Monitoring immunosuppression after liver transplantation : development of individualized Bayesian limited sampling monitoring

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CHAPTER 6

ADVANCED MMF MONITORING STRATEGY IN LIVER TRANSPLANTATION IN PRESENCE OR ABSENCE OF CALCINEURIN INHIBITORS

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ABSTRACT

Background: The immunosuppressive drug mycophenolate mofetil (MMF), with mycophenolic acid (MPA) as active metabolite, is a non-nephrotoxic alternative to calcineurin inhibitors in liver transplant patients. Limited data is available of therapeutic drug monitoring strategies for MMF. Monitoring MMF becomes even more relevant in preventing rejection in CNI-free regimens. We aimed to describe the pharmacokinetic (PK) behaviour of MMF in different immunosuppressive regimens to develop a monitoring strategy for MMF.

Methods: PK data were obtained from stable patients (n=34) and the effect of covariates (liver and kidney function, serum albumin concentration) and CNI co-medication on PK-parameters was studied. A TDM-strategy was developed based on Bayesian estimations, limited sampling models and immunosuppressive co-medication.

Results: A linear relationship between MMF-dose and MPA-AUC was found and a 7-fold apparent clearance range was observed. Significant relationships of albumin concentration and creatinine clearance with MPA-plasma clearance were identified (r^2=0.26, r^2=0.36; $p<0.05$). The model 0+½+1+2h shows good correlation with trapezoidal-AUC0-12h with acceptable bias and precision (with CNI: r^2=0.82, without CNI: r^2=0.85; $p<0.05$).

Conclusion: This study demonstrates the large variability of MPA in liver transplantation, the association of albumin and creatinine clearance with this variability, and illustrates the use of population based monitoring strategies ranked to presence or absence of CNI co-medication.

INTRODUCTION

Mycophenolate mofetil (MMF) is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent. MPA is an inosine monophosphate dehydrogenase (IMPDH) inhibitor and therefore inhibits the de novo pathway of guanosine nucleotide synthesis and thus the proliferative responses of T- and B-lymphocytes[1].
MMF is widely used as immunosuppressant after different types of organ transplantation including liver transplantation (LT). It is often administered in combination with a calcineurin inhibitor (CNI), tacrolimus (TRL) or cyclosporine (CsA), but also without CNI in order to spare renal function, since MMF is not nephrotoxic. Use of MMF may allow CNI dose reduction or discontinuation, with improvement or stabilization of renal function[2].
Different studies in the past years, most in renal and cardiac transplant patients, showed a significant inverse correlation between MPA exposure and the risk of acute rejection[3-6]. Fewer studies were performed in liver transplant patients. Generally, results in terms of patient and graft survival are good if used in combination with a CNI, but a switch to MMF monotherapy after LT can be associated with a rate of 0-20% acute cellular rejection which – if not treated adequately – can lead to chronic rejection and graft loss[7]. However, rejection rates of 10% or more have been reported in MMF-monotherapy after liver transplantation, which may be related to low exposure of MPA[8-11].
In contrast to therapeutic drug monitoring (TDM) for CNIs, at this moment most clinics adhere to a fixed dose of MMF, not based on any individual patient characteristics like age, weight, MPA- or creatinine clearance[12]. Recently, studies have been performed to explore current evidence on the usefulness and clinical relevance of MPA trough level monitoring during MMF therapy in solid organ transplantation[13-14]. Also several limited sampling strategies have been proposed and studied mostly in renal transplant patients, with often 3-5 sampling time points taken in the first 2-6 hours after dosing[15-17]. Le Guellec et al. developed a limited sampling strategy based on Bayesian estimations as a tool for therapeutic drug monitoring in renal transplant patients[18]. However, there is limited information on TDM of MPA in liver transplant patients[19,20]. This becomes even more relevant in CNI free regimens.
Therefore the aim of this study was to describe the pharmacokinetic (PK) behaviour of MPA in liver transplant patients in the context of different co-immunosuppression (with or without CNI). In addition we were aiming at estimating inter-patient variability of MPA clearance in order to develop a TDM-strategy using flexible limited sampling models (LSM) for MPA. We studied factors (covariates) like albumin concentration and creatinine clearance that could have an effect on MPA pharmacokinetics.

MATERIALS AND METHODS

Thirty-four stable patients using MMF who were at least 3 months after OLT were included (median 214 weeks, range 16-630). Apart from MMF seven patients received tacrolimus (± prednisone) as co-medication, fifteen received cyclosporine (± prednisone), and twelve patients received only glucocorticoids (11 prednisone, 1 budesonide) next to MMF. So, 22 patients were on CNI co-medication and 12 patients were without CNI co-medication. Table 1 shows the patients characteristics for different groups of co-medication.

