MONITORING IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION; DEVELOPMENT OF INDIVIDUALIZED BAYESIAN LIMITED SAMPLING MONITORING

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MONITORING IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION; DEVELOPMENT OF INDIVIDUALIZED BAYESIAN LIMITED SAMPLING MONITORING

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CONTENTS

CHAPTER 1

INTRODUCTION

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INTRODUCTION

The first experimental attempts of liver transplantation on dogs were in 1955 by Welch[1]. In 1963 Thomas E. Starzl and colleagues started human liver transplantation[2]. Like the first two of these operations in the Netherlands in Leiden and Arnhem in 1966 and 1968 respectively, these were unsuccesfull auxiliary liver transplantations. Operation technique and medication apparently were not yet ready. After a self-imposed moratorium and more animal experiments Thomas E. Starzl in Denver and also Sir Roy Calne in Cambridge started human orthotopic liver transplantation (OLT) in 1978, and in the Netherlands the fifth center worldwide started in Groningen in 1979 (Gips, Kootstra and Krom). In 1983 at a National Institutes of Health Consensus Development Conference it was decided that liver transplantation was no longer experimental and deserved broader application in clinical practice[3]. Nowadays thousands of OLTs have been performed successfully. The one-year survival is 90% and the 5-year survival over 80% in many centers. This is due to many factors like improved operative technique, better prevention, recognition and treatment of complications, and improved immunosuppression.
The first use of immunosuppressive agents in OLT was in 1966 with a prednisolone and azathioprine schedule derived from the successful kidney transplantations[4]. The breakthrough of the use of immunosuppressive agents in OLT was in 1980, the development of cyclosporine, a calcineurin-inhibitor. Cyclosporine was effective in the prevention of rejection and there was an increase in the survival rate after OLT[5-9]. Later on, other immunosuppressants like tacrolimus (FK-506, another calcineurin inhibitor) and mycophenolate mofetil (MMF) were introduced for prevention of graft-loss due to rejection. With the success of these agents the focus is now shifting towards reduction of side-effects from these drugs, including renal insufficiency from calcineurin inhibitors. Therapeutic drug monitoring (TDM) is an important tool for achieving these goals. This thesis focuses on TDM of cyclosporine, tacrolimus and mycophenolate mofetil after OLT.

Cyclosporine

The drug cyclosporine (Neoral®) is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids. It is produced as a metabolite by the fungus species Beauveria nivea. The effectiveness of cyclosporine results from specific and reversible inhibition of immunocompetent lymphocytes in the G0- and G1-phase of the cell cycle. T-lymphocytes are preferentially inhibited. The T-helper cell is the main target, although the T-suppressor cell may also be suppressed. Cyclosporine also inhibits lymphokine production and release including interleukin-2[10] (Figure 1).

Tacrolimus

Tacrolimus (Prograf®), previously known as FK506, is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e. immunosuppression)[11] (Figure 1).

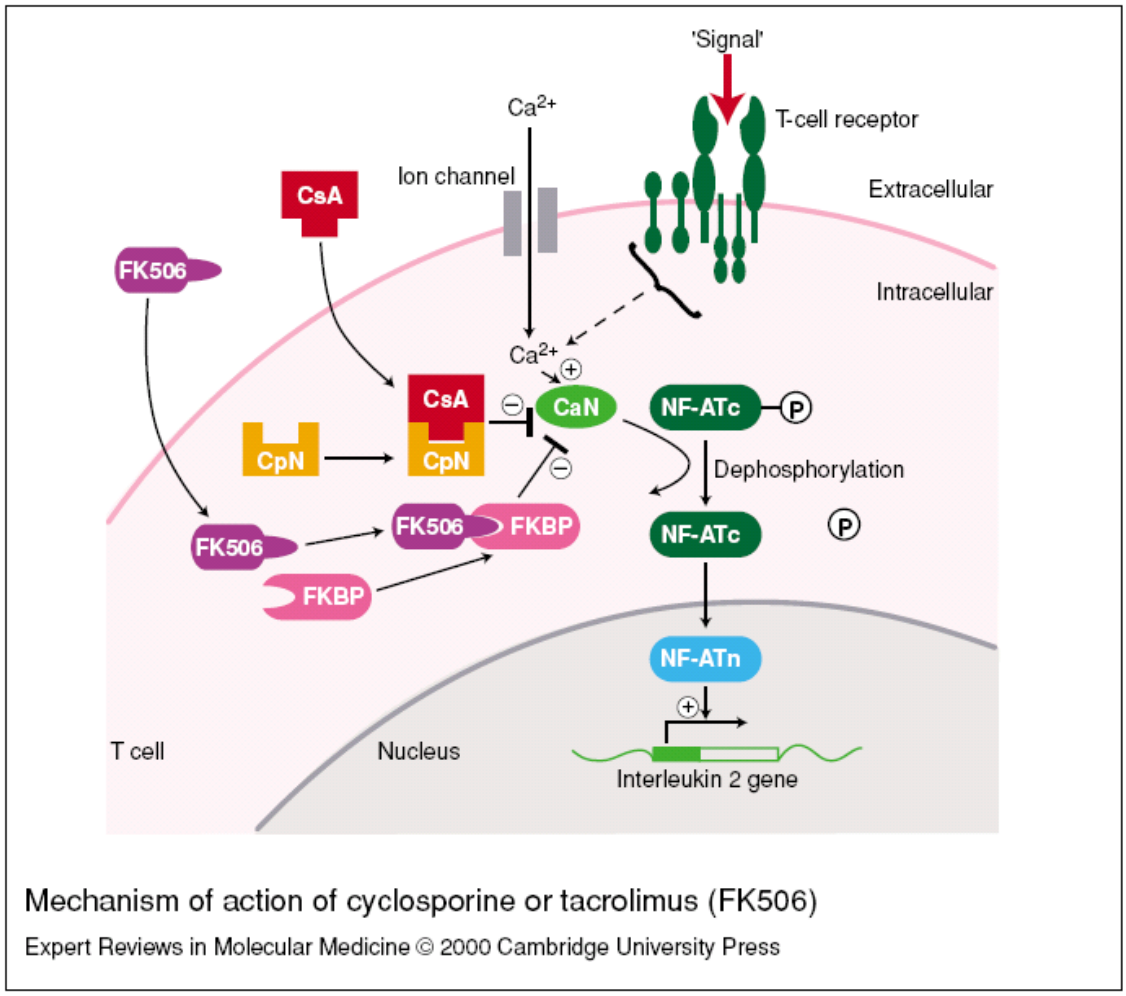

Figure 1: Mechanism of action of cyclosporine and tacrolimus

Mycophenolate mofetil

Mycopheonale mofetil is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent, which is an inosine monophosphate dehydrogenase (IMPDH) inhibitor. Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but did block the coupling of these events to DNA synthesis and proliferation[12] (Figure 2).

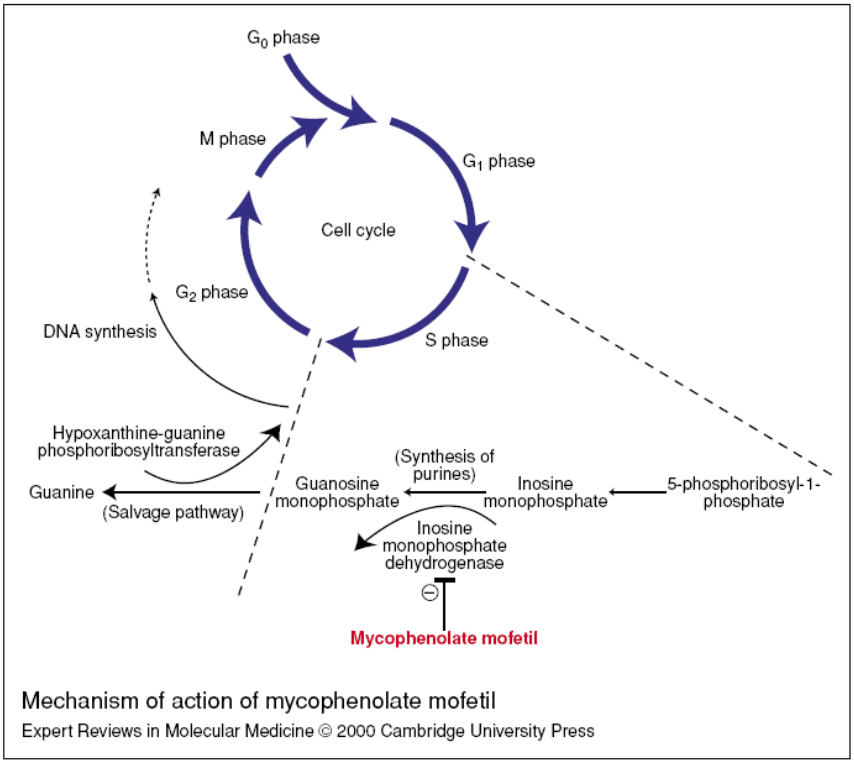

Figure 2: Mechanism of action of mycophenolate mofetil

Therapeutic drug monitoring

Calcineurin inhibitors (cyclosporine and tacrolimus) are characterized by a narrow therapeutic window. Underdosing may lead to acute or chronic rejection of the graft, while overdosing may lead to adverse effects, like elevated blood pressure and nephrotoxicity. Therefore accurate dosing of these drugs is warranted.
When using therapeutic drug monitoring (TDM) dosing is based on measured drug-concentrations in blood. Dependent on these concentrations the dose is adjusted. Especially for medication with a narrow therapeutic range the use of TDM is very useful. This is exactly the reason why in the past decades many studies have been performed to develop different strategies for TDM in organ transplantation.

Trough concentration (C0) monitoring

For many years trough concentration or C0 monitoring was generally accepted as the best way of monitoring cyclosporine and tacrolimus. This means that dose and possible dose adjustments were based on the blood concentration sample just before taking the medication. Both cyclosporine and tacrolimus are mostly dosed twice daily, which means that a predose-level (C0) is taken approximately 12 hours after the last dose. C0-monitoring was proven to be effective in reducing rejections and adverse events. Later, the question arised whether C0-monitoring was the optimal way of therapeutic drug monitoring, particularly for cyclosporine. Studies showed that the correlation of C0 with the area under the concentration time curve for 12 hours (AUC0-12) was poor and that other time sampling points may better reflect systemic exposure of cyclosporine for a dosing interval. Subsequently, a new widely introduced strategy for cyclosporine was C2-monitoring.
For tacrolimus nowadays C0-monitoring is still the common strategy in most clinics.

Fixed dose regimens

In contrast to the calcineurin inhibitors cyclosporine and tacrolimus, there is no consensus on the need for therapeutic drug monitoring of mycophenolate mofetil (MMF). Most centers adhere to fixed dose regimens, which means that dosing is not based on blood concentrations or other clinical properties like weight, co-medication or liver and kidney function. Recently, different strategies were studied including C0-monitoring, but there seemed to be a weak correlation between C0 and AUC.

Limited sampling strategies

The exposition to a drug is determined by the ‘gold standard AUC’. Which is approximated by taking blood samples every hour and integrating these data with the ‘trapezoidal rule’. Next to (fixed) single time points as a basis for therapeutic drug monitoring of immunosuppressive drugs also limited sampling strategies have been

developed in the past decade. This means that multiple blood sampling time points are used in a formula or model as a surrogates for the 'gold standard' AUC0-12h. Most of these strategies are using limited sampling formulas (LSF algorithms). These have the disadvantage that the blood sampling needs to be performed exactly on time, which is difficult in an outpatient clinic.

Modeling based on Bayesian estimation

Few studies have been performed on the development of limited sampling models (LSM) based on Bayesian estimation, a statistical method successfully used in pharmacy but also other fields of medicine. The advantage of these models is that they are flexible, accurate and easy to apply in practice without the need to take blood samples exactly on time. As long as the sampling time is noted, these limited sampling models (LSMs) are accurate, in contrast to the rigid limited sampling formulas (LSFs), if blood is not drawn exactly on time.

Aim of the thesis

In this thesis we try to optimize the therapeutic drug monitoring of cyclosporine, tacrolimus and mycophenolate mofetil in liver transplant patients with limited sampling strategies and modelling, using Bayesian estimation.

Recent literature from studies -more performed in kidney than liver transplantation- suggested that a new way of monitoring cyclosporine in organ transplantation patients (C2-monitoring) better predicted the systemic exposure to the drug over the first 12 hours after dosing than C0-monitoring did, which may lead to improved clinical outcome[13-26]. C2 was then recommended for monitoring cyclosporine. Due to this recommendation in **chapter 2** we switched our stable patients more than 6 months after OLT from C0-monitoring towards C2-monitoring and investigated the influence of this switch on factors as dose, creatinine clearance (CRCL), blood pressure and freedom from rejection and the relationships of C0 and C2 with the gold standard AUC0-12h.

In **chapter 3** we were looking for even better methods for monitoring cyclosporine[27]. We developed and validated an easy to use, accurate and flexible individualized Bayesian population-pharmacokinetic (POP-PK) limited sampling model (LSM) integrating all available information, without the need for fixed blood sampling time points. Different limited sampling models were tested and the correlation of these models with the 'gold standard' AUC0-12h was calculated, in order to predict the systemic exposure of cyclosporine very precisely with a limited number of blood samples.

The limited sampling model with time points 0 + 1 + 2 + 3h was then introduced into our clinic[28]. In **chapter 4** we evaluated the patients who were previously switched from C0 to C2 and now switched to LSM 0,1,2,3h after using this model in our clinic for over

18 months. This allowed us to investigate the feasibility of implementation of LSM in practice, and the potential effects on factors as dose, renal function and rejection rate of the three monitoring strategies, and also inter- and intrapatient variability in pharmacokinetics of cyclosporine using LSM. We determined the required precision of the method used and a new target range for cyclosporine AUC was calculated.

Another frequently used calcineurin inhibitor, tacrolimus, is just as cyclosporine characterized by a narrow therapeutic range. This underlines the need of accurate monitoring to prevent rejection and adverse events for this drug as well. The monitoring of tacrolimus is still based on C0-monitoring in most centres. Recent data showed that other blood sampling time points than C0 may better reflect systemic exposure to tacrolimus[29-32]. In **chapter 5** we examined which single time point or combination of time points best reflect systemic exposure to tacrolimus, estimating the area under the concentration time curve. We calculated limited sampling formulas and developed a new and flexible limited sampling model for monitoring tacrolimus concentration which is easy to apply in the outpatient clinic, as we did earlier for cyclosporine[28].

Mycophenolate mofetil (MMF) is increasingly used after OLT, since in contrast to calcineurin inhibitors (CNI) like cyclosporine and tacrolimus MMF is not nephrotoxic. It may allow CNI reduction or discontinuation, resulting in improvement or stabilization of renal function[33]. Most clinics adhere to a fixed dose of MMF, not based on any individual patient or population characteristics[34]. Recent studies with conflicting results and limitations have been performed to explore current evidence and clinical relevance of TDM (C0 and limited sampling strategies) of MMF[35-40]. Limited information on this is available in liver transplant patients[41-42]. In **chapter 6** we described the pharmacokinetic behaviour of MMF in stable liver transplant patients and looked at possible relationships of albumin concentration, creatinine clearance and co-medication (especially calcineurin inhibitors) with MPA clearance, the active metabolite of MMF. Furthermore we investigated the correlation of C0 with AUC0-12h and possible interpatient variability. Finally we developed different limited sampling models for implementation of therapeutic drug monitoring of MMF in liver transplant patients, with special attention to kidney function in patient selection.

In **chapter 7** we summarize the results of our studies and we discuss the possible role of our findings for clinical practice, now and in the future.

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CHAPTER 2

SWITCHING MONITORING OF EMULSIFIED CYCLOSPORINE FROM TROUGH LEVEL TO 2-HOUR LEVEL IN STABLE LIVER TRANSPLANT PATIENTS

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ABSTRACT

Background: After orthotopic liver transplantation (OLT) many patients use emulsified cyclosporine. Recent data showed that blood levels 2 hours after dosing (C-2) better reflect systemic exposure to the drug (area under the blood concentration time curve) than trough levels (C-0) do.

Methods: We investigated difference in dosage, creatinine clearance (CrCl), blood pressure (BP), freedom from rejection, and relation of C-2, C-0, and AUC while switching 31 stable patients more than 6 months after OLT from C-0 to C-2 monitoring. With C-0 between 90 and 150 ng/ml we collected 24-hour urine, while blood samples were taken at t = 0, 1, 2, 3, 4, 6 and 8 hours after dosing to measure cyclosporine, creatinine, liver tests, and blood pressure and calculated AUC and CrCl. Target AUC was calculated based on C-0. Then the dose was adjusted to two subsequent C-2 values of 600 ng/ml ± 15%, the above was repeated, and the differences were assessed.

Results: Cyclosporine dose was reduced in 21/31 patients (68 %) and remained unchanged in 10/31 (32%) after conversion. Mean lowering was 69 mg daily (26.9 %, $P < 0.0001$). After dose reduction the mean increase of CrCl was 7.93 ml/min (11.6 %, $P = 0.016$). Only systolic and mean morning BP decreased slightly but significantly. C-2 correlated better with AUC0-12 ($r^2=0.75$) than C-0 ($r^2=0.64$). However, 13/21 patients had a second AUC below target AUC and 2 of these 13 patients developed rejection after conversion to C-2 levels.

Conclusion: While C-0 monitoring frequently results in overdosing and more renal dysfunction, C-2 monitoring may lead to episodes of underdosing and rejection. Therefore better ways of monitoring cyclosporine dosing need to be devised.

INTRODUCTION

After orthotopic liver transplantation (OLT) many centers use the microemulsion formulation of cyclosporine (Neoral®) as immunosuppressant[1]. There is a small therapeutic window between too low systemic exposure to the drug, resulting in rejection, and too high systemic exposure, leading to adverse effects such as renal insufficiency and elevated blood pressure. Usually Neoral is given twice daily. Until recently dosage was based on trough-level (C-0) monitoring. Recent data, however, mostly derived from kidney transplantation but also from heart, lung and liver transplantation, show that blood levels 2 hours after dosing (C-2), better than trough levels reflect the systemic exposure over the first 12 hours after dosing (= AUC as gold standard)[2-5]. Based on these and other studies it has been recommended that monitoring based on trough levels should be replaced by monitoring based on C-2 levels both for initial therapy and for maintenance tretment[6,7]. However, only limited data have been published on the results of C-2 monitoring in liver transplantation[8-15]. In the present study we investigated the possible influence of the conversion from C-0 monitoring to C-2 monitoring in stable patients more than 6 months after liver transplantation in the dose, creatinine clearance (CrCl), blood pressure, and freedom from rejection, with the hypothesis that there was no such influence. Furthermore, we calculated the AUC before and after this change in monitoring, and we investigated relationships between blood concentrations at 0 and 2 hours and systemic exposure to the drug.

PATIENTS AND METHODS

The study included 31 stable patients who were at least 6 months post-OLT (21 men, mean age 52, range 31-64 years; 10 women, mean age 39, range 20-58 years). One patient had a biliodigestive (Roux-en-Y) anastomosis, and 30 patients had a duct-to-duct choledochus anastomosis. All patients received Neoral cyclosporine (Neoral) twice daily and were maintained on a stable Neoral dose with two consecutive trough levels (C-0) between 90 and 150 ng/ml before entering the study. Co-medication consisted of mycophenolate mofetil in 9 patients (4 with prednisone), azathioprine in 8 patients (4 with prednisone), and prednisone alone in 8 patients; 6 patients had no immunosuppressive co-medication.

During the day of the AUC, 24-hour urine was collected for measurement of creatinine concentration. Five minutes before the morning dose (approximately 10:00 AM) of Neoral (t = 0), blood samples were taken for liver and kidney function and Neoral concentration.

Further blood samples for Neoral concentration were taken 1, 2, 3, 4, 6 and 8 hours after the morning dose of Neoral. For t = 12 we took the trough level (t = 0), because all our patients were dosed with Neoral twice daily. Blood was taken using an indwelling catheter and was collected in a vacutainer containing EDTA. Whole blood Neoral concentrations were determined by Fluorescence Polarisation Immuno Assay (FPIA, Axsym, Abbott Diagnostics, Abbott Park, IL). In order to avoid an influence (however small) from meals, the patients were instructed to take only a light breakfast with tea and a biscuit on the morning of measuring the AUC, and until the 2-hour sample (C-2) was taken, the patients took no additional food or drinks[16]. Between t = 1 and t = 2 and between t = 6 and t = 8, blood pressure was measured automatically (Dynamap) for one-half hour (morning BP and afternoon BP) with the patient in a reclining chair. Then, according to the recommendations by E. Cole et al.[6], the dose was adjusted to a Neoral level at t = 2 (C-2, peak level) within the target range of 510 and 690 ng/ml (600 ± 15%) using the formula: new dose = old dose x (600/ C-2). Two weeks after the day the first AUC was measured while on C-0 monitoring ("day 1") and the contingent adjustments, the patients came to the clinic for a checkup and a blood sample, which was taken exactly two hours after the morning dose of Neoral (C-2). Further dose adjustments were made using the same formula within weeks. Blood pressure medication was not adjusted during the study. When two subsequent C-2-values were within the target-range, patients were invited for a second day, when the AUC was measured ("day 2") similar to the first "AUC-day" ("day 1"). Again 24-hour urine was collected for the creatinine concentration and blood samples were taken for liver and kidney function tests. The $AUC_{0\text{-}12h}$ of all 62 (2 x 31) curves was calculated using the trapezoidal rule[17], and relationships with C-0 and C-2 were investigated. Differences in second and first C-0, C-2 and AUC and their relation, and changes in renal function, liver functions, and blood pressure were assessed. The "target AUC range" was calculated based on the C-0 range of 90-150 ng/ml, using the linear regression line formula describing the relation of C-0 with $AUC_{0\text{-}12h}$.

