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**Monitoring immunosuppression after liver transplantation :
development of individualized Bayesian limited sampling monitoring**
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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 inpatient 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 inpatient 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 inpatient 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 AUC_{0-12h}. The correlation of the widely used C₀ with AUC_{0-12h} 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 AUC_{0-12h} 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 AUC_{0-12h} 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 AUC_{0-12h}, 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 inpatient 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 \pm 15% range, in 13/21 patients whose dose was lowered the second AUC was below the target AUC of 3380 – 4266 h. μ g/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. μ g/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 recipients³⁻¹⁵. 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 occur¹⁷. In renal transplantation variable cyclosporine levels may contribute to chronic rejection¹⁸. 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 cause¹⁹⁻²². 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 AUC_{0-12h} 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_{0-12h} 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 AUC_{0-12h}.

For cyclosporine the correlation with AUC_{0-12h} 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 AUC_{0-12h}. 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 AUC_{0-12h}²⁸⁻³¹. A high inter-individual variability in cyclosporine pharmacokinetics exists, which seems unrelated to CYP3A polymorphisms³². 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 patients³⁵. 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 inpatient 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 \pm 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 \pm 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 unnecessarily 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 Ctough³⁶. 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 unnecessarily 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 tacrolimus³⁷⁻³⁹.

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/L⁴³. 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 transplantation⁴⁴. 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 clinic⁴³.

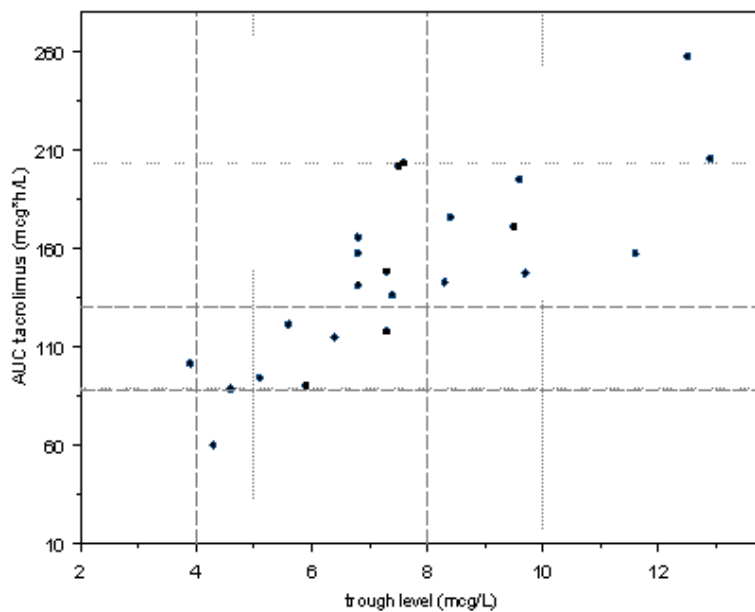


Figure 1: Relationship between trough level (C0) and AUC of all 23 patients, while on C0-monitoring. The thin dotted lines (.....) show the range based on trough-level monitoring of 5-10 µg/l (AUC target 142.5 h*µg/l). The other lines (- - - -) show the proposed AUC range based on trough-level monitoring 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 (Prograf BID) did not have a good correlation with AUC_{0-12h} using LSF ($r^2 = 0.68$) or without using LSF and LSM ($r^2 = 0.69$). Correlation of C₀ with AUC_{0-12h} 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 $\pm 10\%$ (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 AUC_{0-12h}, 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 AUC_{0-12h}, 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 AUC_{0-12h} 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 monitoring⁴⁷. 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 patients⁵². 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 rejection⁵⁸. Furthermore, an increase of C_{max} and AUC of MPA in renal transplant recipients in the months after transplantation is described⁵⁹. This may require dose adjustments.

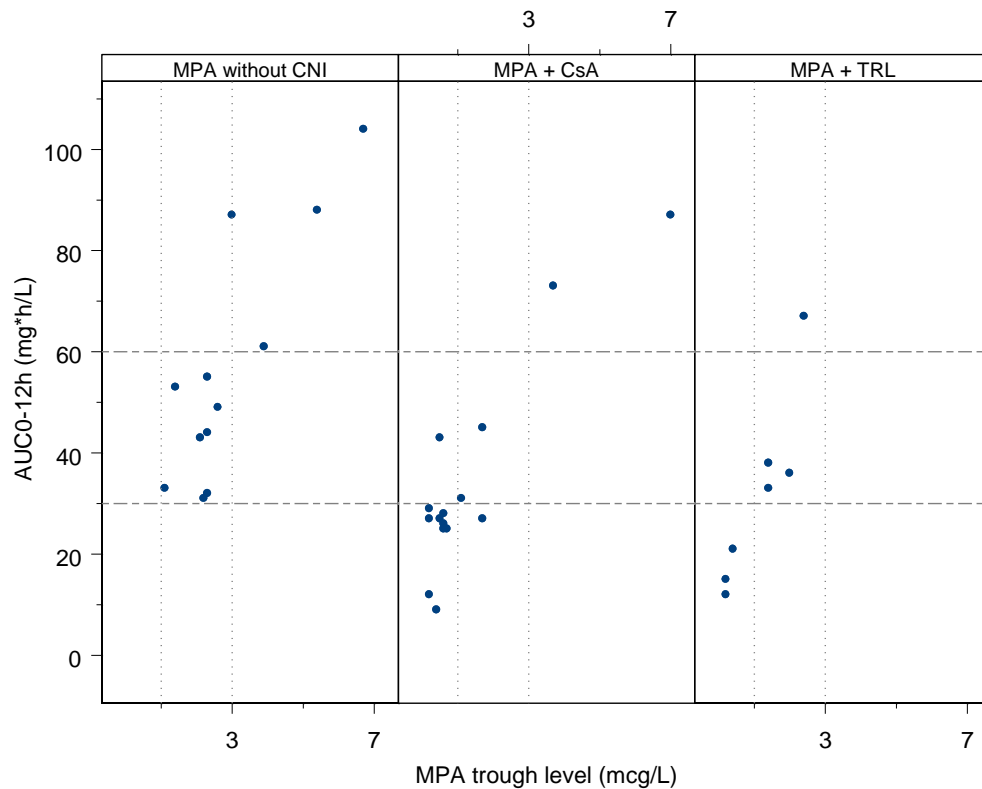


Figure 2: Relationship of MPA trough level with trapezoidal AUC0-12h for different groups of co-medication next to MMF: without CNI, with cyclosporine (CsA) and with tacrolimus (TRL)

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 awaited⁶⁷. 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 levels⁶⁸. Zicheng et al. developed rigid limited sampling algorithms for implementation of MPA-monitoring in liver transplantation necessitating exactly timed blood sampling⁶⁹. 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 AUC_{0-12h} with acceptable predictive performance⁷⁰.

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 exposure⁷⁵. 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 recipients⁷⁶. 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 disturbances⁷⁷. 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 CNI⁷⁰. 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 rejection⁷⁸. For the moment we suggest - in the absence of sufficient data from clinical studies - to use similar targets in liver transplantation as in renal transplantation⁷⁸. 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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