Patient characteristics	**all patients (n=34)**		**MMF without CNI (n=12)**		**MMF + CsA (n=15)**		**MMF + TRL (n=7)**		
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	P
Age (years)	49	12	54	6	50	12	39	16	0.063
Dose twice daily (mg)	720	287	875	311	633	248	643	244	0.085
Weight (kg)	77	19	75	15	78	21	77	22	0.981
Albumine (g/L)	41.5	3.7	42.1	3.2	41.8	3.0	39.9	5.6	0.751
CRCL(mL/min)	72	31	57	31	72	29	96	25	0.032*

Table 1: Patient characteristics of all patients for different groups of co-medication (without CNI; CsA (cyclosporine) and TRL (tacrolimus)). P-values indicate the level of significance of differences between the 3 groups (non-parametric Kruskal-Wallis-test, *=significant).

Mycophenolate mofetil (CellCept®, Roche, Basel, Switzerland) was given twice daily. In our clinic MMF-dosing for liver transplant patients was based on fixed dose regimens. Patients started with 500 mg twice daily and if allowed by absence of leuco- and trombopenia and gastrointestinal side-effects the dose was increased to and kept at 1000 mg twice daily. In three cases a deviant dose of twice daily 250 mg (1 patient), 750 mg (1 patient) or 1500 mg (1 patient) was given.
After informed consent, all patients visited our clinic for one day. Five minutes before administration of the morning dose of MMF (approximately 10.00h AM) blood samples were obtained for liver and kidney function, serum albumin concentration and MPA (C0) concentration. Creatinine clearance (CRCL) was calculated with Cockcroft and Gault formula. Patients were instructed to take their evening dose the night before their visit at 10.00h PM. Further blood samples for MPA concentration were collected at

0.5, 1, 2, 3, 4 and 6 hours after administration of the morning dose of MMF. The missing C=12h was obtained by extrapolation from t=0h to t=12h, assuming steady state condition.
Blood was drawn using an indwelling catheter and collected in a vacutainer containing EDTA. Plasma MPA concentrations were determined using High Performance Liquid Chromatography (HPLC)[43]. In order to lower possible influence from meals the patients were instructed to take only a light breakfast - tea and a biscuit - on the morning of measuring the AUC, and until the 2 hours sample (C2) no additional food or drinks were taken.
Population pharmacokinetic (POP-PK) limited sampling models were developed using the kinpop module of MW\Pharm, version 3.60 (Mediware, Groningen, the Netherlands)[21]. An oral 2-compartment model with first order absorption and lag-time described the data adequately. The best models were selected, based on the log-likelihood-value of MW\Pharm, the correlation with trapezoidal MPA-AUC and precision and bias. A trapezoidal AUC0-12h of all 34 curves was calculated with the trapezoidal rule, using the software package MW\Pharm.
Individualized PK parameters (individualized PK-model based on Bayesian fitting, i.e. *post hoc* values) were obtained. AUCs (mg.h/L) based on MPA clearance on single blood sampling time points and combinations of time points were calculated based on the formula: AUC = (F_po * dose) / clearance, in which *F_po* is bioavailability which was fixed to 1 for MMF since no i.v. data were available[31]. The *dose* (mg) is the morning dose of MMF and *clearance* (L/h) became apparent clearance (CL/F) of MPA in the absence of information on bioavailability. CL/F was estimated for all patients with Bayesian estimation at different time points and combinations of time points (limited sampling models).

Statistics

Statistical analysis on patient data was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± S.D. and as median and range. Potential differences in patient characteristics were tested with non-parametric Kruskal-Wallis-test.
AUCs calculated with the formula AUC = dose / clearance were compared to the trapezoidal AUC0-12h with Pearson correlation coefficient. P-values below 0.05 were considered statistically significant.
The ability to describe the trapezoidal AUC0-12h of the different methods was also investigated by calculating the prediction precision and bias deducted from the paper of Sheiner and Beal[22]. Prediction bias was calculated as the mean prediction error (MPE); that is the mean of differences between AUC0-12h calculated with the formula shown above and the trapezoidal AUC0-12h. Prediction precision was calculated as the mean

absolute prediction error (MAPE); that is the mean of the absolute differences between the calculated AUC0-12h and the trapezoidal AUC0-12h. Smaller values for MPE and MAPE indicate less bias and greater precision respectively.

RESULTS

Pharmacokinetic analysis

There was a linear relationship between MMF dose and trapezoidal MPA area under the curve (Figure 1). There was a wide range in MPA clearance (apparent clearance = Cl/F = dose/AUCtrap) in the population (8.08 – 57.47 L/h). Dividing the total population into 3 groups based on co-medication, the MPA clearance ranges are 8.08 – 31.55 L/h for patients without CNI, 8.27 – 57.47 L/h for those on cyclosporine and 13.66 – 43.10 L/h for those with tacrolimus co-medication.