Statistical Analysis

Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). Results are expressed as mean ± SEM and as median and range (Wilcoxon-test). Potential differences were explored with Paired-Samples T-test, and relationships were investigated using Pearson correlation test and linear regression analysis. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Dose Adjustments

Of the 31 patients 21 (68%) needed a lower dose of Neoral when dosing was based on C-2 monitoring instead of C-0 monitoring. In 10 patients (32%) no change in the dosage of Neoral was necessary and none of the patients required a higher dosage after conversion to C-2 monitoring. In patients in whom the dose was lowered, the dose on day 2 (median 200 mg, range 150-250 mg) was significantly lower than the dose on day 1 (median 250 mg, range 200-350 mg), reduction of 26.9 % of initial dose, $P < 0.0001$, Fig. 1.

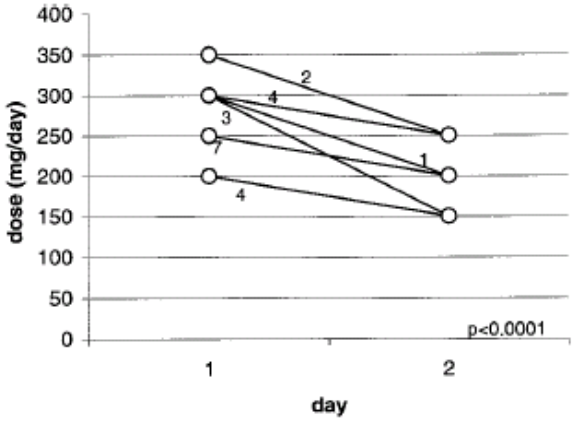

Figure 1. The dose of Neoral on day 1 (269 ± 10 mg) and day 2 (198 ± 9 mg) for patients whose dose was reduced (n = 21). The numbers represent the number of patients per line.

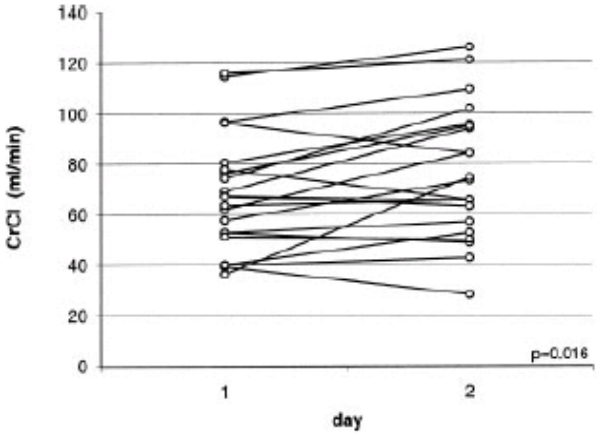

Figure 2. The creatinine clearance (CrCl) on day 1 (68.2 ± 5.1 ml/min) and day 2 (76.1 ± 5.7 ml/min) for patients with dose reduction (n = 21).

Kidney Function and Blood Pressure

Of the 21 patients whose dose was lowered, we calculated the creatinine clearance (CrCl) before (day 1) lowering and after (day 2) lowering of the dose. The mean increase of the CrCl in these patients was 7.93 ± 3.0 ml/min (11.6% of initial CrCl, $P = 0.016$, Fig. 2). The change in systolic blood pressure (morning and afternoon) was – 4.1 ± 1.6 mmHg and +1.52 ± 1.95 mmHg (– 3.1 % and +1.2%, $P = 0.018$ and $P = 0.444$). The change in diastolic blood pressure (morning and afternoon) was – 1.33 ± 0.98 mmHg and +0.048 mmHg ± 1.26 (–1.6 % and 0.00 %, $P = 0.188$ and $P = 0.970$). The differences in the mean arterial pressure (morning and afternoon) were – 2.62 ± 1.09 mmHg and 0.00 ± 1.52 mmHg (– 2.6 % and 0.00 %, $P = 0.026$ and $P = 1.000$) respectively.

Estimation of Systemic Exposure (AUC) while on C-2 Monitoring versus AUC while on C-0 Monitoring

C-2 monitoring correlated better ($r^2 = 0.75$, Fig. 3) than the C-0 monitoring ($r^2 = 0.64$, Fig. 4) with the area under the curve (AUC0-12h). The mean AUC on day 1 was 4588 ± 171 µg.h/L, median 4229 µg.h/L, range 3261–6423 µg.h/L. The mean AUC on day 2 was 3210 ± 117 µg.h/L, median 3195 µg.h/L, range 2380-4096 µg.h/L, $P < 0.0001$ (Fig. 5). Figure 6 shows the difference of C-0 values on the first and the second day ($P < 0.0001$).

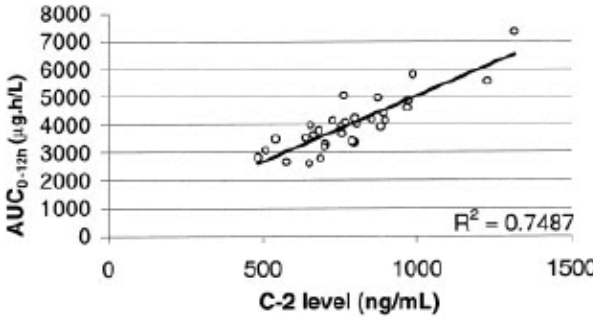

Figure 3. The correlation of the Neoral concentration 2 hours after dosing (C-2) with the area under the curve from 0 to 12 hours after dosing (AUC_{0-12h}) on day 2.

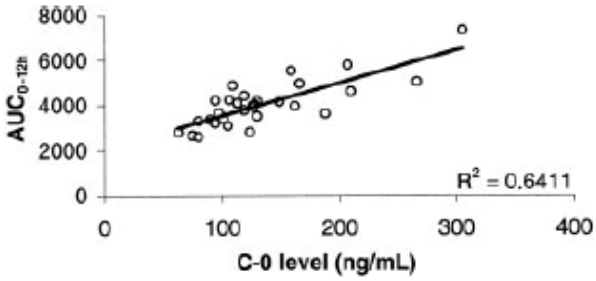

Figure 4. The correlation of the trough-Neoral concentration (C-0) with the area under the curve from 0 to 12 hours after dosing (AUC_{0-12h}) on day 1.

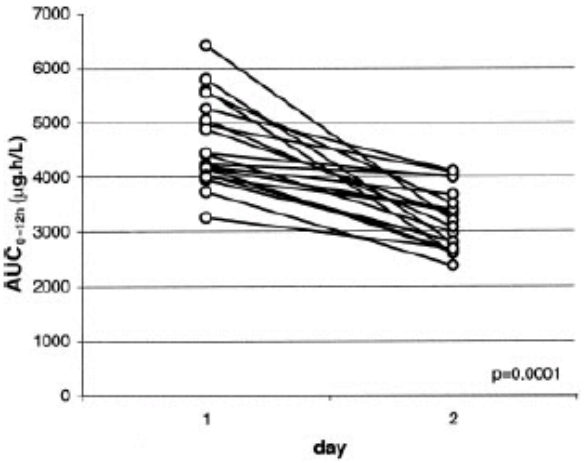

Figure 5. The AUC_{0-12h} of Neoral on day 1 (4588 ± 171 μg.h/L) and day 2 (3210 ± 117 μg.h/L) in patients whose Neoral dose was lowered (n = 21).

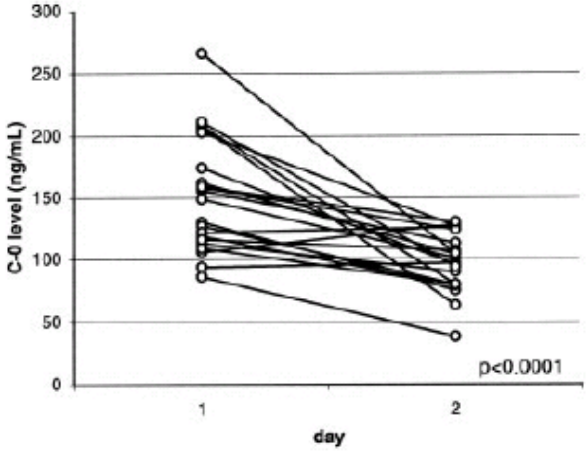

Figure 6. The C-0 of Neoral on day 1 (mean, 151 ± 10 ng/mL) and day 2 (97 ± 5 ng/mL) in patients whose dose was reduced (n = 21).

C-2 Values on Day 1 and Day 2 in Relation to C2 Target Range

As mentioned above, while on C-0 monitoring, C-2 was above the target C-2 in 21/31 patients. In 10/21 patients whose Neoral dose was lowered there were variable C-2 levels; C-2 was outside the target range on day 2 with the same dose after two subsequent C-2 values of 600 ng/mL ± 15%. Mean C-2 value in the 21 patients whose dose was lowered was 666 ± 23 ng/mL (Fig. 7); however, on day 2 just 1/21 of C-2-values was below the target range (C-2 = 485ng/mL) and 9/21 were above the C-2 target range (mean of these 9: 765 ± 20 ng/mL). Also, 7/10 patients with an unchanged Neoral dose had variable C-2 levels with values of C-2 outside the C-2 target range on day 2 (the second "AUC-day").

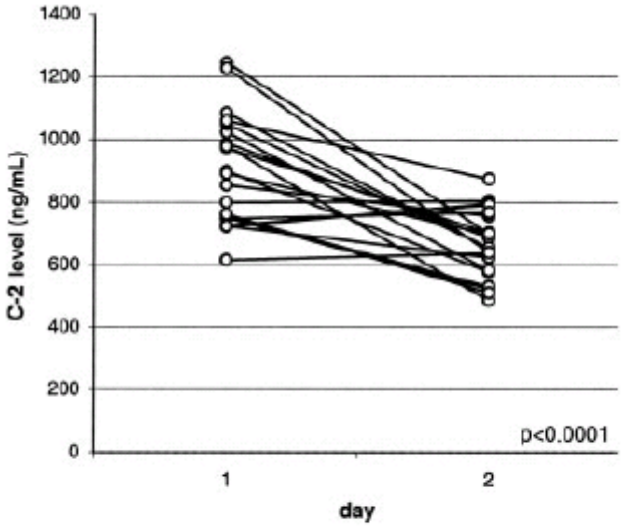

Figure 7. The C-2 of Neoral on day 1 (mean, 898 ± 38 ug/L) and day 2 (666 ± 23 ng/mL) in patients whose dose was reduced (n = 21).

AUC on Day 2 in Relation to Target AUC

We found that 13/21 patients whose Neoral dose was lowered ended below the "target AUC" and were therefore below the lowest exposure on C-0 monitoring. This target AUC is based on the C-trough (C-0) and was calculated with linear regression analysis (Fig. 4). The formula of the line is:

AUC0-12h = 14.75 x C-trough + 2053 (trendline).

The target range of the trough-levels is 90 - 150 ng/mL; therefore, the AUC target range is 3380 - 4266 µg.h/L. The other 8/21 patients showed a second AUC within the range of the target AUC. As expected, no patient whose Neoral dose was lowered had an AUC on day 2 above the highest AUC on day 1.

C-2 and AUC on Day 2 in Relation to Each Other and in Relation to the Target Ranges

Table 1 shows the C-2 and AUC0-12h of Neoral on day 2 in relation to the target ranges of C-2 and AUC0-12h in the patients in whom the dose was lowered (n = 21). Mean AUC on day 2 was 3543 ± 109 µg.h/L. Of those patients in whom the Neoral dose was lowered and whose second AUC was below the target AUC, 2/13 developed acute cellular rejection with aminotransferases up to 500 U/L, requiring additional corticosteroids and an increase in Neoral dose after the second AUC. These 2 patients were 9 and 10 months after OLT; both had prednisone as co-medication and one also had mycophenolate mofetil (MMF) as co-medication. Of the 31 patients, 4 were within 6 - 12 months after OLT; the low AUCs were not limited to these 4 patients. However, the two patients experiencing rejection were among these 4 patients.

Table 1. The C-2 and $AUC_{0\text{-}12h}$ of Neoral on Day 2 in Relation to the Target Ranges of C-2 and $AUC_{0\text{-}12h}$ in the Patients in Whom the Dose Was Lowered (n = 21)

	AUC Below Target Range (<3380 µg.h/L)	AUC in Target Range (3380–4266 µg.h/L)	AUC Above Target Range (>4266 µg.h/L)	Total
C-2 below target range (<510 ng/mL)	1	0	0	1
C-2 in target range (510–690 ng/mL)	8	3	0	11
C-2 above target range (>690 ng/mL)	4	5	0	9
Total	13	8	0	21

In order to reach the subsequent C-2 values of 600 ng/mL ± 15 %, we needed 1.57 ± 0.19 (median 1.00; range 1-3) dose adjustments. Patients with the peak level at 1 hour after dosing had an AUC within the target range as often as did patients with the peak level at 2 hours post-dosing. Table 2 shows the C-2 and AUC0-12h of Neoral on day 2 in relation to the target ranges of C-2 and AUC0-12h in the patients whose dose was not changed (n=10).

Table 2. The C-2 and $AUC_{0\text{-}12h}$ of Neoral on Day 2 in Relation to the Target Ranges of C-2 and $AUC_{0\text{-}12h}$ in the Patients Whose Dose Was Not Changed (n = 10)

	AUC Below Target Range (<3380 µg.h/L)	AUC in Target Range (3380–4266 µg.h/L)	AUC Above Target Range (>4266 µg.h/L)	Total
C-2 below target range (<510 ng/mL)	0	0	0	0
C-2 in target range (510–690 ng/mL)	0	3	0	3
C-2 above target range (>690 ng/mL)	1	3	3	7
Total	1	6	3	10

Differences between Subgroups of Patients

Because only 1 patient with a hepaticojejunostomy was included, no differences between this patient and the other 30 with a duct-to-duct anastomosis could be assessed. No differences in C-2 or AUC of patients with different immunosuppressive co-medications were found, although the number of patients is too small to reliably assess differences between these groups.

Sparse Sampling and AUC0-12h

If AUC is calculated, using the trapezoidal rule, from cyclosporine levels on time points 0, 1, 2, and 3 hours, the correlation with AUC0-12h was $r^2 = 0.96$.

DISCUSSION

During the conversion from C-0 to C-2 cyclosporine monitoring in stable patients more than 6 months after liver transplantation, we saw a significant decrease in cyclosporine dose in two-thirds and an unchanged dose in one-third of the patients. Dose reduction resulted in lower systemic exposure and an improvement of renal function, but only small changes in morning systolic and mean morning blood pressures were observed, with questionable clinical significance. The fact that the kidney function did not improve in all patients may be due to long-term exposure to Neoral, which may have caused a fixed renal insufficiency. Also, further improvement in renal function may require more time. Based on calculating the area under the curve from 0 to 12 hours (cyclosporine blood levels), the correlation of C-2 with AUC was better than the correlation of C-0 with AUC from 0-12 hours. However, in almost one-half of the patients, there was significant intrapatient variability of the C-2 blood levels with the same dose. This made therapeutic drug monitoring with C-2 levels less accurate and may induce many unnecessary subsequent changes in drug dose, which is inconvenient for patients, doctors, and nurses. We found it disturbing that, although two preceding C-2 levels were within the 600 ng/mL ± 15% range, in 13/21 patients whose dose was lowered the second AUC was below the target AUC, while indeed 2 of these 13 patients developed rejection. The fact that these patients were 9 and 10 months post OLT may mean that the dose recommendations of G. Levy and not those of E. Cole should be followed when using C-2 monitoring[6,7]. Further investigations assessing this point may be needed. While on C-2 monitoring, 17/31 patients had a second AUC outside the target AUC. For all patients it may not be necessary to have an AUC within the range of the "target range AUC", but it certainly seems safer if this is the case. Probably the best situation is to have an AUC on day 2 in the lower half of first AUCs, which is

3380 – 3823 µg.h/L. Because 11/13 patients with a second AUC below the target AUC did not develop rejection, some patients may tolerate lower AUCs.
Other studies saw a better correlation of C-2 with AUC when compared to trough-level monitoring in renal and liver graft recipients[3-15]. Most studies in renal transplantation and the limited studies in liver transplantation using C-2 monitoring also showed improved kidney function, and often blood pressure and serum cholesterol also improved. In those studies no rejection occurred despite lower exposure to cyclosporine. However, in the liver transplant studies mentioned AUC was calculated by measuring Neoral blood levels during 4 and 6 hours only, while we used 0-12 hour AUCs. This fact may explain part of the difference between these and our studies. Another explanation may be the lower maintenance levels used in liver transplantation when compared to kidney transplantation: further lowering of the dose may more easily lead to rejection. All samples were taken as recommended[6,7,18] and within 2 minutes from the targeted time (although 10 minutes are allowed); if sampling time would have been more variable (as may be the case in daily practice), an even lower accuracy of C-2 monitoring and inappropriate dose adjustments might occur[19]. In renal transplantation variable cyclosporine levels may contribute to chronic rejection[20]. Although chronic ductopenic rejection has become less common after liver transplantation in the past decade, it forms a continuum with acute cellular rejection; chronic underexposure to cyclosporine can be a cause[21-24]. In renal transplant studies it was shown that absorption profiling over the first 4 hours was superior to trough-level monitoring, with C-2 as the best single-point predictor of AUC[3,25-28]. The clinical superiority of such absorption profiling over C-2 levels has not been examined in those studies. Our data demonstrate that in stable liver transplant patients trough-level monitoring frequently leads to overdosing of cyclosporine, while monitoring by C-2 may cause episodes of underdosing. Therefore, better ways of monitoring cyclosporine dosing in liver transplantation remain to be devised. Because both IL2 blood concentration and 12-hour AUC are related to cyclosporine exposure in the first 4 hours after dosing it seems logical to use a sparse-sampling method over the first hours after dosing. In accordance with others, our data demonstrate that, if AUC is calculated from cyclosporine levels, using the trapezoidal rule, in the first three hours after dosing the correlation with $AUC_{0\text{-}12h}$ is 0.96[25, 29]. Thus use of this method may avoid over- and underdosing and unnecessary changes in dose. A disadvantage is the need for fixed time points. The ideal model should be easy to use and flexible, without the rigid time points used in current multiple-sampling methods, and it should be based both on population kinetics and on individual pharmacokinetics[30-34]. We are currently developing such a model.

In conclusion, while C-0 monitoring frequently results in overdosing and more renal dysfunction, C-2 monitoring may lead to episodes of underdosing and rejection. Therefore, better ways of monitoring cyclosporine dosing need to be devised.

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CHAPTER 3

EASY-TO-USE, ACCURATE AND FLEXIBLE INDIVIDUALIZED BAYESIAN LIMITED SAMPLING METHOD WITHOUT FIXED TIME POINTS FOR CICLOSPORIN MONITORING AFTER LIVER TRANSPLANTATION

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ABSTRACT

Background: New methods to estimate the systemic exposure to ciclosporin such as the level 2 h after dosing and limited sampling formulas may lead to improved clinical outcome after orthotopic liver transplantation. However, most strategies are characterized by rigid sampling times.
Aim: To develop and validate a flexible individualized population-pharmacokinetic model for ciclosporin monitoring in orthotopic liver transplantation.
Methods: A total of 62 curves obtained from 31 patients at least 0.5 year after orthotopic liver transplantation were divided into two equal groups. From 31 curves, relatively simple limited sampling formulas were derived using multiple regression analysis, while using pharmacokinetic software a two-compartment population-pharmacokinetic model was derived from these same data. We then tested the ability to estimate the AUC by the limited sampling formulas and a different approach using several limited sampling strategies on the other 31 curves. The new approach consists of individualizing the mean a priori population-pharmacokinetic parameters of the two-compartment population-pharmacokinetic model by means of maximum a posteriori Bayesian fitting with individual data leading to an individualized population-pharmacokinetic limited sampling model. From the individualized pharmacokinetic parameters, AUC0-12h was calculated for each combination of measured blood concentrations. The calculated AUC0-12h both from the limited-sampling formulas and the limited-sampling model were compared with the gold standard AUC0-12h (trapezoidal rule) by Pearson's correlation coefficient and prediction precision and bias were calculated.
Results: The AUC0-12h value calculated by individualizing the population-pharmacokinetic model using several combinations of measured blood concentrations: 0 + 2 h ($r^2 = 0.94$), 0 + 1 + 2 h ($r^2 = 0.94$), 0 + 1 + 3 h ($r^2 = 0.92$), 0 + 2 + 3 h ($r^2 = 0.92$) and 0 + 1 + 2 + 3 h ($r^2 = 0.96$) had excellent correlation with AUC0-12h, better than limited sampling formulas with less than three sampling time points. Even trough level with limited sampling method ($r^2 = 0.86$) correlated better than the level after 2 h of dosing ($r^2 = 0.75$) or trough level ($r^2 = 0.64$) as single values without limited sampling method. Moreover, the individualized population-pharmacokinetic model had a low prediction bias and excellent precision.
Conclusion: Multiple rigid sampling time points limit the use of limited sampling formulas. The major advantage of the Bayesian estimation approach presented here, is that blood sampling time points are not fixed, as long as sampling time is known. The predictive performance of this new approach is superior to trough level and that after 2 h of dosing and at least as good as limited sampling formulas. It is of clear advantage in busy outpatient clinics.

