maintain a trough blood level between 100 and 300 ng/ml.

After 2 to 3 months, AZA or MMF were withdrawn for rejection-free recipients at low immunological risk (first transplantation with PRA < 30%). Then, these patients received maintenance therapy with CsA and prednisolone. Conversely, re-transplant and/or sensitized patients (high immunological risk), as well as the recipients who had experienced a rejection crisis during the first 2 to 3 months after transplantation, were maintained on a triple-drug therapy consisting of CsA, prednisolone and AZA (patients that had received a transplant before 1997, AZA group) or MMF (patients that had undergone transplantation after 1997, MMF group).

At 1 month, the CsA trough levels were not significantly different between the two groups [142 ± 89 ng/ml in the AZA group and 151 ± 75 ng/ml in the MMF group, not significant (NS)], nor was the dose of prednisolone (29 ± 11 mg/day in the AZA group and 30 ± 12 mg/day in the MMF group, NS).

Treatment protocol for acute rejection

Acute rejection crises were treated with steroid pulses (1 g/day for 3 days). Steroid-resistant rejection episodes

Table 1 Pre-transplantation characteristics of the two groups

Characteristic	AZA group ($n=210$)	MMF group ($n=187$)	P
Donor age (years)	37 ± 12	37 ± 13	NS
Recipient age (years)	39 ± 11	41 ± 12	NS
Recipient gender (male), n (%)	125 (59)	114 (61)	NS
High immunological risk, n (%)[a]	30 (14)	30 (16)	NS
Duration of dialysis (months)	20 ± 20	25 ± 36	NS
No. of HLA matches			
A	0.82 ± 0.62	0.81 ± 0.64	NS
B	0.72 ± 0.62	0.56 ± 0.61	0.017
DR	1.04 ± 0.69	0.98 ± 0.57	NS
Cold ischaemia time (h)	21.6 ± 6.3	19.6 ± 6.0	0.001

[a]PRA > 30% and/or re-transplantation

were treated with OKT3 (5 mg/day for 10 days) or a conversion from CsA to tacrolimus started at 0.2 mg/kg per day then adjusted to maintain a trough blood level between 5 and 15 ng/ml.

Prophylactic and curative treatment of cytomegalovirus infection

Seronegative patients transplanted with a kidney from a cytomegalovirus (CMV)-seropositive donor received prophylactic intravenous anti-CMV-specific immunoglobulin weekly during the first 3 months. No prophylaxis was given to the other patients. CMV diseases, defined as CMV infections proven by positive viraemia or antigenaemia associated with clinical signs, were treated with intravenous ganciclovir. No patient received pneumocystis prophylactic treatment.

Statistical analysis

Results are expressed as mean ± SD. Student's *t*-test was used for unpaired data and the chi-square test for categorical variables. The Kaplan–Meier method was used to determine survival rates, and the log-rank test to compare survival curves. Patient death in the presence of a functioning allograft was considered a graft failure. The proportional hazards regression method (Cox) was used to determine the significance of differences in renal allograft survival when adjusted for various factors. A P value less than 0.05 was considered significant. Statistical analysis was performed with software SPSS, version 9.0 (SPSS, 1989–1999).

Results

Post-transplantation complications

The main complications that occurred in the early post-transplantation period are summarized in Table 2. In the MMF group, we noticed a significant reduction in the incidence of rejection crises (23% vs 35% in the AZA group, $P=0.011$). The proportion of steroid-resistant episodes was numerically lower in the MMF group (7% vs 12% in the AZA group), but this did not reach significance. However, the number of recurrent rejection episodes was significantly less frequent in the MMF group (1.6% vs 6.6% in the AZA group, $P=0.013$). Among the biopsy-proven rejections (32/74 and 36/44 in the AZA and MMF group, respectively), the proportion of vascular injuries (Banff grade ≥2) was identical in each group (48%).

The patients in the MMF group had a lower rate of bacterial sepsis and pneumocystosis, while the incidence of CMV diseases was similar. Consequently, the number of hospitalizations during the first year after transplantation was significantly lower in the MMF group (2.4 ± 1.5 vs 2.9 ± 2.3 in the AZA group, $P=0.011$), as well as the duration of those hospitalizations (28 ± 18 vs 34 ± 21 days in the AZA group, $P=0.006$).