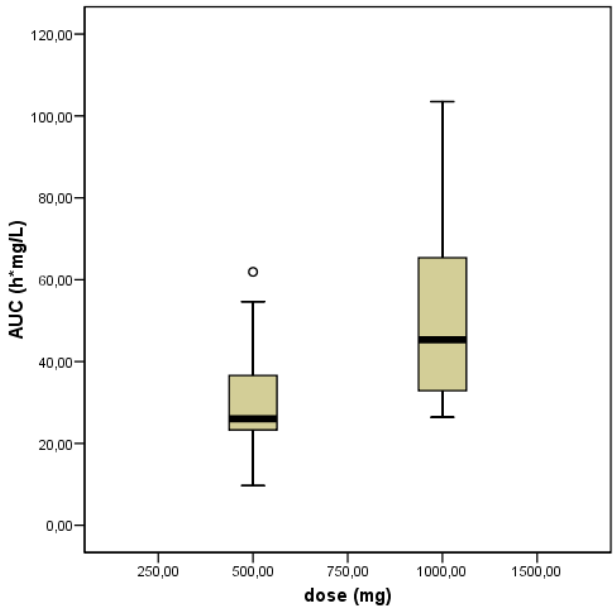

Figure 1: MMF dose versus trapezoidal MPA-AUC relationship of patients with MMF dose 500 mg and 1000 mg twice daily (n=31, dose 500 mg: n=18; dose 1000 mg: n=13)

Looking at possible sources of this variability in MPA clearance, there appeared to be a significant inverse relationship between serum albumin concentration and MPA clearance ($r^2 = 0.26$, $p<0.05$). Specifically, low albumin levels are related to higher MPA clearance. There also was a significant relationship between creatinine clearance and MPA clearance ($r^2 = 0.36$, $p<0.05$). No significant difference in CRCL existed between the two groups with and without calcineurin inhibitors, data not shown.

Co-medication

To explore potential differences in (dose adjusted) MPA-AUC between patients with different co-medication next to MMF, all patients were divided into three groups (cyclosporine, tacrolimus, no calcineurin inhibitors). These non significant differences are shown in Figure 2 (p=0.247). A similar plot could be derived from difference in apparent clearance (data not shown). Based on the comparable dose-adjusted AUCs of patients on tacrolimus or cyclosporine in contrast to group 1 (no calcineurin inhibitors), this led towards further analysis based on two groups, one group with calcineurin inhibitors (cyclosporine or tacrolimus) and one group without calcineurin inhibitors. This classification, based on clinical selection, was used for further development of limited sampling models for therapeutic drug monitoring of MPA.

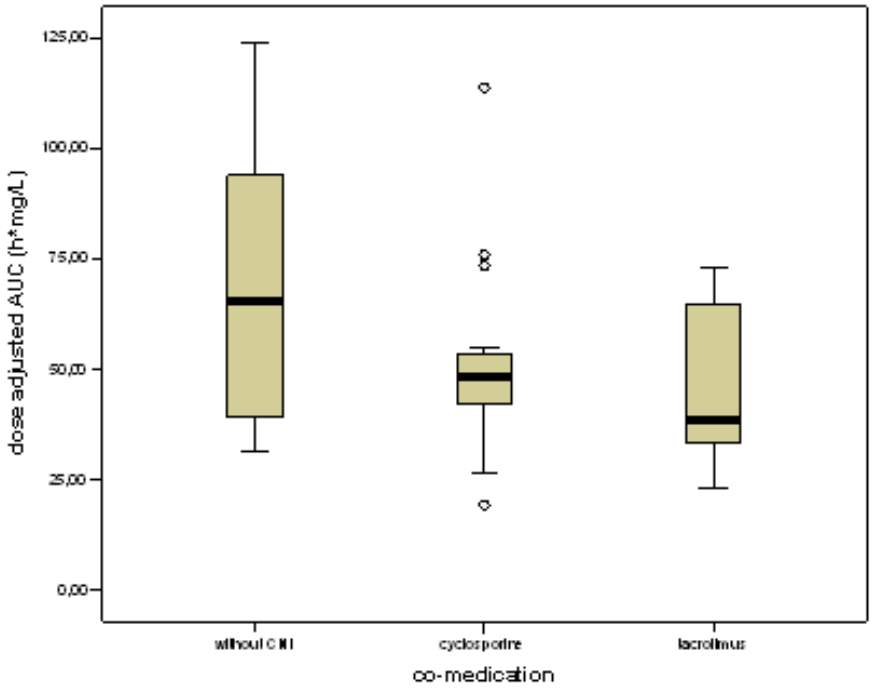

Figure 2: Patients without calcineurin inhibitors; patients with cyclosporine and patients with tacrolimus as co-medication next to MMF and their (non significant) difference in dose adjusted AUC (p=0.247). The circles in the plot indicate individual (cyclosporine) patients outside the range.