INTRODUCTION

After orthotopic liver transplantation (OLT), generally, the microemulsion formulation of ciclosporin (Neoral) (CYCLO) is used as the immunosuppressant[1]. There is a small therapeutic window between too low a systemic exposure to the drug resulting in rejection on the one hand and, too high a systemic exposure, leading to adverse effects like renal insufficiency and elevated blood pressure on the other. Usually CYCLO is given twice daily. Until recently, dosage was based on trough-level (C-0) monitoring. Recent data, however – mostly derived from kidney transplantation but also from heart, lung and liver transplantation – show that blood levels 2 h after dosing (C-2) reflect the systemic exposure over the first 12 h after dosing (AUC as gold standard), better than trough levels[2–5]. Based on these and other studies, it has been recommended to replace monitoring based on trough levels by the one based on C-2 levels both for initial therapy and for maintenance treatment[6,7]. However, only limited data have been published on the results of C-2 monitoring in liver transplantation[8–14]. We recently reported that C-0 monitoring resulted in overdosing in two-thirds of the patients, while conversion to C-2 monitoring may lead to episodes of underdosing and rejection, although the average kidney function improved[15]. In the current study, we develop and validate an easy-to-apply limited sampling method (LSM) based on an individualized Bayesian population-pharmacokinetic (POP-PK) model for monitoring CYCLO dosing after liver transplantation, integrating all available information. In contrast to previously published Bayesian methods and limited sampling formulas (LSFs), sampling times are less fixed in our individualized POP-PK model.

PATIENTS AND METHODS

Thirty-one stable patients who were at least 6 months post-OLT (21 men, mean age 52 years, range 31–64; 10 women, mean age 39 years, range 20–58) were included. One patient had a biliodigestive (Roux-en-Y) anastomosis, and 30 had duct-to-duct choledochal anastomoses. All patients received Neoral (CYCLO; Novartis, Basel, Switzerland) twice daily and were maintained on a stable CYCLO dose with two consecutive trough levels (C-0) between 90 and 150 μg/L before entering the study. Co-medication consisted of mycophenolate mofetil in nine patients (four with prednisone), azathioprine in eight patients (four with prednisone), prednisone alone in eight patients, while six patients had no immunosuppressive co-medication. Five minutes before the morning dose (approximately 10:00 hours) of CYCLO (t = 0), blood samples were analysed for liver and kidney function and CYCLO concentration. Further blood samples for CYCLO concentration were taken 1, 2, 3, 4, 6 and 8 h after the

morning dose of CYCLO. For t = 12, we took the trough level (t = 0), as all our patients were treated with CYCLO twice daily. We previously determined that concentrations at 0 and 12 h were equal in these patients. Blood was taken using an indwelling catheter and was collected in a vacutainer containing ethylenediaminetetraacetic acid (EDTA). Whole-blood CYCLO concentrations were determined by fluorescence polarization immunoassay (FPIA, Axsym; Abbott Diagnostics, Abbott Park, IL, USA). In order to avoid an influence (however small) of meals, the patients were instructed to take only a light breakfast with tea and a biscuit on the morning of measuring the AUC, and until the 2-h sample (C-2) was taken, the patients took no additional food or drinks[16]. The blood pressure was measured once in the morning and once in the afternoon for half an hour. Then, according to the recommendations by Cole et al[6]. the dose was adjusted to a CYCLO level at t = 2 (C-2, peak level) within the target range of 510 and 690 µg/L (600 ± 15%) using the formula: new dose = old dose * (600/C-2). Two weeks after the day the first AUC was measured while on C-0 monitoring (day 1) and the contingent adjustments the patients came to the clinic for checkup and a blood sample was taken exactly 2 h after the morning dose of CYCLO (C-2). Further dose adjustments were made within weeks using the same formula. When two subsequent C-2 values were within the target range, patients were invited for a second AUC measurement (day 2) similar to the first 'AUC day' (day 1). The 'gold standard' AUC0-12h of all 62 (2 x 31) curves was calculated using the trapezoidal rule[17]. Relationships with C-0 and C-2 were investigated. Differences in second and first C-0, C-2 and AUC and their relation, and changes in renal function, liver functions and blood pressure were assessed. The 'target AUC range' was calculated based on the C-0 range of 90–150 µg/L, using the linear regression line formula describing the relation of C-0 with AUC0-12h for all 62 curves.

Development of limited sampling methods

We sorted the 62 curves using AUC and divided them into two groups of 31 curves, based on almost similar values of the AUCs. One group of 31 curves was used for calculation of LSFs and for the development of a POP-PK model with a priori POP-PK parameters. This POP-PK model after individualization was also termed as limited sampling model (LSM). The second group of 31 curves was used for validation of the POP-PK model.

Calculation of limited sampling formulas

Using multiple regression analysis, simple LSFs were calculated from 31 curves based on one or a combination of measured blood concentrations. Their ability to estimate the AUC was tested on the remaining 31 curves. The formulas for 0 h; 1 h; 2 h; 3 h; 0 + 1 h; 0 + 2 h; 0 + 3 h; 0 + 1 + 2 h; 0 + 1 + 3 h; 0 + 2 + 3 h; and 0 + 1 + 2 + 3 h are shown in Table 1.

Table 1. Limited Sampling formulas calculated using multiple regression analysis

Time points blood sampling	Formula
LSF0h	$AUC_{0\text{-}12h} = 1670.891 + 17.551 * C0h$
LSF1h	$AUC_{0\text{-}12h} = 2994.588 + 1.136 * C1h$
LSF2h	$AUC_{0\text{-}12h} = 1380.903 + 3.161 * C2h$
LSF3h	$AUC_{0\text{-}12h} = 1395.147 + 4.805 * C3h$
LSF01h	$AUC_{0\text{-}12h} = 947.219 + 17.035 * C0h + 0.976 * C1h$
LSF02h	$AUC_{0\text{-}12h} = 765.333 + 11.174 * C0h + 2.147 * C2h$
LSF03h	$AUC_{0\text{-}12h} = 1023.365 + 10.544 * C0h + 2.944 * C3h$
LSF012h	$AUC_{0\text{-}12h} = -69.067 + 10.307 * C0h + 1.067 * C1h + 2.249 * C2h$
LSF013h	$AUC_{0\text{-}12h} = -148.704 + 7.783 * C0h + 1.324 * C1h + 3.809 * C3h$
LSF023h	$AUC_{0\text{-}12h} = 746.334 + 10.681 * C0h + 1.966 * C2h + 0.433 * C3h$
LSF0123h	$AUC_{0\text{-}12h} = -253.387 + 8.142 * C0h + 1.202 * C1h + 1.509 * C2h + 1.803 * C3h$

A priori POP-PK parameters

Using the Kinpop module of the pharmacokinetic software package MW\Pharm version 3.33 (Mediware, Groningen, the Netherlands), a population two-compartment model (POP-PK model) with a lag-time and first-order absorption pharmacokinetics was calculated from the CYCLO dosing, body weight and the blood concentration values of the 31 curves. This program uses an iterative two-stage Bayesian procedure, and calculates mean, median and standard deviation values of the pharmacokinetic parameters[18]. During the iterative two-stage Bayesian procedure, pharmacokinetic parameters were set to be distributed log-normally, and bioavailability was fixed at 0.5. A POP-PK model was calculated using the 31 blood concentration–time curves. This a priori model acts as a starting point to calculate values for each patient from the available patient-specific data and the a priori population model, leading towards an individualized PK model, indicated as an a posteriori model. The population model is the PK model based on many measurements in many patients. Combination of the POP-PK model with a limited number of CYCLO blood levels (limited sampling) of each individual patient together with clinical parameters from the same patient (weight, drug dosing, dosing interval, time between dosing and sampling) yields an a posteriori individualized patient-specific pharmacokinetic LSM. Therefore, each patient has his or her specific LSM. The pharmacokinetic parameters of the a priori POP-PK model are shown in Table 2.

Table 2. Population-pharmacokinetic parameters calculated using an iterative two-stage Bayesian procedure

Parameter	Mean (±s.d.)
K_{elm} (/h)	0.34749 ± 0.4922
V1 (L/kg)	0.69815 ± 0.11494
K12 (/h)	0.28554 ± 0.5088
K21 (/h)	0.12886 ± 0.7634
K_a (/h)	5.5685 ± 3.6948
F	0.5 (fixed)
t_{lag} (h)	0.83501 ± 0.5885

A posteriori pharmacokinetic parameters of the individual patients

The calculated mean POP-PK parameters were individualized for each of the remaining 31 AUCs based on their CYCLO dosing and weight and one or a combination of measured blood concentrations (0 h; 1 h; 2 h; 3 h; 0 + 1 h; 0 + 2 h; 0 + 3 h; 0 + 1 + 2 h; 0 + 1 + 3 h; 0 + 2 + 3 h; 0 + 1 + 2 + 3 h) according to the maximum a posteriori (MAP) Bayesian fitting method using the MW\Pharm computer program[19]. Fitting any available information, i.e. a priori population parameters, patient weight, drug dosage regimen, and measured blood concentrations by means of MAP Bayesian method, we estimated the a posteriori pharmacokinetic parameters of the individual patients. These a posteriori pharmacokinetic parameters of the individual patients are the maximum-likelihood estimates obtained by MAP Bayesian fitting, minimizing the deviations of measured and predicted concentrations, and of POP-PK parameters and pharmacokinetic parameters of the individual patient[19]. This LSM approach is very flexible and it ensures an optimal use of available information, both from a population and from the individual patient. From these individualized pharmacokinetic parameters the area under the CYCLO blood concentration–time curve (AUC0-12h) was calculated for each combination of measured blood concentrations. The individualized POP-PK model (LSM) was assessed with several single points of blood sampling and also with different combinations of serial measurements. We compared the various models and verified the correlation of the models with the gold standard AUC0-12h in the second set of 31 curves.

Statistics

Statistical analysis on patient data was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± S.E.M. and as median and range (Wilcoxon test). Potential differences were explored with paired-samples t-test, and relationships were investigated using Pearson correlation test and linear regression analysis. P-values below 0.05 were considered statistically significant. The AUCs calculated by different methods were compared with the gold standard AUC0-12h by linear regression and Pearson correlation coefficient. Predictive performance of the different methods was also investigated by calculating the prediction precision and bias according to Sheiner and Beal[20]. Prediction bias was calculated as the mean prediction error (MPE), that is the mean of differences between the AUC0-12h according to the different methods and the gold standard AUC0-12h. Prediction precision was calculated as the mean absolute prediction error (MAPE), that is the mean of the absolute differences between the AUC0-12h according to the several different methods and the gold standard AUC0-12h. Smaller values for MPE and MAPE indicate less bias and greater precision (acceptable ranges ≤ 10%).

RESULTS

Patients

The results of conversion from C-0 to C-2 monitoring after OLT as far as dose adjustments, renal function, blood pressure, rejection and CYCLO C-0, C-2 levels and AUCs have been reported elsewhere[15]. The dose was lowered in 68% of the patients (reduction of 26.9% of initial dose; $P < 0.0001$) and remained unchanged in 32% of the patients after conversion from C-0 to C-2 monitoring. For those patients whose CYCLO dose was lowered, the mean increase of the creatinine clearance (CRCL) was 7.93 ± 3.0 mL/min (11.6% of initial CRCL; $P = 0.016$). After CYCLO dose lowering blood pressure changes were minimal, blood pressure changes were minimal, with only a significant improvement for systolic and mean blood pressure in the morning. Thirteen of 21 patients whose CYCLO dose was lowered ended below the 'target AUC', and hence below the lowest exposure on C-0 monitoring. This target AUC is based on the trough level (C-0) and was calculated with linear regression analysis. The formula of the line is: AUC0-12h = 14.75 * C-trough + 2053 (trend-line). The target range of the trough levels is 90–150 µg/L, and hence the AUC target range was originally defined as 3380–4266 h*µg/L[15]. Eight of 21 patients showed a second AUC within the range of target AUC. Two of 13 patients in whom the CYCLO dose was lowered and whose second AUC was below the target AUC developed acute cellular rejection with aminotransferases up to 500 U/L, requiring additional corticosteroids and an increase in CYCLO dose after the second AUC (AUCs were 2684 and 3075 h*µg/L, respectively). Significant changes in C-2 were observed intra-individually with the same dose.

Calculation of LSFs

Using multiple regression analysis, LSFs were calculated from 31 curves based on one or a combination of measured blood concentrations. Our results and those from previous studies with Bayesian models indicate the best correlation with the gold standard when the first 3 h after dosing are included and with multiple sampling points when the trough level is included. These results (0 h; 1 h; 2 h; 3 h; 0 + 1 h; 0 + 2 h; 0 + 3 h; 0 + 1 + 2 h; 0 + 1 + 3 h; 0 + 2 + 3 h; 0 + 1 + 2 + 3 h) are shown in Table 1.

A priori POP-PK parameters

The mean POP-PK parameters of the 31 curves of 'group 1' was calculated by an iterative two-stage Bayesian procedure, and their standard deviations are shown in Table 2.

A posteriori pharmacokinetic parameters of the individual patients

Table 3 shows the correlation with the gold standard AUC0-12h, the MPE and MAPE for one-point sampling; one- and multiple-point sampling with MAP Bayesian fitting procedure using the individualized POP-PK model (LSM); and one and multiple-point sampling using the LSFs. AUCs calculated by individualizing the POP-PK model yielding an individualized LSM based on the combinations of measured blood concentrations: 0 + 2 h (r^2 = 0.94), 0 + 1 + 2 h (r^2 = 0.94) (Figure 1), 0 + 1 + 3 h (r^2 = 0.92) (Figure 2), 0 + 2 + 3 h (r^2 = 0.92) and 0 + 1 + 2 + 3 h (r^2 = 0.96) (Figure 3) had excellent correlation with AUC0-12h. Most models without C-0 had r^2 below 0.90 (data not shown). Precision and bias were within acceptable ranges (≤ 10) provided that C-0 with or without one or more additional blood samples was taken in combination with the individualized POP-PK model.

Table 3. Correlation with $AUC_{0\text{-}12h}$, mean prediction error (MPE) and mean absolute prediction error (MAPE) for one-point sampling; one- and multiple-point sampling using limited sampling formulas (LSFs); and one- and multiple-point sampling using an individualized *a posteriori* population-pharmacokinetic (POP-PK) model [limited sampling model (LSM)] based on Bayesian fitting

Time points blood sampling	R^2	MPE (%)	MAPE (%)
Individualized POP-PK model (LSM)			
0	0.86	−8	10
1	0.54	−4	13
2	0.78	−10	12
3	0.78	−7	11
0, 1	0.86	−6	9
0, 2	0.94	−9	9
0, 3	0.89	−7	9
0, 1, 2	0.94	−9	9
0, 1, 3	0.92	−7	8
0, 2, 3	0.92	−8	9
0, 1, 2, 3	0.96	−8	8
Limited sampling formulas			
0 (C-0h)	0.64	1	11
1 (C-1h)	0.28	3	15
2 (C-2h)	0.75	−1	11
3 (C-3h)	0.74	−1	10
0, 1	0.77	1	9
0, 2	0.86	−1	8
0, 3	0.81	0	9
0, 1, 2	0.92	−1	5
0, 1, 3	0.95	−1	4
0, 2, 3	0.87	−1	8
0, 1, 2, 3	0.96	−1	4

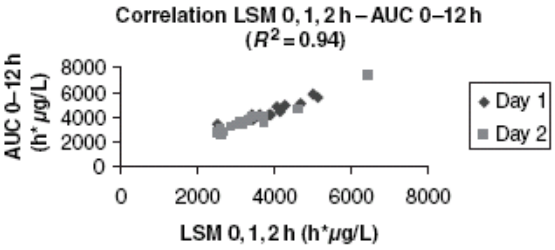

Figure 1. Limited Sampling with the individualized population-pharmacokinetic model at t = 0, 1, 2 h, correlation with $AUC_{0\text{-}12h}$.

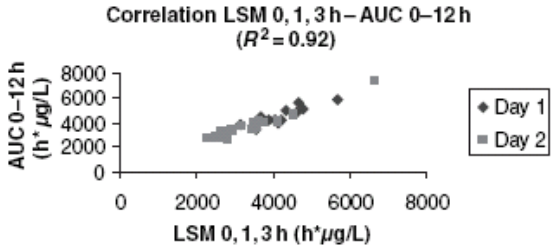

Figure 2. Limited Sampling with the individualized population-pharmacokinetic model at t = 0, 1, 3 h, correlation with $AUC_{0\text{-}12h}$.

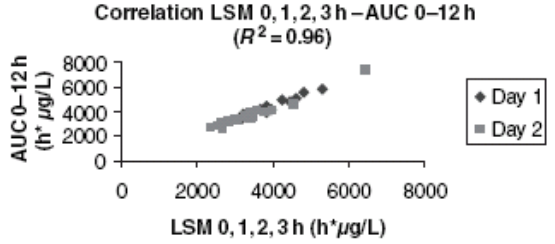

Figure 3. Limited Sampling with the individualized population-pharmacokinetic model at t = 0, 1, 2, 3 h, correlation with $AUC_{0\text{-}12h}$.

DISCUSSION

In the current study we developed a new, accurate, flexible and precise method for CYCLO monitoring in stable patients more than 6 months after liver transplantation based on an individualized limited sampling POP-PK model. This contrasts to most current LSMs that are only based on population pharmacokinetics. Our PK model is based on population pharmacokinetics and Bayesian fitting of limited sampling data from one patient. The method with 0 + 2, 0 + 1 + 2, 0 + 1 + 3, 0 + 2 + 3 or 0 + 1 + 2 + 3 h sampling showed excellent correlation with the gold standard 12-h AUC. Results even for C-0 combined with the model were better than those for simple C-0 or C-2. A major advantage of the new method over current methods based on population kinetics only, such as LSFs, is that sampling time points are more flexible than with, e.g. C-2 monitoring, LSFs or current POP-PK models. Our model is efficient as long as the exact dosing and sampling time, the weight of the patient and the dosing rhythm are registered and sampling time is near the required time after dosing. Both population and individual kinetics are incorporated in our new PK model, making optimal use of available information. Blood concentration data are put into the computer model, which runs on a desktop PC, and the AUC is calculated and a dose modification suggested. It is still necessary to obtain more than one blood concentration of CYCLO during the dosing interval in order to obtain adequate estimates (>90%) of AUC0-12h. While this might be possible in an in-patient setting, applying this method to out-patient practice may be considered difficult and impractical for both the patient and provider. However, as our results show, the correlation with AUC0-12h of the individualized POP-PK model is better than with LSFs, especially when less than three sampling points were used, e.g. when the combination of C-0 and C-2 or the combination of C-0, C-1 and C-2 were taken. The R^2 for C-2 was below 0.80 even with individualized POP-PK model or LSF. The individualized POP-PK model correlated very well (>0.90) with AUC0-12h even with only two time points for 0 + 2 h, and with three sampling points for 0 + 1 + 2, 0 + 1 + 3 and 0 + 2 + 3 h. Indeed, time C-0 almost always needed to be included for a correlation >0.90. These sampling times were less fixed than in LSFs where they need to be exactly on time (otherwise the model is not valid). When, for example, C-1 is forgotten, but C-0, C-2 and C-3 are obtained, individualized POP-PK model can be used with excellent correlation with AUC. Using an individualized POP-PK model with multiple sampling points requires some organization in the clinic but in our experience this is feasible and the advantages are clear. It might be possible to reduce the number of samplings per visit and the number of visits to the clinic in stable patients in the long term and still get sufficient prediction of AUC, but this requires further study. Our current data show that our individualized POP-PK model using multiple sampling points is superior to the other methods.