Table 2 Number and percentage of complications during the first 6 months after transplantation

Complication	AZA group ($n=210$)	MMF group ($n=187$)	P n (%)
Delayed graft function	43 (20)	32 (17)	NS
Acute rejection episodes	74 (35)	44 (23)	0.011
Time post graft (days)	80 ± 210	71 ± 67	NS
Steroid resistant	25 (12)	13 (7)	NS
Recurrent rejection crisis	14 (6.6)	3 (1.6)	0.013
Infections			
CMV diseases	47 (22)	39 (21)	NS
Bacterial sepsis	81 (39)	54 (29)	0.042
Pneumocystosis	16 (8)	2 (1)	0.005

Patient and graft survival

Patient and graft survival rates at 3 years were not significantly different in the two groups (Fig. 1). However,

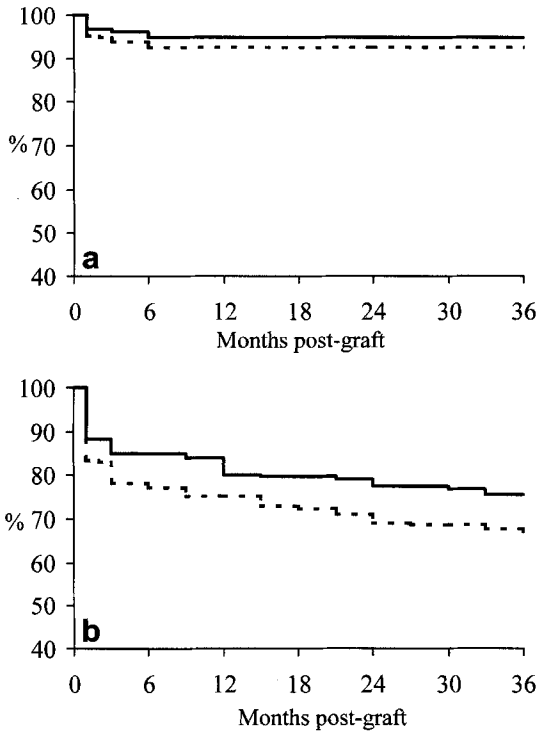

Fig. 1a, b Three-year survival rates. **a** Patient survival at 3 years in the MMF group (*solid line*) and in the AZA group (*dashed line*). Log-rank test was 1.18, $P=0.276$. **b** Graft survival at 3 years in the MMF group (*solid line*) and in the AZA group (*dashed line*). There is a slight but non-significant improvement of the graft survival rate in the MMF group (log-rank test was 3.17, $P=0.075$)

Table 3 Factors influencing graft loss (all patients)

Factor	Univariate analysis		Multivariate analysis[a]	
	RR [95% CI]	*P*	RR [95% CI]	*P*
Age	0.90 [0.52–1.56]	0.711		
Gender	0.88 [0.63–1.23]	0.460		
Immunological risk	1.60 [1.06–2.41]	0.022		
No. of HLA-A mismatches	1.75 [1.11–2.77]	0.015	1.72 [1.09–2.72]	0.019
No. of HLA-B mismatches	0.84 [0.43–1.65]	0.613		
No. of HLA-DR mismatches	1.24 [0.82–1.88]	0.306		
DGF	2.20 [1.53–3.16]	0.001	2.35 [1.63–3.38]	0.001
CMV disease	0.91 [0.64–1.29]	0.586		
Rejection	2.23 [1.60–3.10]	0.001	2.29 [1.64–3.20]	0.001
AZA/MMF	0.72 [0.51–1.03]	0.076		

[a]Only factors remaining significant are indicated for multivariate analysis

we observed a trend towards improvement of the graft outcome in the MMF group (75.3% vs 67.6% in the AZA group, $P=0.075$). Univariate analysis identified four significant risk factors for graft loss: immunological risk, HLA-A mismatches, DGF and rejection (Table 3). The impact of HLA-A matching, DGF and rejection were confirmed in a multivariate analysis (Table 3).