Development of limited sampling models

Different groups of models based on renal function and co-medication were developed in MW\Pharm. For four patients the model building procedure in MW\Pharm could not describe the data adequately according to the population model including the total patient population. Six patients with deviant albumin levels (outside reference range of 40-50 g/L) were excluded when developing the model because MPA concentration levels are positively associated with serum albumin levels[23]. When developing PK models these patients (n=10) were excluded for model building on the condition that the final model should improve the prediction of the apparent clearance for these individuals compared to the base model including their data. The PK models were developed based on the remaining 24 patients.

Population parameters for the CNI as well as the no-CNI group were calculated. Because of nephrotoxicity of CNIs also POP-PK models were developed for groups based on creatinine clearance instead of co-medication. The POP-PK parameters for MMF limited sampling models both for patients with and without CNI co-medication are shown in Table 2. The apparent oral clearance (CL/F) is on average more than 50% higher for the group with CNIs compared to the group without CNIs.

Parameters	**CNI (n=16)**		**Without CNI (n=8)**	
	population	±	population	±
Apparent clearance (L/h/70kg)	17.66	7.15	11.19	4.43
Volume (central) (L/kg)	0.2585	0.2546	0.1476	0.1589
Intercompartimental clearance (L/h/70kg)	22.82	16.37	35.69	10.14
Volume (peripheral) (L/kg)	3.0042	3.4748	2.2672	2.1192
Absorption rate constant (/h)	7.0165	12.2131	33.13	65.03
Oral bioavailability	1	fixed	1	fixed
Lagtime (h)	0.3366	0.1966	0.4893	0.0100

Table 2: Population pharmacokinetic parameters for CNI-group (16 patients) and group without CNI (8 patients)

Based on the individualized PK parameters for both groups with and without CNI, AUCs of different limited sampling models based on one- or multiple point sampling were calculated. Correlations of these calculated AUCs with trapezoidal AUC0-12h including bias and precision for both groups are shown in Table 3.

Blood sampling time points	CNI (n=16)			Without CNI (n=8)		
	r^2	MPE	MAPE	r^2	MPE	MAPE
0	0.89	6	20	0.68	16	20
0-0.5-3	0.87	15	27	0.51	30	31
0-0.5-1-2	0.82	14	24	0.85	14	20
0-1-2-3	0.75	12	29	0.78	19	21
0-0.5-1-2-3	0.69	35	45	0.80	18	19
0-3-4-6	0.93	15	26	0.44	32	34
3-6	0.59	15	29	0.72	4	17
0-0.5-1-2-3-4-6	0.91	6	14	0.86	11	13

Table 3: Correlations of MPA-AUC calculated for models with and without CNI with trapezoidal AUC0-12h (n=24, CNI: n=16, without CNI: n=8)

These time points are a selection of the best of 30 investigated combinations of blood sampling time points. Especially the combination 0-½-1-2h shows very good correlations with trapezoidal AUC0-12h for both models (with and without CNI), with acceptable bias and precision (CNI: r^2=0.82, MPE/MAPE 14/24; without CNI: r^2=0.85, MPE/MAPE 14/20).

The correlations, bias and precision of the groups based on creatinine clearance were inferior to the groups with and without CNI (data not presented).

Correlation of MPA-trough-levels with trapezoidal AUC0-12h for all patients (n=34) without using any limited sampling model was surprisingly good, r^2=0.81 ($p<0.05$). This relationship for the different types of co-medication (without CNI, cyclosporine, tacrolimus) is shown in Figure 3, which underlines our division of co-medication in groups with and without CNI. The correlation of trough level (C0) with trapezoidal AUC0-12h, with the use of limited sampling models, was reasonable (r^2=0.89) in patients on CNI (n=16) versus a lower correlation (r^2=0.68) for patients without CNI (n=8), both $p<0.05$.