The clinical consequences of the improved prediction require further evaluation. Conversion of monitoring CYCLO more than 6 months after OLT from C-0 towards C-2 resulted in dose reduction in two-thirds of the patients, which was associated with improved renal function and marginal improvement in blood pressure. However, significant intrapatient variability of the C-2 blood levels with the same dose and AUCs below the target range in more than half of the patients whose dose was lowered occurred with C-2 monitoring, sometimes resulting in rejection. This was reflected in a R^2 value of only 0.75 for C-2 compared with AUC0-12h (Table 3), which limits the accuracy of therapeutic drug monitoring with C-2 levels and may induce many unnecessary subsequent changes in drug dose, which is inconvenient for the patients, doctors and nurses. Based on the current POP-PK model and generally accepted trough levels of 90–125 μg/L, the AUC range should be 2900–3800 h*μg/L, a range we now adhere to in our clinic, although we cannot exclude that some patients may tolerate lower values. While correlation of C-2 with AUC is better than that of C-0 with AUC, it is far from perfect. Others observed a better correlation of C-2 with AUC when compared with trough-level monitoring in renal and liver graft recipients[3–6]. Most studies in renal transplantation and the limited studies in liver transplantation using C-2 monitoring also showed improved kidney function, and often blood pressure and serum cholesterol also improved. In those studies, no rejection occurred despite lower exposure to CYCLO than while on C-0 monitoring. However, in the reported liver transplant studies, AUC was calculated by measuring CYCLO blood levels during 4 and 6 h only, while we used 0–12 h AUCs. This may explain part of the difference between these and our studies[7–14]. Another explanation may be the lower maintenance levels used in liver transplantation when compared with kidney transplantation; further lowering of the dose may more easily lead to rejection. All samples were taken as recommended[6,7,21] and within 2 min from the target time (although 10 min were allowed for C-2); if sampling time would have been more variable (as may be the case in daily practice) this would have led to an even lower accuracy of C-2 monitoring and inappropriate dose adjustments[22]. This may also be true for LSFs or POP-PK models with fixed sampling time points. In renal transplantation, variable CYCLO levels may contribute to chronic rejection[23]. Although chronic ductopenic rejection has become less common after liver transplantation in the last decade, it forms a continuum with acute cellular rejection and chronic underexposure to CYCLO can be a cause[24–27]. In renal transplant studies it was shown that absorption profiling over the first 4 h was superior to trough level monitoring, with C-2 as the best single-point predictor of AUC[3,12,28–31]. The clinical superiority of such absorption profiling over C-2 levels has not been examined in those studies. Our data demonstrated that in stable liver transplant patients trough level monitoring frequently leads to overdosing of CYCLO, while monitoring by C-2 may cause episodes of underdosing[15]. According to Levy and Cole the long-term benefits of

reduced toxicity caused by C-2 monitoring might well outweigh the development of mild, easily treated rejection[32]. However, it may be better to try to avoid these rejections as well as toxicity. Therefore, better ways of monitoring CYCLO dosing in liver transplantation are required.

As both blood interleukin (IL)-2 concentration and 12-h AUC are related to CYCLO exposure in the first 4 h after dosing, it seems logical to use a sparse-sampling method in the first hours after dosing[33,34]. It had already been shown that using multiple sampling points in the first hours after dosing with Bayesian forecasting results in a better correlation with AUC0-12h[35–38]. A high inter-individual variability in CYCLO pharmacokinetics exists, which seems unrelated to CYP3A polymorphisms[39]. Therefore, the use of multiple sampling models may avoid over- and underdosing and unnecessary changes in dose. A disadvantage of available LSFs and POP-PK models was that multiple samplings were needed on fixed time points. It was previously stated that the ideal model should be easy to use and flexible, without the rigid time points and complicated methods used in current multiple sampling models, and it should be based both on population kinetics and on individual pharmacokinetics[37,38,40,41]. The LSM presented in the current study clearly approximates this goal. A similar model performed well in kidney as well as combined kidney– pancreas transplant patients[42]. However, the effect of its use on clinical outcome remains to be investigated. As our liver LSM model was developed in stable liver transplant patients, it also needs to be evaluated whether graft dysfunction affects the model. We anticipate that use of our model (even with only C-0) will lead to a more stable CYCLO dose with less over- or underdosing than with simple C-0 or C-2 monitoring. Whether this leads to less rejection or renal insufficiency needs to be determined. In conclusion, while C-0 monitoring frequently results in overdosing and more renal dysfunction, C-2 monitoring may lead to episodes of underdosing and rejection and many subsequent dose adjustments. We therefore devised a flexible Bayesian individualized limited sampling POP-PK model for CYCLO monitoring without rigid sampling time points, which is accurate, precise and easy to use in daily practice.

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CHAPTER 4

INDIVIDUALIZED POPULATION PHARMACO-KINETIC MODEL WITH LIMITED SAMPLING FOR CYCLOSPORINE MONITORING AFTER LIVER TRANSPLANTATION IN CLINICAL PRACTICE: C0+C2?

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ABSTRACT

Background: We recently developed and validated limited sampling models (LSMs) for cyclosporine monitoring after orthotopic liver transplantation based on individualized population pharmacokinetic models with Bayesian modelling.

Aim: To evaluate LSM in practice, and to seek optimal balance between benefit and discomfort.

Methods: In 30 stable patients, more than 6 months after orthotopic liver transplantation, previously switched from trough- to 2 h post-dose (C2)-monitoring, we switched to 3-monthly LSM 0,1,2,3 h-monitoring. During 18 months we evaluated dose, creatinine clearance, calculated area under the curve, intra-patient pharmacokinetic variability and ability to assess systemic exposure by several previously validated LSMs.

Results: Within patients, there was variability of cyclosporine-area under the curve with the same dose (CV of 15%). Compared to C2-monitoring, there was no significant difference in dose ($P = 0.237$), creatinine clearance ($P = 0.071$) and number of rejections. Some models showed excellent correlation and precision with LSM 0,1,2,3 h comparing area under the curves (0,2 h: $r^2 = 0.88$; 0,1,3 h: $r^2 = 0.91$; 0,2,3 h: $r^2 = 0.92$, all $P < 0.001$) with no difference in advised dose.

Conclusion: The limited sampling model, with only trough- and 2-h sampling, yields excellent accuracy and assesses systemic exposure much better than C2 with less bias and greater precision. Considering the calculated intra-patient variability, more precision is redundant, so LSM 0,2 h seems the optimal way of cyclosporine-monitoring.

INTRODUCTION

Calcineurin inhibitors like cyclosporine are frequently used after solid organ transplantation such as orthotopic liver transplantation (OLT). However, these drugs are characterized by a narrow therapeutic range with risks of overdosing and underdosing. For this reason systemic exposure of this drug is routinely assessed. Several methods with varying complexity and performance exist. Until recently most clinics used trough-level monitoring (C0) to assess systemic exposure to cyclosporine, but over the last years many centres replaced this method by so-called C2-monitoring, where blood samples were taken exactly 2 h after oral administration of the drug[1-10]. This method has been shown superior in predicting the area under the curve (AUC) and toxicity. In a previous study, in stable patients more than 6 months after OLT, we demonstrated lowering of the dose in two-thirds of the patients with improved kidney function when switching from C0- to C2-monitoring[11]. However, a substantial percentage of underdosing occurred with this method, suggesting the need for even better monitoring methods.

We then developed and validated flexible limited sampling models (LSMs), based on an individualized population pharmacokinetic (PK) model, limited sampling and Bayesian estimations, which was again superior to C2.[12] All patients who were previously switched from C0- to C2- cyclosporine monitoring were now switched to 3-monthly monitoring with this LSM and followed for a period of 18 months. This strategy allowed us to investigate the feasibility of implementation of LSM into daily practice, and the potential effects of the change from C2 to LSM on such factors as dose, renal function, rejection rate and also interpatient variability. Using LSM, it was possible to determine intra-patient variability in PK of cyclosporine. With this, it was possible to determine the required precision of the method used. In addition, a new target range for cyclosporine AUC based on the 95% confidence interval for clearance could be calculated.

PATIENT AND METHODS

Thirty stable patients more than 1 year after OLT (20 men, mean age 54, range 34–66; 10 women, mean age 42, range 22–61) received the micro-emulsion formulation of cyclosporine (Neoral; Novartis Pharmaceuticals, Basel, Switzerland) twice daily as immunosuppressant. The reasons for OLT were cirrhosis due to hepatitis B-virus (four patients), alcoholic liver disease (seven patients), primary biliary cirrhosis (one patient), hepatitis C-virus (five patients), primary sclerosing cholangitis (one patient), Budd Chiari syndrome (two patients), autoimmune-hepatitis (one patient), Wilson's disease (two patients), hepatocellular carcinoma (three patients), neuroendocrine

tumour (one patient), acute fatty liver of pregnancy (one patient) and two patients with acute liver failure with unknown aetiology.

During the study, one patient sometimes had aminotransferases just above the upper limit of normal, probably as signs of reactivation of hepatitis C-virus, but this was not the case on a 3-monthly cyclosporine monitoring day. No cases of hematuria or proteinuria occurred. The mean time of exposure to cyclosporine prior to entry in this study was 46 ± 26 months (range 12–109). Six patients showed rejections between the time of OLT and time of starting this study, but all were stable again when entering the study.

After informed consent, the patients came to the clinic for check-up and blood samples were taken for cyclosporine concentration close to 0 h, 1 h, 2 h and 3 h after the morning dose of cyclosporine, while still on C2- monitoring. Whole blood cyclosporine concentrations were determined by Fluorescence Polarisation Immuno Assay (FPIA, Axsym; Abbott Diagnostics, Abbott Park, IL, USA).

Then cyclosporine dose was adjusted based on AUC calculation of LSM 0,1,2,3 h[12]. AUCs were calculated using the following formula:

*AUC = (F_po * dose * 1000) / clearance*, in which F_po is the bioavailability which is fixed at 0.5 for cyclosporine micro-emulsion, dose is the morning dose of cyclosporine and clearance is the clearance of cyclosporine calculated for the combination of time points 0 + 1 + 2 + 3 h using the PK software package MW\Pharm version 3.50 (Mediware, Groningen, The Netherlands)[13].

After every limited sampling curve, a dose advice was given using the formula:

*Advised dose = (target AUC / calculated AUC) * dose*, in which 3350 is set as value for the target AUC [middle of target-range, (2900 + 3800) / 2], which is based on the range of trough-level monitoring of 90–125 μg/L[11]. If allowed by renal function (CRCL > 50 mL/min) the dose was adjusted to the advised dose. Every 3 months thereafter LSM 0,1,2,3 h was obtained and cyclosporine dose adjusted accordingly. After dose adjustment an extra curve was obtained. From the 30 patients in total 152 LSM 0,1,2,3 h-curves (mean per patient 5 ± 2, range 1–9) were collected over the last 18 months. Four patients changed to other immunosuppressive medication during the course of this study (one because of rejection, two because of renal dysfunction, one because of gum hyperplasia).

Blood samples were also taken for kidney- and liver function. Creatinine clearance (CRCL) was calculated with Cockcroft & Gault formula. As warranted by our liver transplant protocol, a liver biopsy was obtained when rejection was suspected. Moderate-to-severe rejection was treated with additional immune suppression, while in mild rejection the dose of maintenance immune suppression was optimized.

Intra-patient variability in clearance (CV%) was investigated calculating the mean and standard deviation of the clearance of all curves for all patients using the formula: *variation coefficient = (standard deviation / mean clearance) * 100%*.
In order to create a new target-range for the AUC, a 95% confidence interval for clearance was calculated using the formula:
*AUC = (0.5*dose*1000) / (clearance ± 2s.d.)*

Statistics

Statistical analysis on patient data was performed using SPSS 11.0.1 for Windows (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± s.d. and as median and range. Potential differences were explored with Paired Samples *t*-test and relationships were investigated using Pearson correlation test and Pearson chi-squared test. *P*-values below 0.05 were considered to be statistically significant. AUCs were calculated using previously developed and validated LSMs[12].
The calculated AUCs, based on a single-point and combinations of blood sampling time points, were compared with the AUC based on time points 0 + 1 + 2 + 3 h by Pearson correlation test. Predictive performance of this method was also investigated by calculating the prediction precision and bias according to procedures developed by Sheiner and Beal[14]. Prediction bias was calculated as the mean prediction error (MPE), this is the mean of differences between the AUCs of the different models and the AUC based on time points 0 + 1+2 + 3 h. Prediction precision was calculated as the mean absolute prediction error (MAPE), this is the mean of the absolute differences between the AUCs of the different models and the AUC based on time points 0 + 1 + 2 + 3 h. Smaller values for MPE and MAPE indicate less bias and greater precision (acceptable ranges ± 10%).

RESULTS

Time to reach peak concentration

While monitoring cyclosporine concentration in blood there was a difference between patients and also within patients concerning the time to reach peak concentration of cyclosporine. From all the 152 curves we obtained, there were 69 curves (45%) with a peak on C1, 71 curves (47%) on C2 and 12 curves (8%) on C3 (Table 1). Table 2 shows a few examples of the results per patient demonstrating considerable intra-patient variability.
Based on these results we may conclude that monitoring only on C2 is not reflecting the AUC well enough because of intra-patient variation in time after dosing to reach peak concentrations.

Table 1. Time of the peak cyclosporine blood concentration of all 152 limited sampling model 0,1,2,3 h-curves

Sampling point	Number of peaks	(%)
C0	0	0
C1	69	45
C2	71	47
C3	12	8
total	152	100

Table 2. Number of limited sampling model 0,1,2,3 h curves with peak cyclosporine level at C0, C1, C2 or C3 per patient for three patients.

Patient number	Peak C0	Peak C1	Peak C2	Peak C3	Number of curves
3	0	3	2	0	5
9	0	1	5	3	9
21	0	3	3	2	8
Total	0	7	10	5	22

Variation of clearance

Calculating the variation coefficient (CV%) for every patient using the mean clearance and standard deviation of all curves this CV% was 15%. Mean dose of all patients was 109 mg twice daily, so natural variation in one patient is 109*0.15 = 16 mg.
We calculated a 95% confidence interval for the clearance in order to create a new target range, which is based on natural variation. This new target range is 2380–4390 h*µg/L, much wider than the target range we use in our clinic (2900–3800 h*µg/L). Even when we use 1s.d. instead of 2s.d. the target range would be 2680–3620 h*µg/L.

Difference in dose, kidney function and rejection

Before switching from C2-monitoring to LSM 0,1,2,3 h mean cyclosporine dose, while on C2-monitoring, was 207 ± 9 mg daily (range 150–350 mg). After switching, mean daily dose was 218 ± 10 mg (range 100–300 mg). Mean change in dose was 11 ± 9 mg (P = 0.237, median 0.0, range −100 to +100), so there was no significant change of average cyclosporine dose after switching from C2-monitoring to LSM 0,1,2,3 h.
Looking at the individual patient, only two patients once had a daily-dose change of 100 mg, one −100 mg and one +100 mg. The other patients had daily-dose changes of 50 mg or less.
Mean CRCL on C2-monitoring was 77.0 ± 4.5 mL/min (range 40.4–132 mL/min). While using LSM 0,1,2,3 h mean CRCL was 73.0 ± 4.8 mL/min (range 26.6–128.8 mL/min). The difference in CRCL between C2-monitoring and LSM 0,1,2,3 h was

−4.0 ± 2.1 mL/min (P = 0.071) so on average there was no significant change of the kidney function. Looking at the individual patient level, there was a wide variability in CRCL change (range: −30.1 to +17.7, median: −5.4). Even when dividing all patients into three groups (tertiles) based on CRCL in each group there was a comparable variability of CRCL (data not shown).
While using LSM 0,1,2,3 h for 18 months, there were two moderate-to-severe rejections vs. two moderate-to-severe rejections during the previous 18 months on C2-monitoring.

Correlation of other LSMs with LSM 0,1,2,3 h

For the LSM 0,1,2,3 h model and for the models with time points 0 h, 1 h, 2 h and 3 h and the combinations of time points 0,1 h, 0,2 h, 0,3 h, 1,2 h, 1,3 h, 2,3 h, 0,1,2 h, 0,1,3 h, 0,2,3 h and 1,2,3 h we calculated for all 152 curves the AUC and the correlation with LSM 0,1,2,3 h (Table 3). Correlation of AUC calculated with LSM 0,1,2,3 h for other multiple-point models was much better than LSM 0 h and LSM 2 h. Two 2-point-models showed good correlation with our 0,1,2,3 h model:
LSM 0,2 h (r^2 = 0.88) and LSM 0,3 h (r^2 = 0.87) with acceptable bias and precision.
Three 3-point-models also showed good correlation with acceptable bias and precision:
LSM 0,1,2 h (r^2 = 0.84), LSM 0,1,3 h (r^2 = 0.91) and LSM 0,2,3 h (r^2 = 0.92) (Figure 1). Of special interest is the important contribution of the trough-level (C0), which seems to be indispensable for adequate monitoring of cyclosporine in combination with at least one other sampling point.

Table 3. Correlation of other individualized pharmacokinetic (PK) models [limited sampling models (LSMs)] with LSM 0,1,2,3 h

Individualized PK-model (LSM) with sampling on time (h):	r^2	MPE (%)	MAPE (%)
0	0.67	7	16
1	0.12	8	24
2	0.50	−6	13
3	0.66	3	11
0,1	0.69	10	18
0,2	0.88	−2	6
0,3	0.87	5	9
1,2	0.42	−9	16
1,3	0.72	1	9
2,3	0.68	−2	9
0,1,2	0.84	−2	6
0,1,3	0.91	4	6
0,2,3	0.92	2	5
1,2,3	0.67	−4	9
0,1,2,3	1.00	0	0

MPE, mean prediction error; MAPE, mean absolute prediction error.

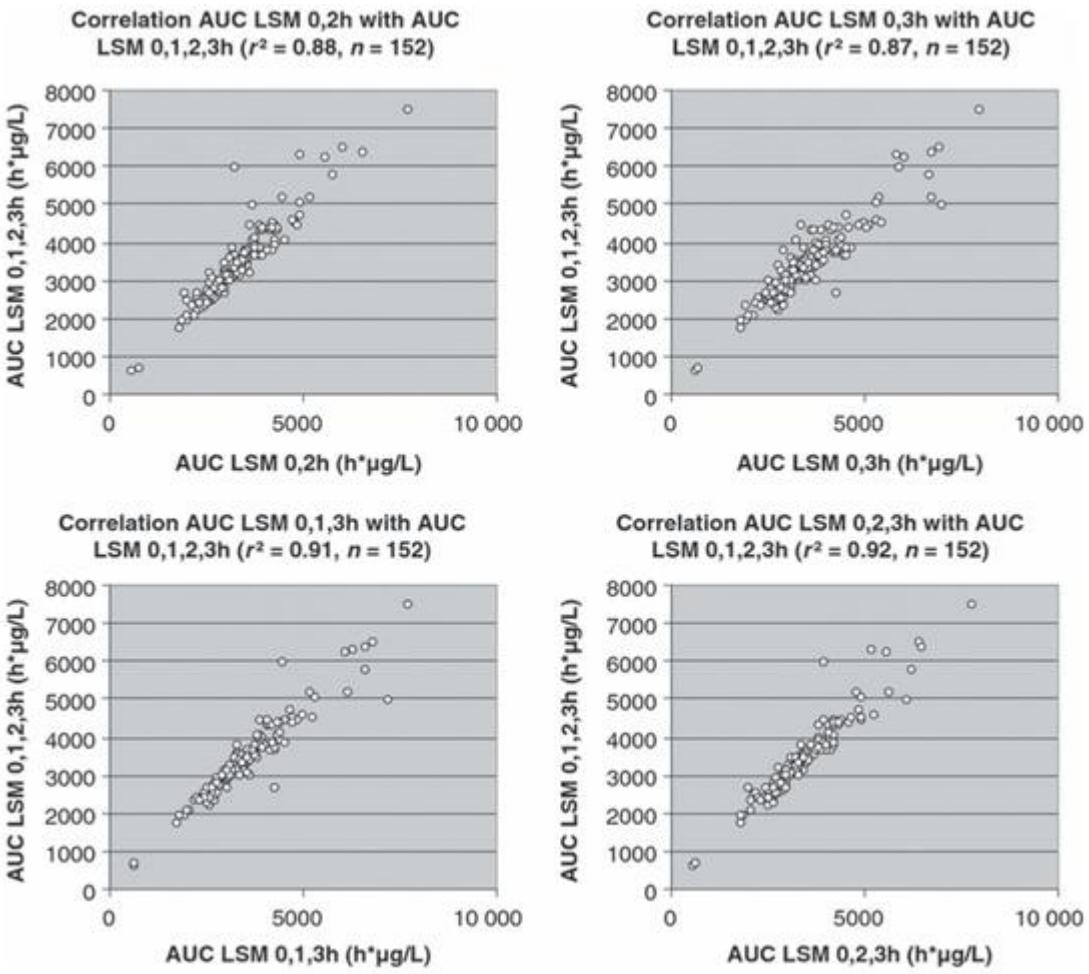

Figure 1. Correlation of area under the curves (AUCs) limited sampling model (LSM) 0,2 h, LSM 0,3 h, LSM 0,1,3 h and LSM 0,2,3 h with AUC LSM 0,1,2,3 h.

We then calculated the correlation of these five two-point and three-point LSMs with LSM 0,1,2,3 h per patient. Looking only at the 20 patients with at least five 0,1,2,3 h curves, in 19/20 patients we see a high and significant correlation of AUCs calculated with LSM 0,1,2,3 h and those AUCs calculated with the models LSM 0,2 h, LSM 0,1,2 h, LSM 0,1,3 h and LSM 0,2,3 h. For the LSM 0,3 h in 16/20 patients there was a good and significant correlation with LSM 0,1,2,3 h (Table 4).

Table 4. Correlation of area under the curve (AUC) calculated with different limited sampling models (LSMs) with AUC calculated with LSM 0,1,2,3 h per patient with five or more curves

LSM (h)	n with $P < 0.05$	r^2 (range)
0,2	19/20	0.81–0.99
0,3	16/20	0.80–1.00
0,1,2	19/20	0.84–1.00
0,1,3	19/20	0.67–1.00
0,2,3	19/20	0.82–1.00

These correlations suggest that these other LSMs with less time points show results comparable to LSM 0,1,2,3 h and that particularly LSM 0,2 h is an accurate, reliable and very practical model with acceptable bias and precision for monitoring cyclosporine, as we saw earlier when developing and validating our LSM 0,1,2,3 h and these other LSMs[12] .

