PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of Mycophenolate Mofetil on Progression of Interstitial Fibrosis and Tubular Atrophy after Kidney Transplantation- A Retrospective Study
AUTHORS	Mihovilovic, Karlo; Maksimovic, Bojana; Kocman, Branislav; Gustin, Denis; Vidas, Zeljko; Bulimbasic, Stela; Ljubanovic, Danica; Matovinovic, Mirjana; Knotek, Mladen

VERSION 1 - REVIEW

REVIEWER	Mark Laftavi SUNY at Buffalo USA
REVIEW RETURNED	18-Mar-2014

GENERAL COMMENTS	In this retrospective study, Dr. Karlo Mihovilović et al. concluded that high dose MMF was associated with less interstitial fibrosis and tubular atrophy. This is a very intriguing topic because IF/TA is a major concern after renal transplantation. I have serious concerns regarding their conclusion. 1. The 79 patients they studied were a mixture of simultaneous pancreas and kidney transplant and kidney transplant from living or deceased donors and thus, the progression of IF/TA in these different cohorts of patients is different. This is non-homogeneous sampling. 2. A dose of four grams of MMF may be difficult to tolerate. Many patients cannot tolerate even the recommended dose of 2 grams due to serious adverse effects. There was no report on how many AEs were seen. Also, what was the rationale for this high dose of MMF in their program? 3. No AUC was performed. The MMF dosing did not correlate with the trough levels. MMF exposure in their patients was not documented. 4. There was no correlation between the MMF dose and rejection or subclinical rejection, showing that high dose MMF did not affect the immunological risk in their patients. 5. It is unusual that there was no correlation in GFR at 12 months between living or and deceased donors. It may be that the deceased donors they studied had very good kidneys to have equal

	eGFR compared to living donors at 12 months. 6. There was no correlation with CNI dose and eGFR, indicating that the CNI dose was not reduced when a high MMF dose was used. 7. Different maintenance immunosuppression regimens were studied, such as steroid free, with steroids and different types of CNI. Cyclosporine interferes with MMF's AUC while tacrolimus does not. Kidney function interferes with MMF's AUC. These important issues were not studied or discussed in this paper. 8. If the authors strongly believe in their findings, then a prospective randomized study in homogeneous renal transplant recipients using same the induction and maintenance immunosuppressive therapy would be the answer.

REVIEWER	Fritz Diekmann Hospital Clínic Nephrology Barcelona, Spain
REVIEW RETURNED	02-Apr-2014

GENERAL COMMENTS	1) How was the MMF dosing selected in the patients? Or was it just the highest tolerated dose? 2) The patients show a surprisingly high DGF rate for standard criteria donors. Are there any reasons for this? Cold ischemia time? 3) Were only the low-risk patients steroid-free and therefore they have better histological scores? 4) safety data like side effects/infections should be reported in order to have a well-balanced report

VERSION 1 – AUTHOR RESPONSE

Answers to reviewer 1 (Dr. Mark Laftavi):

1. We agree that the sampling in our study was not homogenous. However, similar methods of study subject inclusion have been used in the past, e.g. Heilman et al., Clin Transplant 2008;22:309.. Such sampling reflects real life situation in kidney transplant population. We performed multivariate analysis to take into account this heterogeneity. In addition, when performing sensitivity analyses our observation of association of higher average MMF dose with less progression of IF/TA was present in different subgroups of patients (e.g. with respect to type of CNI , type of donor, …).

2. During 2007-2010 MMF was dosed in our center according to C0 monitoring, which led to its dose dispersion (1000-4000 mg/day). Dose adjustments were performed regardless of trough levels for AE. Report of AE is now included in our paper (table 6).

3. We did not perform AUCs for MPA. As our results were derived from routine patient management, no other formal PK testing for MPA exposure, beside MPA trough levels was done. At the time when our study patients were followed during their first posttransplant year, in our centre MPA trough levels were used to guide minimal MMF dose. Similar approach was applied in the Opticept trial (American Journal of Transplantation 2009; 9: 1607). We agree that more precise

knowledge of the relationship between MPA exposure determined by AUC and IF/TA would be informative. However, we believe that lack of this in our study does not substantially diminish the study value. We are currently conducting a prospective trial (NCT01860183) in which MPA AUCs are included. Recently published study analysing data from the Symphony, Opticept and FDCC trials (Transplantation2011;92: 82, ref. 24), completely corroborates our findings. However, our study extends their results by showing better preservation of renal histology with higher dose of MMF.

4. We thank the reviewer for this comment, as indeed we shown in our paper that there was no correlation of higher MMF dose with incidence of acute rejection and it is our belive that there may be significant contribution of nonimmune mechanisms. Also, higher MMF dose might play important role in decreasing total inflammation score which is important predictor of kidney function (data presented separately: Mihovilovic et al. KW2013 FR-PO1057).

5. Indeed, baseline histology (ci and ct score) of the deceased and living donor kidneys was not statistically significant (P value 0.32 and 0.29 respectively, Mann Whitney U test).