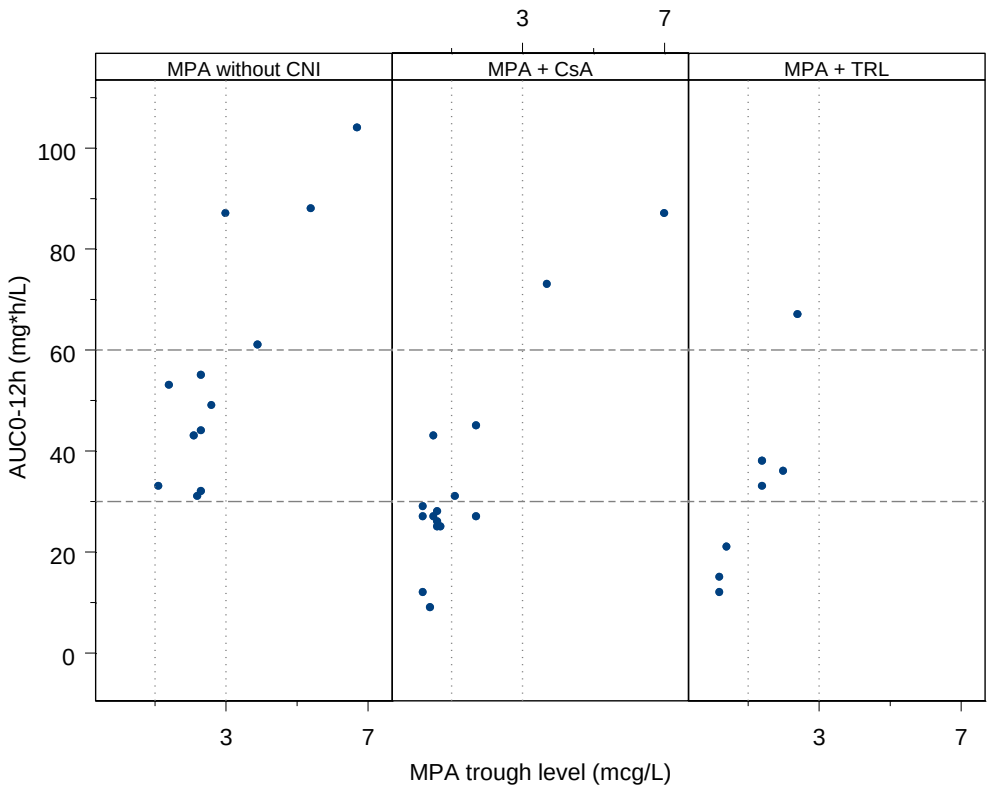

Figure 3: Relationship of MPA trough level with trapezoidal AUC0-12h for different groups of co-medication next to MMF: without CNI, with cyclosporine (CsA) and with tacrolimus (TRL)

DISCUSSION

We could adequately describe the pharmacokinetic profile of MPA in liver transplant patients. There appeared to be a linear relationship between MMF dose and the area under the concentration time curve (AUC) with the remark that a 7-fold variability in MPA apparent clearance was observed. Part of this variability could be associated with the covariates serum albumin concentration and creatinine clearance (CRCL). This analysis was the basis for a proposal to improve TDM in liver transplant patients: we developed limited sampling models for MPA TDM for different groups of patients and depending on co-medication (with and without CNI) or renal function.
Some combinations of time points showed excellent correlation with trapezoidal AUC0-12h, for patients on CNI even with trough level monitoring, when using a limited sampling model. However, with the model of patients without CNI therapy only a moderate correlation of MPA trough level with trapezoidal AUC0-12h was found. Since our Bayesian models have no need for fixed time points they are very flexible and easy to use in daily practice in the outpatient clinic, as we have shown before for cyclosporine monitoring[24].

The trough level without the model demonstrated a nice correlation with trapezoidal AUC, however our dataset is too small to show the imprecision for this method. One could note the possible imprecision for the trough level approach, as is known for the CNI's from Figure 3 (middle plot). A 4-fold difference is observed between trough level and AUC despite the good correlation between trough level and AUC. This large difference in AUC at a measured trough level (i.e. 0.5 mcg/L) is a reflection of the large interpatient variability and is a pitfall in trough level approach. However, for MMF a larger cohort should support these findings.

There are several reasons for introducing therapeutic drug monitoring of mycophenolate mofetil in daily practice. MPA levels are related to efficacy (rejection) and safety (adverse events)[3-6]. A recent article from Yau et al. already concluded that fixed dose regimens of MMF may not be optimal for all patients[25]. Another important reason is the inter-patient variation in MPA pharmacokinetics, due to factors such as renal function, albumin level and (cyclosporine) co-medication[23,26-29]. One third of patients on cyclosporine receiving fixed dose MMF immediately after renal transplantation were underdosed when the AUC was calculated, and this was related to a higher incidence of rejection[30]. Furthermore, an increase of Cmax and AUC of MPA in renal transplant recipients in the months after transplantation is described[31]. This may require dose adjustments.

Calcineurin inhibitors are widely used after organ transplantation. A disadvantage of these drugs is their nephrotoxicity. MMF, in contrast to CNIs, does not cause renal damage. Its use may lead to lowering or even discontinuation of CNI-dosing[32,33]. The discontinuation of CNI may lead to better kidney function in the long term[9,34]. However, conversion to fixed dose MMF monotherapy (or with steroids) after liver transplantation may lead to acute or even chronic rejection in a significant percentage of the patients[8-11]. A solid TDM-based dose guiding strategy for MPA may reduce these risks. In addition, with this approach we can get a clear understanding of the relationship with MPA toxicity in a CNI free regimen in the context of higher MMF doses.