DISCUSSION

This study, in the first place, shows that it is feasible to implement cyclosporine monitoring based on limited sampling and an individualized population PK model in a liver transplant out-patient clinic.
Second, we show that cyclosporine dose, renal function and rejection rate did not change significantly after our switch from C2-based monitoring to LSM 0,1,2,3 h. Third, we show that often we decided not to increase the cyclosporine dose because of renal dysfunction while we were advised to do so because the calculated AUC was below the target range, while usually no rejection followed. This means that apparently the lower limit of the target range was too high. Fourth, a significant intra-patient variation appeared to occur with the same cyclosporine dose. Fifth, several two- and three-point – previously validated- LSMs correlated very well with the four-point LSM 0,1,2,3 h. All include the trough level, which seems indispensable to get an accurate AUC prediction, as we previously showed. Sixth, the LSM 0,2 h seems optimal in terms of accuracy, ease-of-use and intra-patient variability.
Because of the narrow therapeutic range of cyclosporine, assessing the systemic exposure to this drug is mandatory. Ideally, a full AUC is measured on a regular basis. As this is not practical and C0 is a rough estimation of the AUC, for many years monitoring based on trough levels was used. Then many centers switched to monitoring based on C2, after it was shown that C2 correlates better with AUC as Citerrio describes in an article about the evolution of the therapeutic drug monitoring of cyclosporine[15]. This had the disadvantage of a fixed time point after dosing, which is difficult for some patients. Moreover, C2 still does not reflect very well the AUC and according to the review study of Marin *et al.* the best way to individualize therapy is still controversial. Recommendations are made for clinical research that could be done to provide more definitive evidence for the use of C2 or other limited sampling strategies[16]. After C0-monitoring and the more precise C2-monitoring we showed that our LSM 0,1,2,3 h-method more accurately estimates systemic exposure to cyclosporine in OLT patients, based on limited sampling, individualized population PK models and Bayesian estimations with an easy-to-use computer model[12]. LSMs have the advantage

that sampling times are not rigid in contrast to most limited sampling strategies described in a review article of David and Johnston[17]. Switching from C0- via C2-monitoring and subsequently to LSM 0,1,2,3 allowed us to compare the biochemical and clinical effects of these three methods.

There appeared to be considerable intra-patient variability of time to reach the peak-concentration of cyclosporine. This led to the same number of dose adjustments as with C2-monitoring in the 18 months before the C2 to LSM 0,1,2,3 h switch. The intra-patient PK variability may partially be due to interaction with food or other medication. The variation in peak-time is partially responsible for the large intra-patient variation in C2 levels over time in some of the patients. With an LSM with more sampling time points, all important information required for calculating an AUC is obtained and the chance of 'missing' this variability is less, which leads to more accurate AUC estimations.

After more than one-and-a-half-year of using our model for cyclosporine monitoring in the out-patient clinic, 152 LSM 0,1,2,3 h curves from 30 patients were derived. Although this is not a randomized controlled trial these stable patients were their own controls. According to the dose, renal function and rejection on average there was no difference using C2-monitoring or the individualized PK model. However, the target range was based on AUCs while on C0-monitoring. In an earlier study, while on C2-monitoring, we saw two rejections in 13 cases where the AUC dropped below the AUC target range. Apparently, an AUC below 2900 h*µg/L is tolerated in many patients. This was similar for LSM 0,1,2,3 h monitoring: for some patients the dose was not increased as advised after LSM 0,1,2,3 because renal insufficiency did not allow us to do so, but although these patients were at risk of underdosing, usually no signs of rejection occurred.

Although there was no significant change in CRCL between C2-monitoring and LSM 0,1,2,3 h, there seemed to be a trend toward lower CRCL with LSM vs. C2-monitoring (P = 0.071). More data is needed to confirm the usefulness of tailoring cyclosporine dosing by LSM to minimize toxicity.

The current data allow us to investigate the true natural variability in PK of cyclosporine in stable OLT patients. The mean intra-patient variability of the apparent oral clearance of cyclosporine in these stable liver transplantation patients was 15%. This means that a dose adjustment of 16 mg or less (15% of mean dose of 109 mg) is not rational, because this difference is a natural variation, which cannot be avoided. In fact, the lowest possible dose adjustment (25 mg) in practice is relatively close to this natural variation of 16 mg. In case the mean dose of 109 mg and a 95% confidence interval (mean ± 2*s.d.) would be used, a target range of 2380–4390 h*µg/L would be rational. In other words, any AUC value within this range can be explained by natural variability in PK of cyclosporine and may therefore not require a dose adjustment.

In our hospital, a target range of 2900–3800 h*µg/L was used for stable OLT patients, which is narrower, and closer to a mean ± 1*s.d. value of the AUC in this population, which is 2680–3620 h*µg/L. However, to be on the safe side, we until now remain adhering to this narrow range, although we realize that this may be too strict. Based on the current data, a lower range for the AUC than currently used with a target AUC of 2830 h*µg/L (2380–3280 h*µg/L) may be reasonable.

Our data suggest that, considering the natural variability in PK of cyclosporine in stable OLT patients, our method with LSM 0,1,2,3 h may be too accurate in terms of estimating systemic exposure to cyclosporine.

When investigating the correlation between LSMs with only two or three sampling points and the LSM 0,1,2,3 h we see that overall five models showed good correlation when considering both the AUCs and the mean advised dose. These five LSMs were 0,2 h; 0,3 h; 0,1,2 h; 0,1,3 h and 0,2,3 h. Accuracy and bias were acceptable. The trough level is included into all of these models, which illustrates the pivotal role of this sample for assessing systemic exposure to cyclosporine. We are aware of the fact that these five models are abbreviated curves from the already abbreviated 0,1,2,3 h curve, but recently we already noticed a very good correlation of these models with the gold standard $AUC_{0-12\ h}$ (for LSM 0,2 h this was: $r^2 = 0.94$, MPE = −9, MAPE = 9) with less bias and greater precision than e.g. C2 single-point monitoring ($r^2 = 0.78$, MPE = −10, MAPE = 12) or Ctrough[12].

In spite of the fact that LSM 0,1,2 h includes both the common 1- and 2-h peak-level time points, the correlation of this model with LSM 0,1,2,3 h in the patients with five or more curves is not different from LSM 0,2 h (r^2 = 0.84–1.00 vs. 0.81–0.99).

Comparing LSM 0,1,2 h with LSM 0,2 h, the 0,2 h-model has the benefit that it is easier to apply in practice, it is more friendly for the patient and the medical staff, and there is a cost-benefit. Therefore this model seems an optimal balance between benefit and discomfort for the patient. A large randomized controlled trial between C2 and LSM 0,2 h with a target AUC of 2830 h*µg/L (range 2380–3280 h*µg/L) would be of interest.

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CHAPTER 5

FLEXIBLE LIMITED SAMPLING MODEL FOR MONITORING TACROLIMUS IN STABLE PATIENTS HAVING UNDERGONE LIVER TRANSPLANTATION WITH SAMPLES 4 TO 6 HOURS AFTER DOSING IS SUPERIOR TO TROUGH CONCENTRATION

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ABSTRACT

Background: Trough (C0) monitoring is not optimal for therapeutic drug monitoring of tacrolimus. To better estimate systemic exposure of tacrolimus and achieve clinical benefit, an improved therapeutic drug monitoring strategy should be developed.

Methods: The authors examined which single and combination of time points best estimated the empiric "gold standard" $AUC_{0\text{-}12h}$ and developed and validated a new, flexible, and accurate limited sampling model for monitoring tacrolimus in patients having undergone liver transplantation. Twenty-three stable patients with full $AUC_{0\text{-}12h}$ were divided into two groups based on area under the concentration-time curve/dose. With multiple regression analysis, limited sampling formulae were derived and population-pharmacokinetic-based limited sampling models were developed and validated. A regression analysis was performed between either area under the concentration-time curves calculated with formulae or models with the reference trapezoidal $AUC_{0\text{-}12h}$.

Results: Both formulae and models based on single samples C4-C6 (r^2 = 0.94 [MPE/MAPE 0/7]-0.90 [2/8] and 0.97 [0/7]-0.97 [1/5]) showed excellent performance. The calculated area under the concentration-time curve target range for tacrolimus was 90 to 130 h*µg/L. Multiple point sampling performed better, especially when using models (r^2 > 0.94). C0 was a less precise predictor of $AUC_{0\text{-}12h}$ compared with both formulae and models (r^2's 0.68 [5/17] and 0.87 [2/14]).

Conclusion: Trough concentration monitoring is not an accurate method for assessing systemic exposure to tacrolimus in stable patients having undergone liver transplantation. This new limited sampling model, based on single time points C4-C6, shows excellent performance in estimating the $AUC_{0\text{-}12h}$.

INTRODUCTION

The calcineurin inhibitor tacrolimus is widely used for immunosuppression after orthotopic liver transplantation (OLT). Tacrolimus has a small therapeutic window, underexposure can result in rejection whereas overexposure can lead to adverse effects, especially nephrotoxicity. Accurate monitoring of this drug is therefore mandatory to improve clinical outcome[1,2]. For cyclosporine, another calcineurin inhibitor, different methods have been developed to estimate systemic exposure using the area under the concentration-time curve (AUC), which can result in better clinical outcome in terms of reduction of toxicity and improved renal function[3-13].
Monitoring tacrolimus (FK-506, Prograf Astellas Pharma, Stainer, UK) therapy is still based on trough concentration (C0) monitoring in most centers. However, recent data have shown that C0 does not accurately reflect systemic exposure over the first 12 hours after dosing[14]. Patients with similar C0 tacrolimus concentrations can have very different AUCs. Other studies in liver and kidney transplantation have suggested different time points at which better predictions of systemic exposure of tacrolimus can be made than using trough concentrations[14-17].
When better prediction of total systemic exposure of tacrolimus in the first 12 hours after dosing is possible, we may see improved clinical outcome in terms of fewer rejection episodes and lowering of toxicity.
The aim of the present study was to examine which single time point or combination of time points best reflect systemic exposure of tacrolimus by calculating the area under the curve and then to develop and validate a new, flexible, and accurate limited sampling model, which is easy to apply in clinical practice as we have shown previously for cyclosporine[18,19].

PATIENTS AND METHODS

Twenty-three stable patients having undergone liver transplantation from Leiden University Medical Center, who were at least 6 months post-OLT (11 men, mean age 45 years, range 31-73 years; 12 women, mean age 44 years, range 21-70 years) were included. Twenty-two patients received tacrolimus (Astellas Pharma Inc., Deerfield, IL) twice daily and one patient only once daily 0.5 mg in the morning. Mean morning tacrolimus dose was 3.0 ± 0.35 mg (range, 0.50-8.00 mg). In our liver transplant clinic, trough concentration monitoring is used with a target range of 5 to 10 µg/L for patients more than 3 months after OLT[20].
All patients provided informed consent and the study was approved by the Medical Ethical Committee of the Medical Center. Stable patients having undergone liver

transplantation were selected and visited our clinic for 1 day. The patients had no infections or other complications and were not receiving any interacting comedication. Specifically, bilirubin and albumin levels were not outside clinical reference ranges. Five minutes before taking the morning dose of tacrolimus (approximately 10:00 AM), blood samples were taken for liver and kidney function and tacrolimus (C0) concentration. The patients were instructed to take their evening dose of tacrolimus the night before the morning of the study visit at 10:00 pm. Further blood samples for tacrolimus concentration were collected at 1, 2, 3, 4, 6, and 8 hours after administration of the morning dose of tacrolimus. Because these were stable patients, the C12h concentration was taken to be the same as the C0h, assuming steady-state conditions[17]. It was checked by interview that there were no dose changes in the previous week. Blood was drawn using an indwelling catheter and collected in a Vacutainer (Becton Dickinson Diagnostics, Franklin Lakes, NJ) containing EDTA. Whole blood tacrolimus concentrations were determined by Microparticle Enzyme Immuno Assay (IMx; Abbott Diagnostics, Abbott Park, IL). To lower the influence of meals, the patients were instructed to take only a light breakfast-tea and a biscuit-on the morning the AUC was measured, and until the 2 hours sample (C2), no additional food or drinks were taken[21].

$AUC_{0\text{-}12h}$ of all 23 curves were calculated with the trapezoidal rule using the software package MW\Pharm version 3.60 (Mediware, Groningen, The Netherlands)[22,23]. The patients were assigned to a group on the basis of a climbing AUC/dose ratio in a 1:1 fashion. Starting with a low ratio, the first patient entered one group and the second patient entered the other group until all patients were divided among the two groups. Therewith, two groups with a comparable clearance distribution were formed: group 1 (n = 11) and group 2 (n = 12). Data from group 1 were used to calculate limited sampling formulae (LSF) and for the development of a population pharmacokinetic (POP-PK) model. Data from group 2 were used to validate this POP-PK model.

The POP-PK model integrated all available information obtained from PK sampling and generated a population model. This model was used to obtain individualized pharmacokinetic parameters (individualized PK model based on Bayesian fitting) on the basis of new PK information (samples at single or multiple time points) from new patients, allowing individualized dose advice to be given. This Bayesian approach is a flexible alternative to methods using limited sampling formulae that have fixed sampling times[24].

Several single blood sampling time points (C0, C1, C2, C3, C4, C6, and C8) and combinations of these samples were examined, 23 in total. We compared the performance of limited sampling models (LSM) with the more rigid limited sampling formulae. Finally, we performed a validation step and calculated a new target range as a basis for future implementation in clinical practice.

Limited Sampling Formulae

Using multiple regression analysis (SPSS software; SPSS Inc., Chicago, IL) for group 1, relatively simple limited sampling formulae (linear functions) were calculated based on one sample or a combination of measured blood concentrations: 0 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 0 + 1 h, 0 + 2 h, 0 + 3 h, 0 + 1 + 2 h, 0 + 1 + 3 h, 0 + 2 + 3 h, 0 + 1 + 2 + 3 h, 1 + 3 h, 1 + 4 h, 2 + 3 h, 2 + 4 h, 2 + 3 + 4 h, 3 + 4 h, 3 + 4 + 6 h, 3 + 6 h, and 4 + 6 h. Their ability to estimate the AUC was tested on group 2.

Limited Sampling Models

Using the "Kinpop module" of MW\Pharm, a population two-compartment model with first-order absorption pharmacokinetics and without a lag time was calculated from the tacrolimus dosing, body weight, and blood concentration values of group 1. This program uses an iterative two-stage Bayesian procedure and calculates means, medians, and standard deviations of the pharmacokinetic parameters[25]. During this procedure, pharmacokinetic parameters were set to be distributed log-normally, and bioavailability was fixed for tacrolimus at 0.23 as a result of the absence of intravenous data and on the basis of literature values[26].

The calculated mean POP-PK parameters based on group 1 were individualized for the 12 patients of group 2 based on tacrolimus dosing and weight and one or a combination of measured blood concentration as mentioned for LSF.

AUCs (µg/L*h) for group 2 were calculated using the following formula:

*AUC = (F_po * dose * 1000) / clearance* in which F_po is bioavailability, which is fixed at 0.23 for tacrolimus, the dose (mg) is the morning dose of tacrolimus, and clearance (L/h) is the clearance of tacrolimus calculated for any of the 12 patients of group 2 for each time point or combinations of time points as for LSF (Figure 1).

Finally, a regression analysis was performed for both the LSF and the LSM with the reference trapezoidal $AUC_{0\text{-}12h}$.

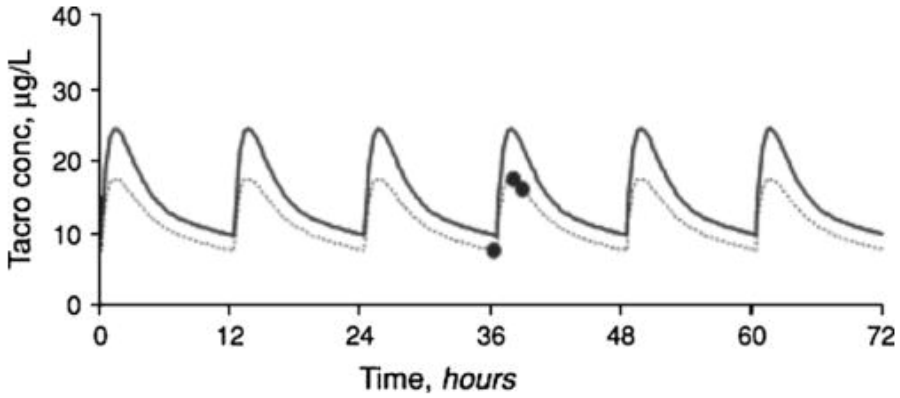

FIGURE 1. Tacrolimus blood concentration-time curve according to the population-based model (continuous line), the measured tacrolimus blood concentrations at t = 0 h, 2 h, 3 h in a patient (•), and the tacrolimus blood concentration-time curve according to the model after fitting the population parameters to the measured concentrations (dotted line) after which the $AUC_{0\text{-}12h}$ is calculated by the model.

Statistics

Statistical analysis of patient data was performed using SPSS 13.0 for Windows. Results are expressed as mean ± standard deviation and as median and range.

AUCs calculated by the different methods were compared with the trapezoidal calculated $AUC_{0\text{-}12h}$ by linear regression analysis (MW\Pharm) and Pearson correlation coefficient. P values below 0.05 were considered statistically significant.

Predictive performance of the different methods was also investigated by calculating the prediction precision and bias, which is deduced from the paper by Sheiner and Beal[27]. Prediction bias was calculated as the mean prediction error (MPE), that is the mean of differences between the $AUC_{0\text{-}12h}$ according to the different methods and the gold standard $AUC_{0\text{-}12h}$. Prediction precision was calculated as the mean absolute prediction error (MAPE), that is the mean of the absolute differences between $AUC_{0\text{-}12h}$ according to the different methods and the gold standard $AUC_{0\text{-}12h}$. Smaller values for MPE and MAPE indicate less bias and greater precision (practical clinical range based on smallest possible dose adjustment: ±10%).

RESULTS

Using multiple regression analysis, LSFs were calculated from 11 curves (group 1) based on one or a combination of measured blood concentrations. A few examples are shown in Table 1. The results of the performance in estimating the gold standard $AUC_{0\text{-}12h}$ (derived by the trapezoidal rule) of these LSFs are shown in the lower part of Table 2.

Time points blood sampling	Formula
LSF 0 h	AUC0–12h = 40.858 + 12.53*C0
LSF 4 h	AUC0–12h = -0.083 + 11.555*C4
LSF 6 h	AUC0–12h = 18.694 + 11.967*C6
LSF 8 h	AUC0–12h = 17.399 + 13.386*C8
LSF 0, 1, 3 h	AUC0–12h = 5.023 + 4.27*C0 + 1.24 * C1 + 5.04*C3
LSF 0, 2, 3 h	AUC0–12h = 11.884 + 5.842*C0 + 1.252*C2 + 3.841*C3
LSF 1, 4 h	AUC0–12h = –4.9 + 0.843*C1 + 10.556*C4

TABLE 1. Examples of Limited Sampling Formulae (LSF) Derived Using Multiple Regression Analysis.

Time Points Blood Sampling	r^2	MPE (%)	MAPE (%)
Without model or formula			
0	0.69		
Individualized POP-PK model (LSM)			
0	0.87	2	14
4	0.97	0	7
6	0.97	1	5
8	0.88	0	8
0, 2	0.96	3	6
0, 1, 3	0.99	3	5
0, 2, 3	0.98	2	5
1, 3	0.98	2	10
1, 4	0.96	4	7
3, 4	0.95	−1	9
4, 6	0.98	1	4
Limited sampling formulas (LSFs)			
0	0.68	5	17
4	0.94	0	7
6	0.90	2	8
8	0.93	2	8
0, 2	0.89	1	8
0, 1, 3	0.98	0	4
0, 2, 3	0.95	1	6
1, 3	0.94	1	7
1, 4	0.96	0	5
3, 4	0.94	0	6
4, 6	0.94	0	7

MPE, mean prediction error; MAPE, mean absolute prediction error.

TABLE 2. Performance of Estimation of $AUC_{0\text{-}12h}$ of Limited Sampling Models (LSMs) and Limited Sampling Formulae (LSFs).

The best single point markers for tacrolimus monitoring in terms of predicting systemic exposure (gold standard $AUC_{0\text{-}12h}$) to tacrolimus using LSF were C4 (r^2 = 0.94 [MPE/MAPE 0/7]), C6 (r^2 = 0.90 [2/8]), and C8 (r^2 = 0.93 [2/8]), all $P < 0.05$.

Precise multiple-point combinations using LSF were, for example, C1 + C4 (r^2 = 0.96 [0/5]), C0 + C2 + C3 (r^2 = 0.95 [1-6]), and C0 + C1 + C3 (r^2 = 0.98 [0/4]), all $P < 0.05$.

The calculated mean POP-PK parameters based on group 1 are shown in Table 3. The upper part of Table 2 shows the performance of the individualized POP-PK model (LSM) in estimating the gold standard $AUC_{0\text{-}12h}$, the MPE and MAPE for single- and multiple-point sampling, validated on 12 patients (group 2).

Parameter	Mean (± Standard Deviation)
CLm (L/h/1.85 m^2)	3.9834 ± 2.0629
V1 (L/kgLBMc)	0.2469 ± 0.0898
K12 (/h)	0.5661 ± 0.2311
K21 (/h)	0.1464 ± 0.0620
Ka (/h)	1.2595 ± 1.1188
F_po	0.23 (fixed)

CLm, apparent clearance; V1, volume (central); K12, distribution rate constant (central to peripheral compartment); K21, distribution rate constant (peripheral to central compartment); Ka, absorption rate constant; F_po, oral bioavailability.