A further survival analysis, focused on rejection, revealed that MMF treatment was associated with a better graft outcome among patients who had experienced a rejection crisis (74 and 44 in the AZA and MMF group, respectively). In this subgroup of patients that remained on a triple-drug therapy (AZA/CsA/prednisolone or MMF/CsA/prednisolone) the 3-year graft-survival rate was higher for those on maintenance therapy with MMF (70.4% in the MMF group vs 47.3% in the AZA group, $P=0.009$, Fig. 2a). Conversely, the probability of graft survival was independent of the initial immunosuppressive protocol for the rejection-free patients that were converted to a dual therapy (CsA/prednisolone) after 2 to 3 months (Fig. 2b).

Univariate and multivariate analyses revealed that DGF and treatment with MMF vs AZA were the only determining factors for graft survival among patients who had experienced rejection (Table 4). By contrast, in rejection-free patients, multivariate analysis confirmed that the initial immunosuppressive protocol did not influence the graft outcome. For these patients the significant risk factors for graft loss were the number of HLA-A mismatches and the occurrence of DGF (data not shown).

Discussion

Acute rejection remains a major risk factor for the development of chronic rejection. Some authors have reported a recent increase in the negative prognostic significance of acute rejection that could partially explain the lack of improvement in the long-term renal graft survival rate, despite a significant reduction in the incidence of acute rejection [7].

In accordance with previously published studies [1, 2, 3], our results show that the replacement of AZA by MMF, in a sequential immunosuppressive regimen with ATG, CsA and prednisone, is followed by a lower incidence of acute rejection. The probability of graft survival remained the same for rejection-free patients whether they received AZA or MMF. However, MMF significantly improved the outcome of transplantation for the patients that experienced acute rejection.

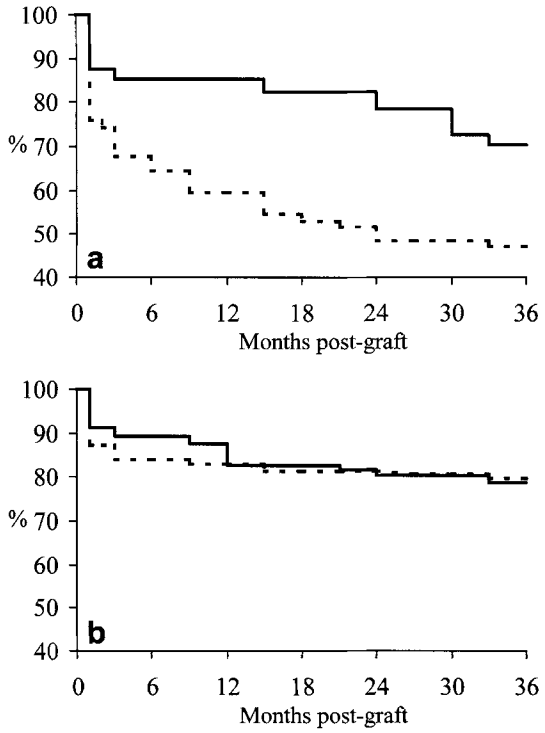

Fig. 2a, b Three-year graft survival rates stratified by rejection. **a** Patients who experienced a rejection episode (74 in the AZA group and 44 in the MMF group). Graft survival rate is significantly improved in the MMF group (*solid line*) compared with the AZA group (*dashed line*); log-rank test was 6.73, $P=0.009$. **b** Rejection-free patients have equivalent graft survival rates, whether they initially received MMF (*solid line*) or AZA (*dashed line*), log-rank test was 0.15, $P=0.700$

Table 4 Factors influencing graft loss among patients who experienced rejection

Factor	Univariate analysis		Multivariate analysis[a]	
	RR 95% CI]	*P*	RR [95% CI]	*P*
Age	0.18 [0.03–1.32]	0.092		
Gender	1.07 [0.65–1.75]	0.797		
Immunological risk	1.59 [0.89–2.84]	0.118		
No. of HLA-A mismatches	1.01 [0.46–2.22]	0.981		
No. of HLA-B mismatches	1.26 [0.57–2.76]	0.566		
No. of HLA-DR mismatches	0.85 [0.43–1.68]	0.648		
DGF	1.97 [1.08–3.57]	0.025	1.96 [1.08–3.56]	0.026
CMV disease	0.88 [0.51–1.50]	0.633		
AZA/MMF	0.46 [0.25–0.84]	0.011	0.46 [0.25–0.84]	0.012

[a]Only factors remaining significant are indicated for multivariate analysis

In our study maintenance therapy with MMF, instead of AZA, after a first rejection crisis, induced a significant decrease in the incidence of recurrent rejection episodes that could have contributed to this better outcome. Previous reports have demonstrated that the addition of MMF to the immunosuppressive regimen of patients experiencing a first rejection crisis may prevent recurrent rejections and increases the rate of reversal of acute rejection episodes [8, 9, 10, 11]. However, the 3-year graft survival rates were not significantly improved [12].