6. The reviewer's conclusion is correct. MMF dose was guided by MPA trough levels, independently of CNI dosing, which was also based on the target trough level. However, when we performed a three-way analysis, we observed that best renal function was present in patients treated with highest average MMF dose and lowest average tacrolimus concentration (data not shown in this paper, but were presented at the ASN 2013 Kidney Week; SA-PO1015).

7. Although different maintenance regimens were used, observed study results were similar when patients were divided in groups with respect to steroid use or with respect to type of CNI (cyclosporine vs. tacrolimus). In both, tacrolimus and cyclosporine treated patients MMF dose was related to better graft histology (less IF/TA). These results persisted in the multivariate analysis, as shown in Table 3.
8. Indeed, we have initiated a multicenter, randomized, prospective trial that would investigate findings of these retrospective study. (NCT018600183)

Answers to reviewer 2 (Dr. Fritz Diekmann).
1. At the time period encompassed by the study, MMF was dosed in our centre based on trough level according to available evidence on minimal target MPA levels in protocols with cyclosporine, or with tacrolimus. Highest MMF dose was 4 g daily, regardless of the achieved trough level. This is similar to the Opticept trial (American Journal of Transplantation 2009; 9: 1607). MMF dose was reduced in case of side effects, irrespective of trough levels.
2. Indeed, incidence of DGF is high and it cannot be explained by the cold ischemia time, which is not too long within Eurotransplant area. Warm ischemia time was also within expected time frame in our centre. However, majority of kidney donors originated in Croatia, where brain death is frequently confirmed by the cerebral angiography, which might induce contrast nephropathy, manifesting after transplantation as DGF. This requires further testing, and is subject of a separate study. Nevertheless, in a recent retrospective study in European population, with similar characteristics to our population, incidence of DGF was 32.4 % (Transplantation. 2012;93:52), which is close to the DGF rate observed in our study.

3. Patients with steroid free immunosuppression were the low immunological risk patients but this was accounted for by a multivariate analysis as it was presented in table 4.

4. As it was answered to second question of the first reviewer (dr.Mark Laftavi) during 2007-2010 MMF was dosed in our center according to C0 monitoring, which led to its dose dispersion (1000-4000 mg/day). Dose adjustments for AE were performed regardless of trough levels. Report of AE is now included in our paper in table 6.

VERSION 2 – REVIEW

REVIEWER	Mark Laftavi State University of new York at Buffalo, buffalo, NY, USA
REVIEW RETURNED	02-Jun-2014

GENERAL COMMENTS	In a retrospective study, Knotek et. al. reported that the MMF dose was correlated with higher rate of IF and TA. The association of low dose MMF with more graft loss has been reported before and is not new to the transplant community. The authors retrospectively reviewed the biopsies from 79 patients after renal transplantation. I have serious concerns regarding this report: 1. In this study, different immunosuppression regimens were used, which may impact on IF/TA. Steroid withdrawal has been reported to be associated with more allograft fibrosis. Thus, exaggerated IF/TA in their patients could be caused by steroid withdrawal. The authors did not discuss this issue in their paper. 2. Cyclosporine interferes with MMF pharmacokinetics but tacrolimus does not. Therefore, combining cyclosporine and tacrolimus treated patients in one group is misleading. In one study, co-administration of cyclosporine instead of tacrolimus resulted in a significant increase of median (range) of the ratio of dose-to-concentration 0.92 (0.11-8.33) (n=167) versus 0.38 (0.11-14.28) (n = 66); $p<0.0001$. The choice of maintenance immunosuppression plays a significant role in IF/TA development so including patients with different types of immunosuppression regimen in one small group of 79 patients will provide a misleading conclusion.

REVIEWER	Fritz Diekmann Hospital Clinic Nephrology Barcelona, Spain
REVIEW RETURNED	30-May-2014

GENERAL COMMENTS	Reviewers' concerns were addressed. No further comments.

VERSION 2 – AUTHOR RESPONSE

We agree with Dr.Laftavi that steroid use may have affected IF/TA progression. However according to our protocol only patients with low immunologic risk and without delayed graft function are candidates for rapid steroid withdrawal. As we have stated on page 10, line 55 to page 11 line 4 steroid-free imunosuppression did not adversely affect progression of ci (0.47 ± 0.7 vs. 1.09 ± 0.87; p=0.002). This effect persisted in a multivariate analysis (Table 7.). While reviewing the present version of our papaer we noticed an error in Table 6 regarding description of the row with steroid use results (it should have been written “no vs yes”) which we corrected in this new revision. Concerns regarding steroid-free imunosuppression originate from a landmark ASTELAS trial (Woodle at all, Ann Surg 2008; 248:564) where incidence of CAN was 5.8 percent higher in steroid withdrawal group. However, 70 percent of patients had CAN diagnosed on their first allograft biopsy. Since that study did not include protocol biopsies and implant biopsies, it was not possible to exclude preexisting donor kidney disease as the etiology of CAN.

As we analyzed our data we had similar concerns as Dr. Laftavi regarding different interaction of cyclosporin and tacrolimus with MMF exposure. However, effect of increased average MMF dose with lower progression of ci and ct score was present regardless of type of CNI inhibitor (now included on page 11 line 5 of the revised manuscript).

We hope that we have addressed all of the reviewer’s concerns and that our paper may be now suitable for publication in the BMJ Open.