TABLE 3. Population Pharmacokinetic Parameters Calculated Using an Iterative Two-Stage Bayesian Procedure.

The best single point samples in terms of estimating systemic tacrolimus exposure using LSM appeared to be C4 and C6, which show excellent performance with the gold standard $AUC_{0\text{-}12h}$ (both $r^2 = 0.97$, $P < 0.05$) with excellent precision and bias (MPE/MAPE 0/7 and 1/5) (Figure 2).

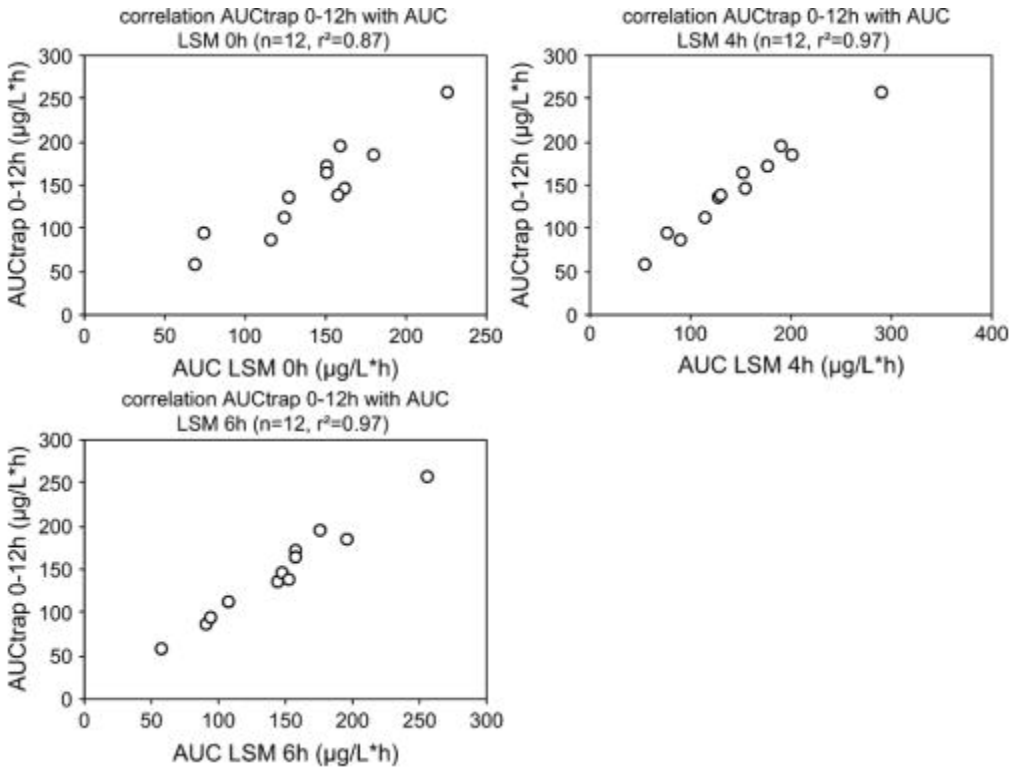

FIGURE 2. Performance of area under the concentration-time curves limited sampling model (LSM) 0 h, LSM 4 h, and LSM 6 h with "gold standard" $AUC_{0\text{-}12h}$.

Except for LSM 0 + 1 h, all examined multiple-point LSMs showed excellent performance in estimating the gold standard $AUC_{0\text{-}12h}$ (Table 2; r^2 = 0.94 or higher, not all data shown).

The widely used C0 showed poorer performance with the gold standard $AUC_{0\text{-}12h}$ both for LSF and LSM (r^2 = 0.68 and 0.87). More importantly, prediction precision for both methods was relatively high (MAPE 17% and 14%). Without using a model or formula, the r^2 of C0 with $AUC_{0\text{-}12h}$ was 0.69.

Based on the C0 target range of 5 to 10 µg/L for patients more than 3 months after OLT, we calculated an AUC target range with the use of the pharmacokinetic software package MW\Pharm. This range is 95 to 190 h*µg/L (target = [95 + 190]/2 = 142.5 h*µg/L).

The range can also be derived from Figure 3. This figure visualizes the relationship between the tacrolimus trough concentrations and AUC for this population of patients undergoing OLT. A wide range of AUC values is observed corresponding to the C0 monitoring range of 5 to 10 µg/L.

From this figure, possible other (lower) AUC target ranges can be deduced from trough concentration ranges (inserted range; see figure), eg, 4 to 8 µg/L (see "Discussion").

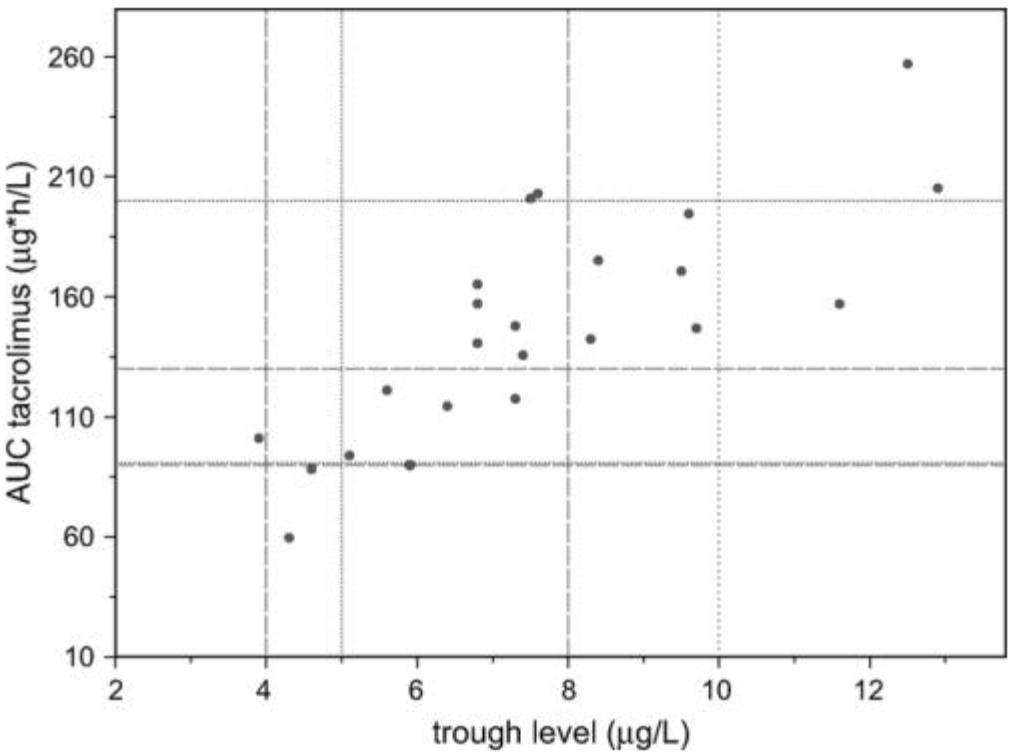

FIGURE 3. Relationship between trough concentration (C0) and area under the concentration-time curve (AUC) of all 23 patients while on C0 monitoring. The thin dotted lines (.....) show the range based on trough concentration monitoring of 5 to 10 µg/L (AUC target 142.5 h*µg/L). The other lines (- - - - -) show the proposed AUC range based on trough concentration monitoring of 4 to 8 µg/L, which is 80% lower than 5 to 10 µg/L (AUC target 110 h*µg/L; range, 90-130 h*µg/L).

DISCUSSION

In this study, we demonstrated that C0 monitoring for tacrolimus after liver transplantation is not precise and does not accurately reflect systemic exposure. We developed and validated individualized POP-PK models based on C4 or C6, which appear to accurately reflect systemic exposure of tacrolimus with excellent precision and bias. Recent studies on tacrolimus monitoring have suggested that trough concentrations, as currently used in most centers for therapeutic drug monitoring of tacrolimus, are not the best estimators of systemic exposure of this drug. These studies have involved different types of organ transplantation and vary in time after transplantation[28-30]. In our study, C0 monitoring did not have a good performance in estimating $AUC_{0\text{-}12h}$ without using LSF and LSM (r^2 = 0.69), or with using LSF (r^2 = 0.68 [MPE/MAPE 5/17]). Performance of C0 with $AUC_{0\text{-}12h}$ using LSM seems to be acceptable (r^2 = 0.87), but concentrating on MPE and MAPE, we conclude that the prediction precision (MAPE) is not in an acceptable range of ± 10% (MAPE 14%). Figure 3, which illustrates all 23 patients while on C0 monitoring, already showed a wide range of AUC values corresponding to the (currently accepted) C0 range of 5 to 10 µg/L. This confirms that trough concentrations do not adequately reflect systemic exposure of tacrolimus. Our finding that sampling between 4 and 6 hours postdosing seems optimal is in line with two other studies that suggested C4 and C5 sampling, respectively[15,16]. Our model has the advantage that it is very flexible. Others also found C0 insufficient in different patient populations[16,17]. Likewise, in cyclosporine monitoring, C0 and even C2 monitoring did not appear to be optimal, and several methods for optimizing therapeutic drug monitoring were developed by our group and others[3,6,7,13,19].

A limitation of our models and formulae is that these were developed and validated in two small independent groups of stable patients more than 6 months after liver transplantation. Given the considerable changes in tacrolimus kinetics shortly after transplantation, we cannot recommend using these models in less stable patients or early posttransplantation. For the period early after OLT, new models would need to be developed and validated.

The results concerning correlation with $AUC_{0\text{-}12h}$ for both LSF and LSM were satisfying with slightly better results for the model. The advantage of this model over LSF is that the model is flexible and no fixed time points are needed in contrast to the rigid formulae. As long as the exact time of blood sampling is noted, it is possible to use this time (and blood concentration) in the model as a result of the fact that this approach is based on Bayesian estimation. The AUC is calculated after estimating the individual clearance and dose advice is given.

Comparing single and multiple point monitoring, the latter group showed, in most cases, a slightly better performance in estimating $AUC_{0\text{-}12h}$.

However, despite this slightly better performance, LSM C4 and LSM C6 already had r^2's of 0.97 (MPE/MAPE 0/7 and 1/5). Therefore, these single point LSMs seem sufficient. For practical reasons, both the C4 and the C6 model seem feasible. Patients can take their medication at home, visit the hospital for checkups, and blood can be drawn 4 to 6 hours after the morning dose, not interrupting the medication schedule. There is no need to take the blood sample exactly on time as long as the dosing and blood sampling time are recorded. These factors, in combination with the adequate performance of the model in the outpatient setting, which is normally a source of variability, provides a tool for adequate monitoring of tacrolimus.

The calculated AUC target range based on C0 monitoring (90-195 h*µg/L) is rather wide, which also suggests that C0 monitoring is not the optimal way for therapeutic drug monitoring of tacrolimus. In kidney transplantation in our clinic, for stable patients, a target AUC of 125 h*µg/L is adhered to (range, 100-150 h*µg/L), corresponding to a trough concentration of 7.5 µg/L[17].

Currently, in the field of OLT, a trend with regard to reduction in calcineurin inhibition is noticeable. In a review article from Staatz et al, lower targets are described for liver transplantation compared with kidney transplantation[31]. With respect to this trend, and after observing Figure 3, we decided to adopt a new target, slightly lower than used for kidney transplantation, in the stable period more than 6 weeks posttransplantation[17] and also lower than the range corresponding with C0 = 5 to 10 µg/L, which we were using in our clinic.

Thus, for the last 6 months, we have lowered the C0 range from 5 to 10 µg/L to the arbitrary range of 4 to 8 µg/L, which is 80% of the original range, without rejection (data not shown). We now calculate a new AUC target and AUC target range, which is 80% of the original AUC target (142.5 µg/L) and which is based on the lowest possible dose adjustment of 0.5 mg, which would be, respectively, 110 h*µg/L for the target and 90 to 130 h*µg/L for the range. The new target AUC of 110 h*µg/L is based on the C0 concentration of (4 + 8)/2 = 6 µg/L. The new range (90-130 h*µg/L) is wider than the lowest possible dose adjustment of 0.5 mg, which makes it practical in daily use. The new target is visualized in Figure 3 and the clinical consequences will be studied prospectively.

The current trend toward lower target ranges underlines the need for precise monitoring, because tacrolimus underexposure should be avoided with respect to the prevention of rejection episodes. High tacrolimus exposure should be avoided as well, especially in the stable phase post-OLT, with regard to clinical toxicity such as nephrotoxicity, which could have a clear negative impact on patient and graft survival[1,2].

With more accurate prediction of systemic exposure of tacrolimus in the first 12 hours after dosing with the individualized LSMs C4 or C6, we have developed we expect

improvement in clinical outcome such as decrease in rejection rate, less (nephro)toxicity, and fewer infections. We are planning further validation with a prospective, randomized, controlled trial comparing C0 and LSM 4 h (or 6 h) monitoring, which includes clinical outcome parameters such as renal function, blood pressure, rejection, and laboratory parameters.

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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.

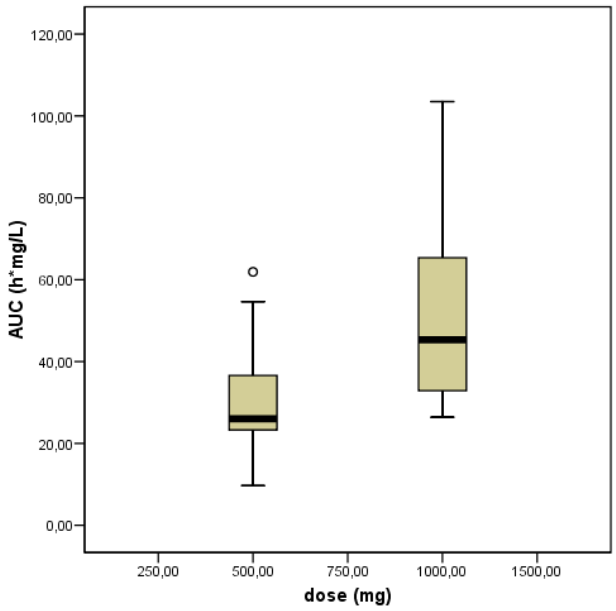

Figure 1: MMF dose versus trapezoidal MPA-AUC relationship of patients with MMF dose 500 mg and 1000 mg twice daily (n=31, dose 500 mg: n=18; dose 1000 mg: n=13)

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.

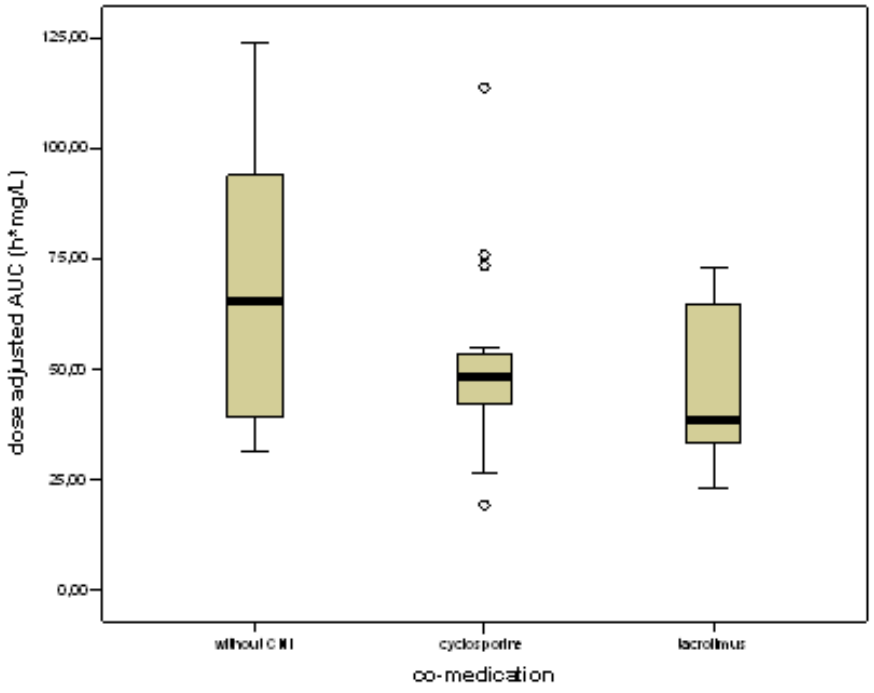

Figure 2: Patients without calcineurin inhibitors; patients with cyclosporine and patients with tacrolimus as co-medication next to MMF and their (non significant) difference in dose adjusted AUC (p=0.247). The circles in the plot indicate individual (cyclosporine) patients outside the range.

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 $p<0.05$.

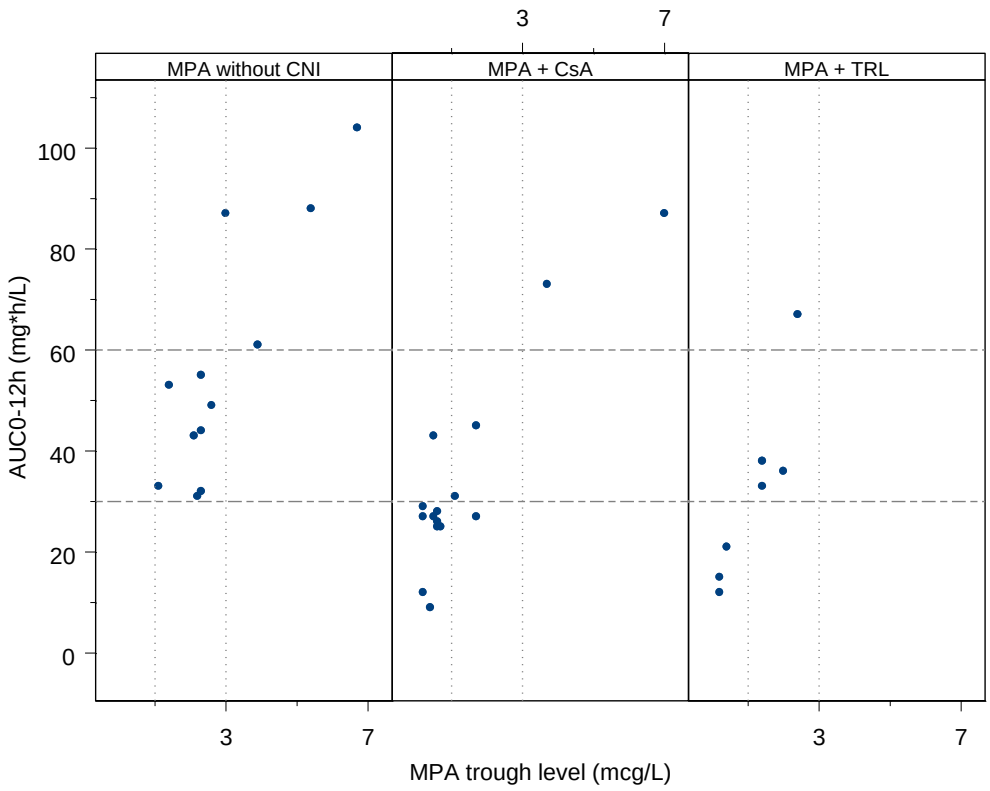

Figure 3: Relationship of MPA trough level with trapezoidal AUC0-12h for different groups of co-medication next to MMF: without CNI, with cyclosporine (CsA) and with tacrolimus (TRL)

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.

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CHAPTER 7

SUMMARY AND DISCUSSION

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SUMMARY

After using C0-monitoring as the tool for therapeutic drug monitoring of cyclosporine for many years, studies suggested that C2-monitoring might be better in terms of predicting systemic exposure to cyclosporine. After switching 31 liver transplant patients using cyclosporine from C0 to C2 monitoring in **chapter 2** in 21/31 patients (68%) the cyclosporine dose was lowered and in the other patients the dose remained unchanged. For patients whose dose of cyclosporine was lowered, improvement of renal function and some decrease in mean- and systolic morning blood pressure was observed. C2 correlated better ($r^2 = 0.75$) than C0 ($r^2 = 0.64$) with the area under the curve after the first 12 hours after dosing (AUC0-12h). A problem we observed was the significant intrapatient variability. In 13/21 patients whose dose was lowered the second AUC was below the target range but only 2/13 developed rejection.
Because of the problem of overdosing with C0-monitoring and episodes of underdosing with C2 monitoring in **chapter 3** we developed new, accurate and flexible limited sampling strategies to optimize therapeutic drug monitoring of cyclosporine. We developed (rigid) limited sampling formulas (LSF) and flexible limited sampling models (LSM). The models showed even better correlations with AUC0-12h than the formulas. Combinations of blood sampling time points 0+2h ($r^2 = 0.94$); 0+1+2h ($r^2 = 0.94$); 0+1+3h ($r^2 = 0.92$); 0+2+3h ($r^2 = 0.92$) and 0+1+2+3h ($r^2 = 0.96$) showed excellent correlation with AUC0-12h with acceptable precision and bias.
When evaluating in **chapter 4** the LSM 0+1+2+3h model that best correlated with AUC0-12h in the 18 months after introduction there was no significant change in average cyclosporine dose and creatinine clearance, compared to the previous C2-monitoring. Also the number of rejections was comparable. There was wide inter- and intrapatient variability in the time to reach peak concentrations of cyclosporine after dosing. The variation coefficient of clearance based on all patients was 15%. When investigating the required precision, the correlation of two 2-point and three 3-point models with LSM 0+1+2+3h were very good with acceptable bias and precision: LSM 0+2h ($r^2 = 0.88$); LSM 0+3h ($r^2 = 0.87$); LSM 0+1+2h ($r^2 = 0.84$); LSM 0+1+3h ($r^2 = 0.91$) and LSM 0+2+3h ($r^2 = 0.92$). We also calculated these correlations per patient and these results show that other limited sampling models with less time points show comparable results as LSM 0+1+2+3h. Especially LSM 0+2h was optimal in terms of accuracy, ease-of-use and intrapatient variability.
When optimizing tacrolimus monitoring after calculating limited sampling formulas (LSF) in **chapter 5** different single point and multiple-point combinations showed good correlations with AUC0-12h: LSF 4h ($r^2 = 0.94$); LSF 6h ($r^2 = 0.90$); LSF 8h ($r^2 = 0.93$); LSF 1+4h ($r^2 = 0.96$); LSF 0+2+3h ($r^2 = 0.95$) and LSF 0+1+3h

(r^2 = 0.98). The best single point calculation in terms of estimating systemic tacrolimus exposure using limited sampling models (LSM) were LSM 4h (r^2 = 0.97) and LSM 6h (r^2 = 0.97). Also, multiple-point LSMs showed excellent correlation with AUC0-12h. The correlation of the widely used C0 with AUC0-12h was not as good for both LSF and LSM (r^2 = 0.68 and 0.87), both also with relatively high prediction precision errors (MAPE 17% and 14%). The new calculated AUC target range for tacrolimus was 95-190 h.µg/L.