A second explanation for the improvement of graft survival after a first rejection crisis could be the reduction in the incidence of chronic rejection and the prevention of the progression of chronic allograft nephropathy under MMF therapy, which have been reported in several single-centre studies with a smaller number of patients [13, 14, 15, 16]. Furthermore, the report by Ojo et al., based on the data collected by the US Renal Transplant Scientific Registry, showed a 27% reduction in the relative risk of developing chronic allograft failure with MMF. In this study, the decrease in the incidence of acute rejection partly account for this result, since rejection-free patients had also benefited from the MMF therapy [17]. Recently, the demonstration that MMF protects against the long-term deterioration of renal allograft function has suggested that this drug has a beneficial impact on chronic allograft nephropathy [18]. Two others studies support these results and show a significant reduction in late acute rejection risk [19] and long-term graft loss with MMF [20].

Nevertheless, if MMF really is an efficient treatment for chronic rejection, the highest improvement in graft survival rates with this drug might be reasonably expected for patients who experienced acute rejection, which remains the strongest risk factor for chronic allograft nephropathy. This hypothesis is supported by a re-analysis, after a 3-year follow-up period, of the three pivotal trials conducted in Australia, North America and Europe that showed a particular beneficial effect of MMF 2 g in patients that had suffered delayed graft function and acute rejection [21]. These data highlight the fact that MMF could be an especially attractive treatment for patients with a very high risk of developing chronic allograft nephropathy.

Conversely, in the context of sparing immunosuppression, initial treatment with MMF can be safely removed, without increasing the risk of acute rejection, in stable renal transplant recipients early after transplantation [22, 23].

This approach is not only relevant, to limit the consequences of potent immunosuppressive protocols, but might also reduce the cost of immunosuppression in the long term. For the first year after transplantation, the cost effectiveness of MMF has been demonstrated by different studies [24, 25] and was mainly related to the reduction of early acute rejection episodes. In our present report we also noticed a significant decrease in the number and duration of hospitalization in the patients initially treated with MMF instead of AZA, mainly related to the reduction in the rate of rejection during the first 6 months after transplantation.

On the other hand, maintenance therapy with MMF for a longer period could be required, as previously mentioned, to improve the long-term results [18, 19, 20]. However, maintenance with MMF, with a 50% CsA reduction after the first year post-transplantation, could be an alternative cost-effective strategy [26].

In conclusion, in this retrospective study, the introduction of MMF in our immunosuppressive regimen was followed by a reduction in the incidence of acute rejection. The improvement in the 3-year graft survival rate of patients that remained on MMF instead of AZA, because of a first acute rejection, suggests that MMF may weaken the deleterious impact of acute rejection on graft outcome and could influence the course of chronic allograft nephropathy.