A recent review article from Kaplan concluded that the contribution of TDM for MMF in the investigated studies remains unproven and that results of large randomized controlled trials are awaited[14]. Another review article from Arns et al. concluded that there still was no clear support for a substantial clinical benefit of TDM, but that MPA area under the curve might be more reliable than predose (C0) MPA levels[13]. Zicheng et al. developed rigid limited sampling algorithms for implementation of MPA-monitoring in liver transplantation necessitating exactly timed blood sampling[20]. In the roundtable meeting of Van Gelder et al. also different limited sampling strategies, mostly algorithms, for monitoring MPA were described as good estimators of AUC0-12h with acceptable predictive performance[35]. Based on the MPA AUCs in our patients on tacrolimus, cyclosporine or without CNI it appeared necessary to divide the liver

transplant patients in one group with calcineurin inhibitors (no difference between tacrolimus or cyclosporine) and another group without calcineurin inhibitors and to develop two separate LSMs for these two groups.
The program used for Bayesian estimations is a two stage approach which is able to predict PK parameters adequately in strictly defined populations. The studied population of liver transplant patients displays large inter-individual variability with a 7-fold apparent clearance difference. Therefore we had to make a patient selection (*i.e.* albumin selection) which at first sight seems to indicate bias and would not reflect the clinical situation. However, with this selection we were able to build a model with more degrees of freedom which has the advantage to estimate individual (*post hoc*) PK parameters more accurately and precise. This is reflected and justified by the fact that these excluded patients - both groups of 4 patients who did not adequately described the data during model building and the 6 patients with deviant albumin levels - fitted better in the newly developed model. However, this does indicate that the model should be validated on a larger dataset before introduction in clinical practice.
One should note that the CNI free group demonstrated low CRCL, which is an artefact caused by rather late conversion of patients with deteriorated kidney function to a CNI free regimen. Also, the correlations, MPE and MAPE of the groups based on creatinine clearance were inferior to the groups with and without CNI. When the trend evolves to minimize or discontinue CNIs, our MPA classification provides an excellent tool for continuation of therapeutic drug monitoring of MMF.
The distinction between cyclosporine/no-cyclosporine as co-medication of MMF is described in different studies[26,36-39]. Cyclosporine has an influence on MPA clearance by disrupting the enterohepatic cycle, leading to lower MPA exposure[40]. However, we did not find a difference in MPA AUCs between patients on tacrolimus and those on cyclosporine. A limitation of our study is the absence of blood sampling time points between 6 and 12 hours after dosing MMF, exactly the time in which the enterohepatic recirculation may occur. Due to these missing values we could not take the enterohepatic cycle into account, which may mean that the MPA AUCs in patients using cyclosporine may be slightly higher than calculated in our study. However, the absence of a difference in trough levels between the CNI groups (same dose range) indicates that this effect might not be relevant for MPA in liver transplant patients. Because of possible disturbances in bile production and flow the influence of the enterohepatic cycle might be different in liver transplant patients compared to renal transplant recipients[41]. Figure 3 suggests that both CNIs may cause a higher CL/F of MPA and therewith a lower MPA exposure than in patients without CNI. However, as earlier mentioned, this could also be biased by kidney function or by albumin concentration. Because the models we developed are based on a limited number of patients, we are planning to validate these models.

In addition, we will implement limited sampling models with more time points than may be needed to achieve more information during this prospective validation. Also the role of trough level-monitoring in combination with a POP-PK model, which appeared to be reliable in patients on CNI according to our findings, and the clinical relevance, need further validation on a larger dataset. The LSM seems excellent with sampling at 0-½-1-2h for both groups with and without CNIs, with good correlations with trapezoidal AUC0-12h and acceptable bias and precision.

No target ranges for the MPA AUC especially for liver transplantation patients have been developed yet. In the scarce literature about TDM of MPA after liver transplantation Tredger et al. suggests a therapeutic range of 1 to 3.5 mg/L for trough-level monitoring in order to prevent acute rejection and to lower adverse effects, like infection, leucopenia and gastrointestinal disturbances[19]. For renal transplantation in the early post-transplant period, an AUC0-12h range of 30-60 mg.h/L is adhered to in the presence of a CNI[35]. De Fijter et al. suggests that a target AUC of 75 mg.h/L (range 60-90 mg.h/L) for kidney transplant recipients allows cyclosporine withdrawal, and with this target range very few patients developed acute rejection[42]. For the moment we suggest - in the absence of sufficient data from clinical studies - to use similar targets in liver transplantation as in renal transplantation[42]. Especially for the patients without CNI with increased risk of (chronic) rejection, the lower side of the AUC range (60 mg.h/L) seems to be more important than the danger of (reversible) toxicity from high levels, which is easier to recognize and usually rapidly responds to dose lowering.