During the study of the pharmacokinetic behaviour of MMF in **chapter 6** we found a linear relationship between MMF dose and trapezoidal MPA area under the curve. There was a wide range in MPA clearance in the population (8.08 – 57.47 L/h). 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, $p<0.05$). There also was a significant relationship between creatinine clearance and MPA clearance (r^2 = 0.36, $p<0.05$).
Based on clinical selection, two groups (with and without calcineurin inhibitors) were used for further development of limited sampling models for therapeutic drug monitoring of MPA.
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. The combination 0-½-1-2h showed 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).
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 ($p<0.05$).
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 $p<0.05$.

DISCUSSION

Cyclosporine

Switching cyclosporine monitoring from C0- via C2-monitoring and subsequently to LSM 0+1+2+3h allowed us to compare the biochemical and clinical effects of these three methods.

During the conversion from C0 to C2 cyclosporine monitoring in stable patients more than 6 months after liver transplantation, we saw a significant decrease in cyclosporine dose in two-thirds and an unchanged dose in one-third of the patients. Dose reduction resulted in lower systemic exposure and an improvement of renal function, but only small but significant changes in morning systolic and mean morning blood pressures were observed, with questionable clinical significance. The fact that the kidney function did not improve in all patients who had a dose reduction may be due to long-term exposure to cyclosporine, which may have caused a fixed renal insufficiency. Also, further improvement in renal function might require more time. Based on calculating the area under the concentration time curve from 0 to 12 hours (cyclosporine blood levels), the correlation of C2 with AUC0-12h was better than the correlation of C0 with AUC0-12h.

However, in almost one-half of the patients, there was significant intrapatient variability of the C2 blood levels with the same dose. This made therapeutic drug monitoring with C2 levels less accurate and may induce many unnecessary subsequent changes in drug dose, which is inconvenient for patients, doctors and nurses. We found it disturbing that, although two preceding C2 levels were within the 600 ng/mL ± 15% range, in 13/21 patients whose dose was lowered the second AUC was below the target AUC of 3380 – 4266 h.µg/L, although only 2 out of these 13 patients developed rejection. The fact that these patients were 9 and 10 months post OLT may mean that the dose recommendations of G. Levy and not those of E. Cole should be followed when using C2 monitoring[1,2].

While on C2 monitoring, 17/31 patients had a second AUC outside the AUC target range. Not all patients may need to have an AUC within the range of the 'target range AUC'. It seems safer if the value is within the target range, but this may lead to an unnecessary worse renal function. A compromise would be to have an AUC on day 2 in the lower half of AUCs while on C0, which is 3380 – 3823 h.µg/L. Because 11/13 patients with an AUC below the target AUC while on C2 monitoring did not develop rejection, many patients may tolerate lower AUCs.

Other studies saw a better correlation of C2 with AUC when compared to trough-level monitoring in renal and liver transplant recipients[3-15]. Most studies in renal transplantation and the limited studies in liver transplantation using C2 monitoring also showed improved kidney function. Often blood pressure and serum cholesterol also

improved. In those studies no rejection occurred despite lower exposure to cyclosporine. However, in the liver transplant studies mentioned AUC was calculated by measuring multiple cyclosporine blood levels during 4 and 6 hours post-dosing only, while we used 0-12 hour AUCs. This fact may explain some of the difference between these and our studies. Another explanation for the difference with the kidney studies may be the lower maintenance levels used in liver transplantation when compared to kidney transplantation: further lowering of the already low dose after liver transplantation may more easily lead to rejection.

In our study all cyclosporine concentration blood samples were taken as recommended [1,2,16] and within 2 minutes from the targeted time (although 10 minutes are allowed); if sampling time would have been more variable (as may be the case in daily practice), an even lower accuracy of C2 monitoring and inappropriate dose adjustments might occur[17]. In renal transplantation variable cyclosporine levels may contribute to chronic rejection[18]. Although chronic ductopenic rejection has become less common after liver transplantation in the past decade, it forms a continuum with acute cellular rejection; chronic underexposure to cyclosporine can be a cause[19-22]. In renal transplant studies it was shown that absorption profiling over the first 4 hours was superior to trough-level monitoring, with C2 as the best single-point predictor of AUC[3,23-26]. The clinical superiority of such absorption profiling over C2 levels has not been examined in those studies. Our data demonstrated that in stable liver transplant patients trough-level monitoring frequently leads to overdosing of cyclosporine, while monitoring by C2 may cause episodes of underdosing in some patients. Therefore, better ways of monitoring cyclosporine dosing in liver transplantation were awaited. Because both IL2 blood concentration and AUC0-12h are related to cyclosporine exposure in the first 4 hours after dosing it seems logical to use a sparse-sampling method over the first hours after dosing. In accordance with others, our data demonstrated that, if AUC is calculated from cyclosporine levels, using the trapezoidal rule, in the first 3 hours after dosing the correlation with $AUC_{0\text{-}12h}$ is 0.96[23,27]. Thus use of a sparse sampling method may avoid over- and underdosing and unnecessary changes in dose.

We then developed a new, accurate, flexible and precise method for cyclosporine monitoring in stable patients more than 6 months after liver transplantation based on an individualized population pharmacokinetic (PK) limited sampling model. This contrasted to most limited sampling strategies in that the other strategies were only based on population pharmacokinetics, while our PK model is based on population pharmacokinetics as well as Bayesian fitting of limited sampling data from one patient. A major advantage of the new method over methods based on population kinetics only was that sampling time points are more flexible than with C2 monitoring, limited sampling formulas (LSFs) or current POP-PK models. Our model is efficient as long as

the exact dosing and sampling time, the weight of the patient and the dosing rhythm are registered and sampling time is near the required time after dosing. Both population and individual kinetics are incorporated in the model, making optimal use of all available information. Blood concentration data are put into the computer model, which runs on a desktop PC, the AUC is calculated and a dose modification is suggested. It is still necessary to obtain more than one blood concentration of cyclosporine during the dosing interval in order to obtain adequate estimates (>90%) of AUC0-12h.

For cyclosporine the correlation with AUC0-12h of the individualized POP-PK model was better than with LSFs, especially when less than three sampling points were used. The models with sampling time points 0+2h; 0+1+2h; 0+1+3h; 0+2+3h and 0+1+2+3h showed excellent correlation ($r^2 > 0.90$) with the gold standard AUC0-12h. Results even for C0 combined with the model were better than those for simple C0 or C2. The r^2 for C2 was below 0.80 even with an individualized POP-PK model or LSF. It was almost always necessary to include a trough blood sample in the LSMs in order to achieve a correlation (r^2) > 0.90.

Based on the developed POP-PK model and generally accepted cyclosporine trough levels of 90–125 µg/L, the AUC range should be 2900–3800 h.µg/L. We introduced this target range into our clinic, although from the previous studies we knew that some patients may tolerate lower values.

Using an individualized POP-PK model with multiple sampling points requires some organization in the clinic but in our experience this is feasible and the advantages are clear.

It had already been shown that using multiple sampling points in the first hours after dosing with Bayesian forecasting results in a better correlation with AUC0-12h[28–31]. A high inter-individual variability in cyclosporine pharmacokinetics exists, which seems unrelated to CYP3A polymorphisms[32]. Therefore, the use of multiple sampling models may avoid over- and underdosing and unnecessary changes in dose. A disadvantage of available LSFs and POP-PK models was that multiple samplings were needed on fixed time points. It was previously stated that the ideal model should be easy to use and flexible, without the rigid time points and complicated methods used in current multiple sampling models. Ideally it should be based both on population kinetics and on individual pharmacokinetics[30,31,33,34]. The LSM 0+1+2+3h we presented clearly approximated this goal. A similar model performed well in kidney as well as combined kidney–pancreas transplant patients[35]. Because of the superiority of LSM 0+1+2+3h ($r^2 = 0.96$) we introduced this model into our clinic.

Next, in stable patients it might in the long term be possible to reduce both the number of samplings per visit and the number of visits to the clinic while still getting sufficient prediction of AUC. We therefore evaluated our model after using it for more than

18 months. We showed that our LSM 0+1+2+3h-method accurately estimated systemic exposure to cyclosporine in OLT patients. However, there appeared to be considerable intra-patient variability in the time to reach the peak-concentration of cyclosporine. This led to the same number of dose adjustments as with C2-monitoring in the 18 months before the switch from C2 to LSM 0+1+2+3h. The intrapatient pharmacokinetic variability may partially be due to interaction with food or other medication. The variation in peak-time is partially responsible for the large intra-patient variation in C2 levels over time in some of the patients. Using a limited sampling model with more sampling time points all important information required for calculating an AUC is obtained and the chance of ´missing´ this variability is less, which leads to more accurate AUC estimations.

After more than 1,5 year of using our model for cyclosporine monitoring in the outpatient clinic 152 LSM 0+1+2+3h curves from 30 patients were derived. Although this was not a randomized controlled trial these stable patients were their own controls. According to the dose, renal function and rejection on average there was no difference using C2-monitoring or the individualized PK-model. However, the target range was based on AUCs while on C0-monitoring. In the first study, while on C2-monitoring, we saw two rejections in 13 cases where the AUC dropped below the AUC target-range. Apparently an AUC below 2900 h.µg/L was tolerated in most of these patients. This was similar for LSM 0+1+2+3h monitoring: for some patients the dose was not increased because of renal insufficiency if LSM 0+1+2+3h gave an AUC below the target range , but in spite of that usually no signs of rejection occurred.

Although there was no significant change in creatinine clearance between C2-monitoring and LSM 0+1+2+3h there seemed to be a trend toward lower CRCL with LSM versus C2-monitoring ($p=0.071$), despite the fact that the same target range for AUC was used. More data is needed to confirm that cyclosporine dosing by LSM may lead to less toxicity than C2-based dosing.

The current data allowed us to investigate the true natural variability in PK of cyclosporine in stable OLT-patients. The mean intra-patient variability of the apparent oral clearance of cyclosporine in these stable liver transplantation patients was 15%. This means that a dose-adjustment of 16 mg or less (15% of mean dose of 109 mg) is not rational, because this difference is a natural variation which cannot be avoided. In fact, the lowest possible dose adjustment (25 mg) in practice is relatively close to this natural variation of 16 mg. In case the mean dose of 109 mg and a 95% confidence interval (mean ± 2.SD) would be used, a target range of 2380-4390 h.µg/L would be rational. In other words, any AUC-value within this range can be explained by natural variability in PK of cyclosporine and may therefore not require a dose adjustment. In our hospital a target-range of 2900-3800 h.µg/L was used for stable OLT patients, which is narrower, and closer to a mean ± 1.SD value of the AUC in this population,

which is 2680-3620 h.µg/L. However, to be on the safe side until now we remain adhering to this narrow range, although we realize that this may be too strict. Based on the current data, a lower range for the AUC than currently used with a target AUC of 2830 h.µg/L (2380-3280 h.µg/L) may be reasonable. Our data suggest that, considering the natural variability in PK of cyclosporine in stable OLT patients, our method with LSM 0+1+2+3h may be unnecessary accurate in terms of estimating systemic exposure to cyclosporine.

When investigating the correlation between LSMs with only two or three sampling points and LSM 0+1+2+3h we see that overall five models showed good correlation when considering both the AUCs and the mean advised dose. These five LSMs were 0+2h; 0+3h; 0+1+2h; 0+1+3h and 0+2+3h. Accuracy and bias were acceptable. The trough level is included into all of these models, which (again) illustrates the pivotal role of this trough sample for assessing systemic exposure to cyclosporine, although models on C0 only are inaccurate. When developing the model we already noticed a very good correlation of these models with the gold standard AUC0-12h (for LSM 0+2h this was: r^2=0.94, MPE=-9, MAPE=9) with less bias and greater precision than e.g. C2 single-point monitoring (r^2=0.78, MPE=-10, MAPE=12) or Ctrough[36]. In spite of the fact that LSM 0+1+2h includes both the common 1- and 2-hour peak-level time points, the correlation of this model with LSM 0+1+2+3h in the patients with five or more curves is not different from LSM 0+2h (r^2= 0.84-1.00 vs 0.81-0.99). Comparing LSM 0+1+2h with LSM 0+2h, the 0+2h-model has the benefit that it is easier to apply in practice, it is more friendly for the patient and the medical staff, and there is a cost-benefit. Therefore this model seems to be an optimal balance between patient benefit and discomfort. A large randomized controlled trial between C2 and LSM 0,2h with a target AUC of 2830 h.µg/L (range 2380-3280 h.µg/L) would be of interest.

In conclusion, while cyclosporine C0-monitoring frequently results in overdosing and more renal dysfunction, C2-monitoring may lead to episodes of underdosing but rejection in only some of these patients and it may lead to many subsequent dose adjustments. We therefore devised and introduced a flexible Bayesian individualized population pharmacokinetic limited sampling model for cyclosporine monitoring, without rigid sampling time points. This model is accurate and easy to use in daily practice. After using LSM 0+1+2+3h for more than 18 months we showed the feasibility of implementation of this method. Considering the natural variability in pharmacokinetics of cyclosporine LSM 0+1+2+3h may be unnecessary accurate in terms of estimating systemic exposure of cyclosporine. Reducing the numbers of samplings per visit to LSM C0+C2 seems to be an optimal balance between patient benefit and discomfort.

Tacrolimus

Therapeutic drug monitoring of tacrolimus in many clinics is based on trough-level (C0) monitoring. Recent studies including patients with varying time after transplantation and different types of organ transplantation showed that C0 might be not the best estimator of systemic exposure of tacrolimus[37-39].

In our study we demonstrated that indeed C0 monitoring is not very precise for tacrolimus monitoring after OLT and that this time point does suboptimally reflect systemic exposure to tacrolimus in the first 12 hours after dosing. We investigated strategies for tacrolimus monitoring and developed and validated individualized population pharmacokinetic (POP-PK) models based on blood sampling time points C4 or C6, which appeared to very accurately reflect systemic exposure of tacrolimus with excellent precision. Our finding that sampling between 4 and 6 hours after dosing seems optimal was in line with two other studies that suggest C4 and C5 sampling respectively[40,41]. Others also found C0 to be not very accurate in different patient populations[42,43].

In our study the results concerning correlation with AUC0-12h for both calculated limited sampling formulas (LSF) and LSM were satisfying, with slightly better results for the model. The advantage of the formula is the simplicity of the calculation. The advantage of the model above LSF is that the model is flexible and no fixed time points are needed, in contrast to the rigid formulas.

Comparing single- and multiple-point monitoring the latter group showed in most cases an almost perfect correlation with AUC0-12h. But, in spite of this slightly better correlation, LSM C4h and LSM C6h already had r^2's of 0.97. Therefore, single-point LSMs seem sufficient. For practical reasons both the C4 and the C6 model seem feasible. Patients can take their medication at home at the normal time, visit the hospital for checkup and blood is taken 4-6 hours after taken the morning dose. In contrast to C0 monitoring this new method does not interrupt the regular dosing, improving compliance and reducing error in measuring levels. Because the model is based on Bayesian estimation, there is no need to take the blood sample exactly on time, as long as the dosing and blood sampling time are recorded. The measured blood concentration is introduced in the model and after estimating the individual clearance the AUC is calculated and a dose advice is given. These factors in combination with the adequate performance of the model in the outpatient setting, which is normally a source of variability, provides with a tool for improved monitoring of tacrolimus.

A limitation of our models and formulas is that these were developed and validated in two small independent groups of stable patients more than 6 months after OLT (11 and 12 patients). Given the considerable changes in tacrolimus kinetics shortly after transplantation, we do not recommend using these models in less stable patients

early post transplant. For these patients new models need to be developed and validated.

The calculated AUC target range based on C0 monitoring (90 - 195 h.µg/L) is very wide, which also suggests that C0 monitoring is not the optimal way for therapeutic drug monitoring of tacrolimus. In kidney transplantation in our clinic for stable patients a target AUC of 125 h.µg/L is adhered to (range 100 - 150 h.µg/L), corresponding to a target trough-level of 7.5 µg/L[43]. Currently, in the field of OLT a trend towards reduction in (nephrotoxic) calcineurin inhibition is noticeable. Moreover, in a review article from Staatz et al. also lower targets are described for liver transplantation compared to kidney transplantation[44]. With respect to this trend and after observing Figure 1 depicting our data we decided to adopt a new target range, which is slightly lower than used for stable kidney transplantation patients more than 6 weeks after transplantation, and also lower than the range corresponding with C0 = 5 - 10 µg/L which we were using in our clinic[43].

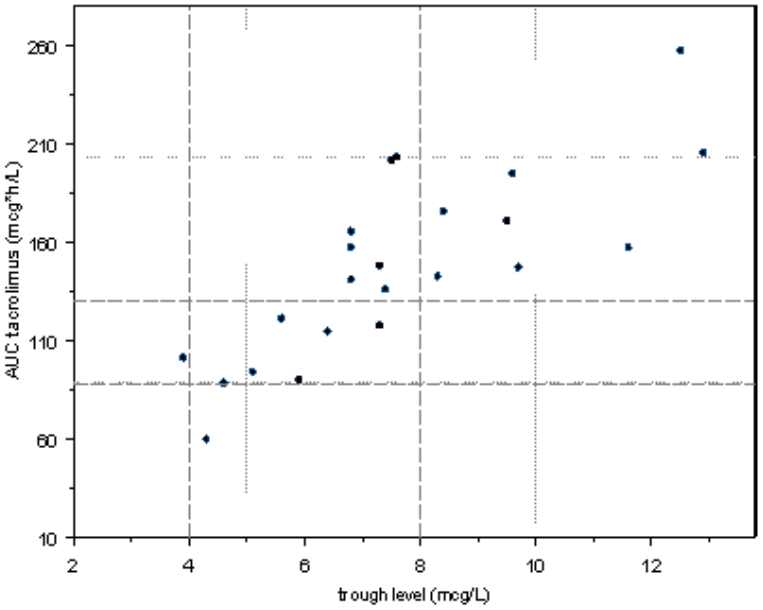

Figure 1: Relationship between trough level (C0) and AUC of all 23 patients, while on C0-monitoring. The thin dotted lines (……) show the range based on trough-level monitoring of 5-10 µg/l (AUC target 142.5 h*µg/l). The other lines (- - - - -) show the proposed AUC range based on trough-level monitoring of 4-8 µg/l which is 80% lower than 5-10 µg/l (AUC target 110 h*µg/l, range 90-130 h*µg/l).

We lowered the C0-range from 5 - 10 µg/L to the (arbitrary) range of 4 - 8 µg/L, which is 80% of the original range. When calculating a new AUC target and AUC target range we calculated 80% of the original AUC target (142.5 h.µg/L) and based the target range on the lowest possible dose-adjustment of 0.5 mg, which would be respectively 110 h.µg/L for the target and 90 - 130 h.µg/L for the range. The new target AUC of 110 h.µg/L is based on the C0-level of (4 + 8) / 2 = 6 µg/L. The new range (90 - 130 h.µg/L) is wider than the lowest possible change due to a dose adjustment of 0.5 mg, which makes it practical in daily use. The new target is visualized in Figure 1 and the clinical consequences of C4 monitoring with this range are currently being studied prospectively.

High tacrolimus exposure should be avoided in the stable phase post OLT since clinically relevant toxicity, such as nephrotoxicity, can have a clearly negative impact on patient and graft survival[45,46]. The current trend towards lower target ranges underlines the need for precise monitoring, since tacrolimus underexposure and rejection should be avoided.