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References

1. European Mycophenolate Mofetil Cooperative Study Group. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for the prevention of acute rejection. Lancet 1995; 345:1321
2. Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. Transplantation 1996; 61:1029
3. Sollinger HW, for the US Renal Transplant Mycophenolate Mofetil Study Group. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. Transplantation 1995; 60:225
4. Mathew TH, for the Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. Transplantation 1998; 65:1450
5. US Renal Transplant Mycophenolate Mofetil Study Group. Mycophenolate mofetil in cadaveric renal transplantation. Am J Kidney Dis 1999; 34:296
6. European Mycophenolate Mofetil Cooperative Study Group. Mycophenolate mofetil in renal transplantation: 3-year results from the placebo-controlled trial. Transplantation 1999; 68:391
7. Meier-Kriesche HU, Ojo AO, Hanson JA, Cibrik DM, Punch JD, Leichtman AB, et al. Increased impact of acute rejection on chronic allograft failure in recent era. Transplantation 2000; 70:1098
8. The Mycophenolate Mofetil Acute Renal Rejection Study Group. Mycophenolate mofetil for the treatment of a first acute renal allograft rejection. Transplantation 1998; 65:235
9. Triemer HL, Pearson TC, Odom KL, Larsen CP. Analysis of a single-center experience with mycophenolate mofetil based immunosuppression in renal transplantation. Clin Transplant 2000; 14:413
10. Arnold AN, Wombolt DG, Whelan TV, Chidester PD, Restaino I, Gelpi B, et al. Mycophenolate mofetil, with cyclosporine and prednisone, reduces early rejection while allowing the use of less antilymphocytic agent induction and cyclosporine in renal recipients with delayed graft function. Clin Transplant 2000; 14:421
11. Vasquez EM, Sifontis NM, Pollak R, Benedetti E. Impact of mycophenolate mofetil on recurrent rejection in kidney transplant patients. Clin Transplant 2001; 15:253
12. The Mycophenolate Mofetil Acute Renal Rejection Study Group. Mycophenolate mofetil for the treatment of a first acute renal allograft rejection: three-year follow-up. Transplantation 2001; 71:1091
13. Kliem V, Boeck A, Eisenberger U, Petersen R, Radermacher J, Hiss M et al. Treatment of chronic renal allograft failure by addition of mycophenolate mofetil: single-center experience in 40 patients. Transplant Proc 1999; 31:1312
14. Campistol JM, Mazuecos A, Segura J, Osuna A, Herrero JC, Andres A, et al. Mycophenolate mofetil slows the decline of renal function in patients with biopsy-proven chronic rejection: a collaborative pilot study. Transplant Proc 1999; 31:2267
15. Park JH, Park JH, Yang CW, Kim YS, Kim SY, Moon IS, et al. Mycophenolate mofetil prevents the progression of chronic kidney allograft nephropathy. Transplant Proc 2000; 32:1747
16. Jirasiritham S, Sumethkul V, Mavichak V, Chalermsanyakorn P. The treatment of chronic rejection with mycophenolate mofetil versus azathioprine in kidney transplantation. Transplant Proc 2000; 32:2040
17. Ojo AO, Meier-Kriesche HU, Hanson JA, Leichtman AB, Cibrik D, Magee JC, et al. Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. Transplantation 2000; 69:2405
18. Meier-Kriesche HU, Stefen BJ, Hochberg AM, Gordon RD, Liebman MN, Morris J, et al. Mycophenolate mofetil versus azathioprine therapy is associated with a significant protection against long-term renal allograft function deterioration. Transplantation 2003; 75:1341
19. Meier-Kriesche HU, Steffen BJ, Hochberg AM, Gordon RD, Liebman MN, Morris JA, et al. Long term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. Am J Transplant 2003; 3:68
20. Cherikh WS, Kaufman HM, Maghirang J, Bleyer AJ, Johnson CP. A comparison of discharge immunosuppressive drug regimen in primary cadaveric kidney transplantation. Transplantation 2003; 76:463
21. Cho S, Hodge E, Navarro M. Mycophenolate mofetil improves long-term graft survival following renal transplantation in patients experiencing delayed graft function. Transplant Proc 1999; 31:322
22. Kaplan B, Meier-Kriesche HU, Vaghela M Kaplan B, Meier-Kriesche HU, Vaghela M, et al. Withdrawal of mycophenolate mofetil in stable renal transplant recipients. Transplantation 2000; 69:1726
23. ter Meulen CG, Gregoor PJ, Weimar W, Hilbrands LB. Elective withdrawal of mycophenolate mofetil in renal transplant recipients treated with mycophenolate mofetil, cyclosporine, and prednisone. Transpl Int 2001; 14:99
24. Sullivan SD, Garrison LP Jr, Best JH. The cost effectiveness of mycophenolate mofetil in the first year after primary cadaveric transplant. J Am Soc Nephrol 1997; 8:1592
25. Wuthrich RP, Weinreich T, Ambuhl PM, Schwarzkopf AK, Candinas D, Binswanger U. Reduced kidney transplant rejection rate and pharmacoeconomic advantage of mycophenolate mofetil. Nephrol Dial Transplant 1999; 14:394
26. Pascual M, Curtis J, Delmonico FL, Farrell ML, Williams WW, Kalil R, et al. A prospective, randomized clinical trial of cyclosporine reduction in stable patients greater than 12 months after renal transplantation. Transplantation 2003; 75:1501