In conclusion, with our two flexible and accurate Bayesian limited sampling models for MMF (e.g. with sampling times 0-½-1-2h) based on co-medication with or without calcineurin inhibitors we developed a tool for improving therapeutic drug monitoring based dose guiding of MMF in liver transplant patients. This becomes especially important when one wants to avoid rejection while lowering or discontinuing calcineurin inhibitors in order to improve renal function. Prospective validation and assessment of clinical relevance of our models is planned.

REFERENCES

1. Allison AC, Eugui EM. The design and development of an immunosuppressive drug, mycophenolate mofetil. Springer Semin Immunopathol. 1993;14(4):353-80. Review.
2. Reich DJ, Clavien PA, Hodge EE; MMF Renal Dysfunction after Liver Transplantation Working Group. Mycophenolate mofetil for renal dysfunction in liver transplant recipients on cyclosporine or tacrolimus: randomized, prospective, multicenter pilot study results. Transplantation. 2005 Jul 15;80(1):18-25.
3. Kiberd BA, Lawen J, Fraser AD, Keough-Ryan T, Belitsky P. Early adequate mycophenolic acid exposure is associated with less rejection in kidney transplantation. Am J Transplant. 2004 Jul;4(7):1079-83.
4. Pillans PI, Rigby RJ, Kubler P et al. A retrospective analysis of mycophenolic acid and cyclosporin concentrations with acute rejection in renal transplant recipients. Clin Biochem. 2001 Feb;34(1):77-81.
5. DeNofrio D, Loh E, Kao A et al. Mycophenolic acid concentrations are associated with cardiac allograft rejection. J Heart Lung Transplant. 2000 Nov;19(11):1071-6.
6. Yamani MH, Starling RC, Goormastic M et al. The impact of routine mycophenolate mofetil drug monitoring on the treatment of cardiac allograft rejection. Transplantation. 2000 Jun 15;69(11):2326-30.
7. Wiesner R, Rabkin J, Klintmalm G et al. A randomized double-blind comparative study of mycophenolate mofetil and azathioprine in combination with cyclosporine and corticosteroids in primary liver transplant recipients. Liver Transpl. 2001 May;7(5):442-50.
8. Bilbao I, Castells L, Rojas L et al. Immunosuppression based on mycophenolate mofetil in stable liver transplanted patients. Int Immunopharmacol. 2006 Dec 20;6(13-14):1977-83.
9. Moreno Planas JM, Cuervas-Mons Martinez V, Rubio Gonzalez E et al. Mycophenolate mofetil can be used as monotherapy late after liver transplantation. Am J Transplant. 2004 Oct;4(10):1650-5
10. Fairbanks KD, Thuluvath PJ. Mycophenolate mofetil monotherapy in liver transplantation: a single center experience. Liver Transpl 2004 Sep;10(9):1189-94.
11. Reich DJ, Clavien PA, Hodge EE; MMF Renal Dysfunction after Liver Transplantation Working Group. Mycophenolate mofetil for renal dysfunction in liver transplant recipients on cyclosporine or tacrolimus: randomized, prospective, multicenter pilot study results. Transplantation. 2005 Jul 15;80(1):18-25.
12. van Gelder T, Hilbrands LB, Vanrenterghem Y et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. Transplantation. 1999 Jul 27;68(2):261-6.
13. Arns W, Cibrik DM, Walker RG et al. Therapeutic drug monitoring of mycophenolic acid in solid organ transplant patients treated with mycophenolate mofetil: review of the literature. Transplantation. 2006 Oct 27;82(8):1004-12.