In conclusion, in our study C0-monitoring of tacrolimus (Prograft BID) did not have a good correlation with AUC0-12h using LSF ($r^2 = 0.68$) or without using LSF and LSM ($r^2 = 0.69$). Correlation of C0 with AUC0-12h using LSM seems to be acceptable ($r^2 = 0.87$) but concentrating on MPE and MAPE we have to conclude that prediction precision errors (MAPE) are not in our range of ±10% (MAPE 14%). This confirms that trough-levels do not very well reflect systemic exposure of tacrolimus. Limited sampling models and limited sampling formulas based on sampling time points 4h or 6h showed excellent correlation with AUC0-12h, with acceptable bias and precision. We are currently further validating C4 monitoring in a randomized controlled trial.

Mycophenolate mofetil

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 indirectly 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[47]. 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 2 (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) [48-51]. An article from Yau et al. already concluded that fixed dose regimens of MMF may not be optimal for all patients[52]. Another important reason is the inter-patient variation in MPA pharmacokinetics, due to factors such as renal function, albumin level and (cyclosporine) co-medication[53,54-57]. 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[58]. Furthermore, an increase of Cmax and AUC of MPA in renal transplant recipients in the months after transplantation is described[59]. This may require dose adjustments.

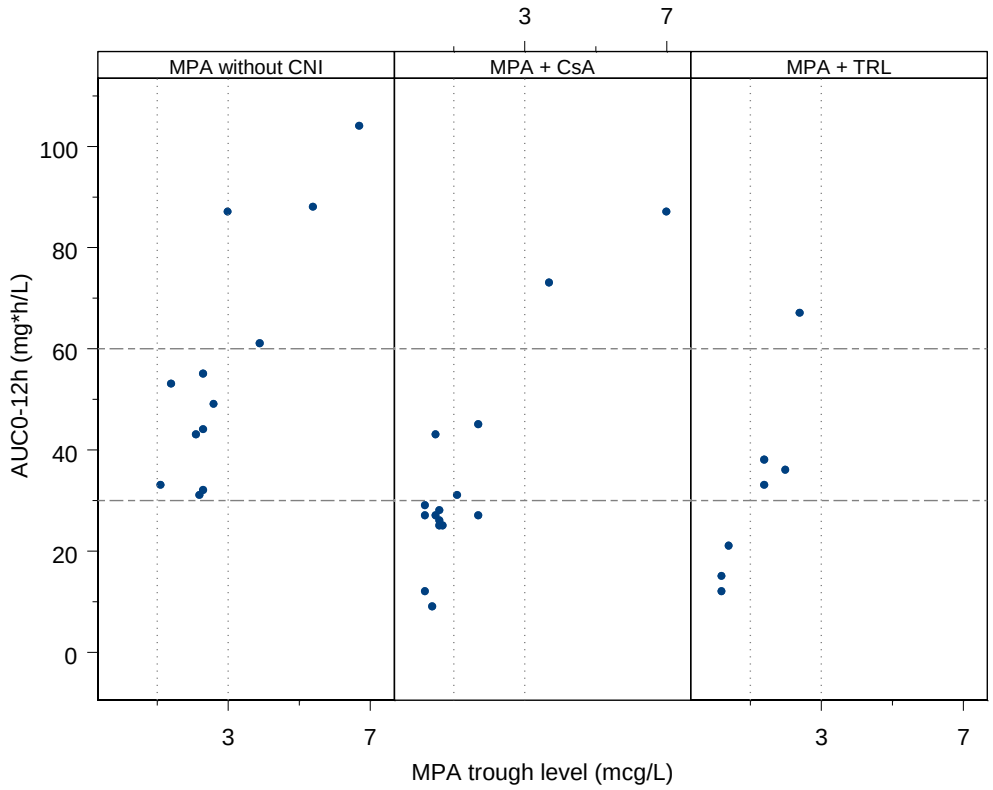

Figure 2: Relationship of MPA trough level with trapezoidal AUC0-12h for different groups of co-medication next to MMF: without CNI, with cyclosporine (CsA) and with tacrolimus (TRL)

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[60,61]. The discontinuation of CNI may lead to better kidney function in the long term[62,63]. 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[62,64-66]. 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 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[67]. 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[68]. Zicheng et al. developed rigid limited sampling algorithms for implementation of MPA-monitoring in liver transplantation necessitating exactly timed blood sampling[69]. 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[70].

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 four patients who did not adequately described the data during model building and the six 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[54,71-74]. Cyclosporine has an influence on MPA clearance by disrupting the enterohepatic cycle, leading to lower MPA exposure[75]. 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[76]. Figure 2 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[77]. 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[70]. 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[78]. 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[78]. 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.

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NEDERLANDSE SAMENVATTING

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Na orthotope levertransplantatie worden afweeronderdrukkende medicijnen (immuunsuppressiva) gegeven om afstoting van de donor-lever tegen te gaan. Deze medicamenten hebben hun werking de afgelopen decennia bewezen door een verlaging van het aantal (acute) afstotingen (rejecties) na transplantatie.
Een groep van deze immuunsuppressiva is die van de calcineurine-remmers, waartoe cyclosporine en tacrolimus behoren. Een kenmerk van deze medicijnen is hun nauwe therapeutische breedte. Onderdosering kan leiden tot rejectie en overdosering kan leiden tot het optreden van bijwerkingen, waarvan nierfunctiestoornissen de belangrijkste zijn. Adequate dosering van deze medicijnen is daarom uiterst belangrijk.
De dosering van cyclosporine en tacrolimus gebeurt op basis van het prinicipe van "therapeutic drug monitoring" (TDM). Dit houdt in dat op basis van de gemeten concentratie van het medicijn in het bloed de dosering zonodig wordt aangepast.
De monitoring van cyclosporine en tacrolimus gebeurde voornamelijk op basis van de dalspiegel van de medicijnen. Dit is de concentratie gemeten net voor inname van de medicijnen (C0), welke dus laag is. Voor mycophenolate mofetil is men het er nog niet over eens of het principe van TDM een duidelijke meerwaarde op zou leveren of niet.

Cyclosporine
De afgelopen jaren verschenen artikelen in de literatuur dat het beter zou zijn om cyclosporine niet te monitoren op dalspiegel (C0) maar op basis van de concentratie 2 uur na inname van dit medicijn (C2). Dit tijdspunt zou de totale systemische blootstelling van cyclosporine over de periode van 12 uur na inname van dit medicijn beter weerspiegelen. Vanwege deze aanbevelingen besloten wij onze levertransplantatie patiënten om te zetten van C0 naar C2 monitoring **(hoofdstuk 2)**. Na deze omzetting bleek 68% van de patiënten een lagere dosis cyclosporine nodig te hebben en bij de overige 32% bleef de dosis gelijk. Er werd een significante verbetering van de nierfunctie waargenomen en kleine veranderingen in systolische- en gemiddelde ochtend-bloeddrukken bij degenen bij wie de dosis verlaagd was.
De correlatie van C2 met de oppervlakte onder de concentratie-tijd-grafiek (area under the curve, AUC) was beter dan van C0 met de AUC ($r^2 = 0.75$ versus $r^2 = 0.64$). Wat opviel was dat er een duidelijke variabiliteit was binnen patiënten (intra-patiënt variabiliteit), terwijl patiënten op dezelfde dosering cyclosporine stonden. Hierdoor wordt de C2-monitoring bemoeilijkt en dit kan leiden tot onnodige dosisaanpassingen, wat niet goed is voor patiënten, maar ook een onnodige belasting van artsen en verpleegkundigen. Het was storend dat 13 van de 21 patiënten van wie de dosering verlaagd was, nadat ze 2 keer met een C2 binnen de target range zaten, met dezelfde dosis een AUC hadden onder de AUC target range. Hiervan ontwikkelden er 2 zelfs een rejectie. Het feit dat 11 van de 13 patiënten geen rejectie ontwikkelden geeft aan dat veel patiënten lagere AUCs dan geadviseerd tolereren. Totaal hadden 17 van de 31

patiënten AUCs buiten de range. De AUC target range die wij hanteren in de kliniek, gebaseerd op de algemeen geaccepteerde cyclosporine dalspiegel concentraties van 90 – 125 µg/L, is 2900 – 3800 h.µg/L.
Onze gegevens lieten zien dat C0 monitoring vaak leidt tot overdosering, maar dat C2 monitoring kan leiden tot perioden van onderdosering en zelfs soms rejectie. Omdat de concentratie in het bloed en 12-uurs AUC gerelateerd zijn aan cyclosporine blootstelling in de eerste 4 uur na dosering lijkt het logisch om een verkorte curve te maken waarin deze concentratiepunten voorkomen teneinde overdosering en onderdosering te voorkomen. Een nadeel van deze methode is dat er vaste tijdstippen nodig zijn. De ideale situatie zou zijn om een model te hebben dat flexibel is, zonder de noodzaak van deze vaste punten en dat dit model gebaseerd is op populatie farmacokinetische parameters en op individuele farmacokinetiek.
Vanwege de noodzaak om de monitoring van cyclosporine te verbeteren hebben wij in **hoofdstuk 3** verschillende strategieën ontwikkeld. Eén strategie was met behulp van formules voor tijdstippen en combinaties van tijdstippen waarop bloed werd afgenomen, verkregen met behulp van regressie analyses (limited sampling formulas, LSF). De andere strategie was het ontwikkelen van een model, dat gebaseerd is op verkorte curves met een beperkt aantal tijdspunten (limited sampling models, LSM). Dit model is gebaseerd op geïndividualiseerde populatie farmacokinetiek en op Bayesiaanse kansen. De modellen met tijdspunten 0+2h, 0+1+2h, 0+1+3h, 0+2+3h en 0+1+2+3h lieten zeer goede correlaties zien met de gouden standaard AUC van 0-12 uur. Het model met alleen tijdspunt C0 was ook nog beter dan de 'gewone' C0 en C2, zonder gebruik te maken van het model. Opvallend was dat het tijdspunt C0 bijna altijd nodig was om een goede correlatie te krijgen.
Het model gebruikt dosering, de tijd van bloedafname, het gewicht van de patiënt en de doseringsintervallen. Zowel de kinetiek van de populatie als van het individu zitten in het model. De gegevens worden in de computer ingevoerd en een eventuele dosiswijziging wordt geadviseerd. Dit model is veel flexibeler dan de limited sampling formulas (LSF), waarbij de bloedafname exact op tijd moet gebeuren.
Het lijkt erop dat dit model met verschillende tijdspunten veel tijd en organisatie kost, maar we hebben de ervaring dat dit in de praktijk erg mee valt en dat de voordelen van deze methode duidelijk zijn. Het model met de tijdspunten 0+1+2+3h was superieur boven de andere modellen ($r^2 = 0.96$) en dit model hebben we geïntroduceerd in onze kliniek.
Omdat het wellicht mogelijk zou zijn om bij een toenemend aantal waarnemingen per patiënt het aantal bloedafnames per bezoek te verkleinen op de lange termijn, zonder daarbij betrouwbaarheid te verliezen, hebben we in **hoofdstuk 4** ons model 0+1+2+3h geëvalueerd, na dit gedurende 18 maanden te hebben gebruikt. Wat opviel was dat er zowel tussen als binnen patiënten een duidelijk verschil was in de tijd tot het bereiken

van de hoogste cyclosporine concentratie (piek-concentratie). Deze variatie kan mogelijk worden veroorzaakt door voedsel of door andere medicatie. Met behulp van ons model met meerdere bloedafname momenten is de kans op het missen van deze variabiliteit en dus op een verkeerd berekende AUC een stuk kleiner.
Na de wijziging van C2-montoring naar LSM 0+1+2+3h was er gemiddeld gezien geen verschil in cyclosporine dosis, nierfunctie (creatinine klaring) en aantal rejecties. Een aantal patiënten eindigde onder de ondergrens van de 'target-AUC', maar ondanks het niet verhogen van de dosis vanwege een slechte nierfunctie volgde er meestal geen rejectie.
De verzamelde data stelden ons in staat om de ware variabiliteit in farmacokinetiek van cyclosporine te onderzoeken, welke 15% was. De gemiddelde dosis was 109 mg, wat betekent dat een gemiddelde dosisaanpassing van 109 * 15% = 16 mg kan worden veroorzaakt door natuurlijke variatie die niet te vermijden is. Als op basis van de gemiddelde dosis met een 95% betrouwbaarheidsinterval de AUC target range zou worden berekend dan zou deze 2380 – 4390 h.µg/L zijn. Met andere woorden: elke AUC waarde binnen deze range zou verklaard kunnen worden door variabiliteit en zou daarom niet moeten leiden tot een dosiswijziging. De range die wij aanhouden in de kliniek (2900 – 3800 h.µg/L) is veel kleiner en op basis van de huidige gegevens zou het in ieder geval theoretisch beter zijn om een andere range aan te nemen, namelijk 2380 – 3280 h.µg/L. De LSM 0+1+2+3h is dus gezien genoemde variabiliteit mogelijk zelfs te nauwkeurig in het schatten van de systemische blootstelling aan cyclosporine. Daarom onderzochten we de correlatie tussen modellen met 2 of 3 tijdspunten en 5 modellen kwamen er goed uit qua correlatie met AUC0-12h en nauwkeurigheid: 0+2h; 0+3h; 0+1+2h; 0+1+3h and 0+2+3h. De dalspiegel (C0) komt in alle modellen voor, wat opnieuw het belang van deze waarde aangeeft, maar alleen in combinatie met de andere tijdspunten. Bij het ontwikkelen van de modellen zagen we al eerder een goede relatie van deze 2- en 3-puntsmodellen met de gouden standaard 12-uurs AUC. Ondanks het feit dat LSM 0+1+2h zowel de piek-concentratiepunten 1 en 2 in zich heeft, is de correlatie van dit model niet anders dan LSM 0+2h (r^2 = 0.84-1.00 vs 0.81-0.99). Vanwege het feit dat LSM 0+2h gemakkelijker is toe te passen in de praktijk en vriendelijker is voor de patiënten, doktoren en verpleegkundigen lijkt dit model een optimale balans tussen doelmatigheid en belasting wat betreft de monitoring van cyclosporine.

Tacrolimus

Het monitoren van tacrolimus is in de meeste klinieken, net als aanvankelijk cyclosporine, gebaseerd op het meten van de dalspiegel. Wij hebben in **hoofdstuk 5** laten zien dat C0 de systemische blootstelling aan tacrolimus in de eerste 12 uur na dosering niet optimaal weergeeft, wat in lijn was met eerdere onderzoeken. Net als

voor cyclosporine hebben we ook voor tacrolimus een farmacokinetisch model ontwikkeld, gebaseerd op populatie gegevens en individuele gegevens van patiënten. De modellen C4 en C6 lieten goede correlaties zien met de gouden standaard 12-uurs AUC in combinatie met uitstekende precisie.
Het voordeel van onze modellen is dat ze flexibel zijn, in tegenstelling tot andere methoden waarbij de bloedafnamen exact op tijd moeten gebeuren, zoals bij de limited sampling formulas (LSF). De LSFs lieten in onze studie overigens, net als de modellen (LSM), goede resultaten zien, maar de modellen waren net wat beter. Een LSF is wel snel met een eenvoudige formule te berekenen. De modellen met meerdere tijdspunten waren over het algemeen genomen iets beter dan de modellen met slechts 1 tijdspunt van bloedafname, maar omdat LSM 4h en LSM 6h beiden een correlatie van $r^2 = 0.97$ lieten zien was een meerpunts-model niet nodig. Groot voordeel van zo'n 1-punts model is dat het erg praktisch is, patiënten kunnen thuis de medicatie innemen op de gebruikelijke tijd en zich 4 tot 6 uur later laten prikken in het ziekenhuis. In theorie leidt dit tot minder fouten bij dosering en meting.
Een beperking van onze studie is dat het model is ontwikkeld op basis van 2 kleine groepen patiënten. Vanwege de aanzienlijke schommelingen in de kinetiek van tacrolimus net na de transplantatie raden we niet aan om deze modellen te gebruiken voor patiënten kort na transplantatie.
De berekende AUC target range, gebaseerd op C0 (90 – 195 µg/L) is erg breed, wat ook aangeeft dat C0-monitoring niet ideaal is. Bij niertransplantatie wordt een nauwere range aangehouden (100 – 150 µg/L). Momenteel is er in het veld van de levertransplantaties een trend die neigt naar verlaging van de dosis van de (nier aantastende) calcineurineremmers, waarvan tacrolimus er een is. Mede op basis van onze data hebben we de 'AUC targetrange' verlaagd naar 90 – 130 h.µg/L (gebaseerd op een
C0 range van 4 – 8 µg/L). De neiging om over te gaan op lagere ranges onderstreept nog eens het belang van precieze monitoring van tacrolimus. Momenteel verrichten we een studie waarbij de gangbare C0-methode wordt vergeleken met de nieuwe 4-uurs monitoring, met als uitkomstmaten onder andere de nierfunctie, bloeddruk, afstoting en laboratoriumparameters.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is een afweer onderdrukkend medicament dat veel wordt gebruikt na orgaantransplantatie, waaronder levertransplantatie. In tegenstelling tot cyclosporine en tacrolimus heeft MMF geen schadelijke bijwerkingen voor de nieren. Het gebruik van MMF kan de dosering van tacrolimus en cyclosporine mogelijk verlagen of zelfs geheel overbodig maken. In tegenstelling tot de calcineurineremmers cyclosporine en tacrolimus wordt MMF niet gedoseerd op basis van concentraties van

het medicament in het bloed, maar geldt een vaste dosis, waarbij geen rekening wordt gehouden met bijvoorbeeld albumineconcentratie of creatinine klaring. In de literatuur kwam naar voren dat het mogelijk toch beter zou zijn in plaats van de vaste doseringen over te gaan op TDM.
In **hoofdstuk 6** hebben we het farmacokinetisch gedrag van MMF nader bestudeerd. Tijdens deze studie vonden we een lineaire relatie tussen MMF dosis en de trapezoidale AUC van MPA, de actieve metaboliet van MMF. Er bleek een grote variatie te zijn in de klaring van MPA tussen de patiënten, tot een factor 7 (8.08 – 57.47 L/h).
Tijdens het onderzoeken van deze variabiliteit bleek er een significante inverse relatie te bestaan tussen serum albumine concentratie en klaring van MPA ($r^2 = 0.26$, $p<0.05$). Dit betekent dat lage albumine concentraties gerelateerd waren aan een toegenomen klaring van MPA. Ook was er een significante relatie tussen creatinine klaring en klaring van MPA ($r^2 = 0.36$, $p<0.05$).
Op basis van klinische selectie hebben we de beschikbare patiëntenpopulatie verdeeld in 2 groepen. Groep 1 bestond uit patiënten die MMF gebruikten met een calcineurine remmer (CNI) en groep 2 uit patiënten die MMF gebruikten zonder daarnaast een calcineurine remmer te gebruiken. Deze twee groepen zijn gebruikt voor verdere ontwikkeling van limited sampling modellen. Het model met de combinatie van concentratiebepaling van MPA na 0-½-1-2h liet in beide groepen een goede correlatie zien met de trapezoidale AUC (met CNI: $r^2=0.82$, MPE/MAPE 14/24;
zonder CNI: $r^2=0.85$, MPE/MAPE 14/20).
Verrassend was de goede correlatie van dalspiegels van MPA met de trapezoidale AUC voor alle patiënten, zonder het gebruik van een limited sampling model, $r^2=0.81$ ($p<0.05$). Met gebruik van de modellen was de correlatie met de trapezoidale AUC redelijk in groep 1 ($r^2=0.89$), maar in groep 2 was de correlatie minder sterk ($r^2=0.68$).
Met de ontwikkelde modellen op basis van de momenten van bloedafname 0-½-1-2 uur na inname van MMF en het onderscheid in de groepen met/zonder CNI hebben we een goede methode om de dosering van MMF nauwkeuriger te sturen. Dit is met name belangrijk om afstoting van het transplantaat te voorkomen tijdens het verlagen van de dosering van tacrolimus en cyclosporine of zelfs het stoppen van deze CNI's.
Prospectieve validatie van onze modellen en de klinische relevantie van deze modellen worden nader onderzocht.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 23 maart 1978 te Goirle. In 1995 behaalde hij het VWO-diploma aan het Paulus Lyceum te Tilburg. In datzelfde jaar begon hij met de studie Geneeskunde aan de Universiteit van Antwerpen. In 1996 stapte hij over naar de Universiteit Leiden, om daar de studie Geneeskunde te vervolgen. In 1997 werd de propedeuse behaald. In 2002 werd onder supervisie van dr. B. van Hoek een aanvang gemaakt met een onderzoek op de afdeling Maag-, Darm- en Leverziekten van het Leids Universitair Medisch Centrum, dat de basis is geweest voor dit proefschrift. In 2002 werd het doctoraal examen behaald en na de coassistentschappen werd in 2004 het artsexamen behaald te Leiden. Van 2005 tot 2007 was de auteur huisarts in opleiding aan het Huisartsinstituut Leiden (LUMC), waarna hij besloot de richting te kiezen van de beleidsmatige- en organisatorische kant van de gezondheidszorg. Na een jaar te hebben doorgebracht aan de faculteit Beleid en Management in de Gezondheidszorg (BMG) aan de Erasmus Universiteit te Rotterdam is hij sinds 2009 werkzaam als accountmanager bij het bedrijf ZorgDomein Nederland B.V., waarbij de visie van verbinden van zorgverleners met als doel de efficiëntie binnen de zorg te vergroten centraal staat.

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