14. Kaplan B. Mycophenolic acid trough level monitoring in solid organ transplant recipients treated with mycophenolate mofetil: association with clinical outcome. Curr Med Res Opin. 2006 Dec;22(12):2355-64. Review.
15. Pawinski T, Hale M, Korecka M, Fitzsimmons WE, Shaw LM. Limited sampling strategy for the estimation of mycophenolic acid area under the curve in adult renal transplant patients treated with concomitant tacrolimus. Clin Chem. 2002 Sep;48(9):1497-504.
16. Shaw LM, Holt DW, Oellerich M, Meiser B, van Gelder T. Current issues in therapeutic drug monitoring of mycophenolic acid: report of a roundtable discussion. Ther Drug Monit. 2001 Aug;23(4):305-15.
17. Filler G. Abbreviated mycophenolic acid AUC from C0, C1, C2, and C4 is preferable in children after renal transplantation on mycophenolate mofetil and tacrolimus therapy. Transpl Int. 2004 Mar;17(3):120-5.
18. Le Guellec C, Bourgoin H, Büchler M et al. Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. Clin Pharmacokinet. 2004;43(4):253-66.
19. Tredger JM, Brown NW, Adams J et al. Monitoring mycophenolate in liver transplant recipients: toward a therapeutic range. Liver Transpl. 2004 Apr;10(4):492-502.
20. Zicheng Y, Weixia Z, Hao C, Hongzhuan C. Limited sampling strategy for the estimation of mycophenolic acid area under the plasma concentration-time curve in adult patients undergoing liver transplant. Ther Drug Monit. 2007 Apr;29(2):207-14.
21. Proost JH. Adaptive control of drug dosage regimens using maximum a posteriori probability Bayesian fitting. Int.J.Clin.Pharmacol.Ther. 1995; 33: 531-536.
22. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J.Pharmacokinet.Biopharm. 1981; 9: 503-512.
23. Borrows R, Chusney G, James A et al. Determinants of mycophenolic acid levels after renal transplantation. Ther Drug Monit. 2005 Aug;27(4):442-50.
24. Langers P, Cremers SC, den Hartigh J et al. Easy-to-use, accurate and flexible individualized Bayesian limited sampling method without fixed time points for ciclosporin monitoring after liver transplantation. Aliment Pharmacol Ther. 2005 Mar 1;21(5):549-57.
25. Yau WP, Vathsala A, Lou HX, Chan E. Is a standard fixed dose of mycophenolate mofetil ideal for all patients? Nephrol Dial Transplant. 2007 Jul 19;[Epub ahead of print].
26. van Hest RM, Mathot RA, Pescovitz MD, Gordon R, Mamelok RD, van Gelder T. Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. J Am Soc Nephrol. 2006 Mar;17(3):871-80.
27. van Hest RM, van Gelder T, Vulto AG, Mathot RA. Population pharmacokinetics of mycophenolic acid in renal transplant recipients. Clin Pharmacokinet. 2005;44(10):1083-96.
28. Kuriata-Kordek M, Boratynska M, Falkiewicz K et al. The influence of calcineurin inhibitors on mycophenolic acid pharmacokinetics. Transplant Proc. 2003 Sep;35(6):2369-71.

29. Cattaneo D, Gaspari F, Ferrari S et al. Pharmacokinetics help optimizing mycophenolate mofetil dosing in kidney transplant patients. Clin Transplant. 2001 Dec;15(6):402-9.
30. de Fijter JW, Mourer JS, den Hartigh J, Mallat MJ, Berger SP. Concentration-controlled systemic exposure provides improved safety after CNI or MMF withdrawal. Presented at American Transplant Congres 2007, San Francisco, USA. Abstract 238.
31. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet. 1998 Jun;34(6):429-55.
32. Flechner SM. Minimizing calcineurin inhibitor drugs in renal transplantation. Transplant Proc. 2003 May;35(3 Suppl):118S-121S.
33. Créput C, Blandin F, Deroure B et al. Long-term effects of calcineurin inhibitor conversion to mycophenolate mofetil on renal function after liver transplantation. Liver Transpl. 2007 Jul;13(7):1004-10.
34. Pfitzmann R, Klupp J, Langrehr JM et al. Mycophenolatemofetil for immunosuppression after liver transplantation: a follow-up study of 191 patients. Transplantation. 2003 Jul 15;76(1):130-6.
35. van Gelder T, Le Meur Y, Shaw LM et al. Therapeutic drug monitoring of mycophenolate mofetil in transplantation. Ther Drug Monit. 2006 Apr;28(2):145-54.
36. van Gelder T, Klupp J, Barten MJ, Christians U, Morris RE. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. Ther Drug Monit. 2001 Apr;23(2):119-28.
37. Smak Gregoor PJ, van Gelder T, Hesse CJ, van der Mast BJ, van Besouw NM, Weimar W. Mycophenolic acid plasma concentrations in kidney allograft recipients with or without cyclosporin: a cross-sectional study. Nephrol Dial Transplant. 1999 Mar;14(3):706-8.
38. Filler G, Zimmering M, Mai I. Pharmacokinetics of mycophenolate mofetil are influenced by concomitant immunosuppression. Pediatr Nephrol. 2000 Feb;14(2):100-4.
39. Patel CG, Harmon M, Gohh RY, Akhlaghi F. Concentrations of mycophenolic acid and glucuronide metabolites under concomitant therapy with cyclosporine or tacrolimus. Ther Drug Monit. 2007 Feb;29(1):87-95.
40. Hesselink DA, van Hest RM, Mathot RA et al. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. Am J Transplant. 2005 May;5(5):987-94.
41. Cremers S, Schoemaker R, Scholten E et al. Characterizing the role of enterohepatic recycling in the interactions between mycophenolate mofetil and calcineurin inhibitors in renal transplant patients by pharmacokinetic modelling. Br J Clin Pharmacol. 2005 Sep;60(3):249-56.
42. de Fijter, JW. Maintenance of total immunosuppression during CNI minimisation. Satellite symposium "Ensuring adequate overall immunosuppression in CNI minimisation protocols", 13th Congress of the European Society for Organ Transplantation, 2007, Prague, Czech Republic. Abstract book p. 10-12.