

Objective: develop a pharmacokinetic model for mycophenolate acid (MPA) and its main active metabolite, phenolic glucuronide metabolite (MPAG), in patients given mycophenolate mofetil in association with low doses of tacrolimus and steroids to prevent graft rejection after hepatic transplantation.

CONTEXT

- Solid organ transplantation
 - risk of organ rejection (acute or chronic)
 - long-term treatment with immunosuppressive drugs
 - * calcineurin inhibitors (CNI): tacrolimus, cyclosporin
 - * generally associated with corticosteroids
 - * main toxicities: renal dysfunction, arterial hypertension, diabetes mellitus, causing long-term mortality
 - mycophenolate mofetil (MMF) given in association with CNI
 - * T and B-cell specific immunosuppressive agent
 - * hydrolysis to mycophenolic acid (MPA), the active component, in the gastro-intestinal tract
 - * almost complete absorption after oral administration
 - * therapeutic drug monitoring recommended due to large variability, to limit risk of rejection associated with low concentrations
- MMF-FK trial: study the benefits of co-administering MMF to reduce daily doses of tacrolimus in liver transplant recipients
 - multicentre prospective randomised clinical trial carried out by INSERM CIC 1414 (Rennes University Hospital)
 - * arm 1: tacrolimus therapy in conventional doses
 - * arm 2: half doses of tacrolimus in combination with oral MMF, given as 1.5 g twice daily for 6 weeks, then 1 g twice daily
 - evidence for reduction of both renal dysfunction and risk of rejection in the reduced dose arm [1]
- Ancillary study of MMF pharmacokinetics (PK) in a subgroup of patients in the reduced dose arm
 - measurements of MPA and its glucuronidated (inactive) metabolite, mycophenolic acid glucuronide (MPAG)
 - compare PK 3 weeks and 3 months after transplantation

DATA AND METHODS

Data

- 27 patients included in the PK study
 - adult recipients of primary deceased donor whole-liver allograft transplantation
 - undergoing surgery in the Pontchaillou hospital in Rennes
 - Kehr drain implanted in the bile duct after surgery
- PK of MMF recorded during 2 periods
 - PK1: 3 weeks after transplantation
 - PK2: 3 months after transplantation
 - PK sampled at 0 (predose) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hr after MMF intake
 - evening dose skipped on the day of the PK sampling
 - Kehr drain declamped at PK1 and clamped at PK2
- Analytical methods: MPA and MPAG plasma concentrations measured by HPLC-UV

Statistical models

Nonlinear mixed effect model for y_{ij} , the j th observation taken in subject i ($1 \leq i \leq N$, $1 \leq j \leq n_i$):

$$y_{ij} = f(x_{ij}, \psi_i) + g(x_{ij}, \psi_i, \xi) \epsilon_{ij} \quad (1)$$

- f denotes the structural model
- x_{ij} are regression variables, or design variables, assumed to be known
- ψ_i is a vector of individual parameters, written as

$$\psi_i = H(\mu, c_i, \eta_i)$$

where c_i is a known vector of covariates,

μ is an unknown vector of fixed effects

η_i is an unknown vector of normally distributed random effects:

$$\eta_i \sim i.i.d. \mathcal{N}(0, \Omega)$$

- residual errors (ϵ_{ij}) $\sim \mathcal{N}(0, 1)$
- variance defined by the function g and parameters ξ

Parameter estimation

- Population PK analysis performed using Monolix [2] version 4.2.2
 - SAEM algorithm
 - models written in MLXTRAN
- Model building
 - nested models selection: log-likelihood ratio test, comparison of estimation errors
 - models compared through likelihood-based criteria as well as model estimates and stability
- Different models tested, combining:
 - disposition models for MPA and MPAG
 - absorption model for MPA
 - enterohepatic recirculation (EHR)

Covariate model building

- Two steps
- Covariate exploration for each parameter separately
 - model with all available covariates
 - selection of candidate covariates: Wald test, threshold $p=0.3$
 - when several correlated covariates ($\rho \geq 0.8$), only one retained based on clinical relevance
- Stepwise model building
 - inclusion of all candidate covariates in the model
 - backward elimination of all covariates for which the p-value of the Wald test is $p \geq 0.3$ (several steps, until all p-values remaining in the model are smaller than 0.3)
 - backward elimination of remaining non-significant covariates one by one, starting with the one with the highest p-value

RESULTS

Study population

Baseline characteristics of the population are summarised in table 1.

Variable	Mean (SD)	Min-Max
Height (cm)	174.6 (5.1)	[164-187]
Weight (kg)	79.9 (11.3)	[65-105]
Age (yr)	49.5 (9.8)	[18-62]
BMI (kg/m ²)	26.0 (4.3)	[20-37]
Gender	Men:21	Women: 6
Child score	A: 9	B: 8 C: 9

Variable	Mean (SD) PK1	Mean (SD) PK2
Leukocyte count (10 ⁹ /mL)	9.6 (3.2)	5.5 (2.5)
AST (IU/L)	51.9 (47.6)	23.9 (17.8)
ALT (IU/L)	219.9 (232.6)	39.3 (45.8)
ALP (IU/L)	447.4 (231.7)	214.4 (186.5)
GGT (IU/L)	335.8 (231.9)	118.7 (201.9)
Bilirubine T (μmol/L)	41.4 (64.3)	9.8 (3.8)
Bilirubine C (μmol/L)	17.3 (32.3)	3.3 (3.3)
TP (%)	86.4 (10.6)	91.5 (12.1)
Albuminemia (g/L)	39.2 (5.3)	38.7 (8.1)
Plasma creatinin (μmol/L)	98.5 (53.4)	90.6 (14.9)
Urea (mmol/L)	9.1 (6.7)	6.4 (2.4)
Sodium (mmol/L)	135.2 (4.3)	140.6 (1.9)
Potassium (mmol/L)	4.3 (0.5)	4.3 (0.5)
Calcium (mmol/L)	2.2 (0.2)	2.4 (0.1)
Creatinin clearance (mL/min) ¹	105.3 (35.5)	93.3 (22.8)

¹ computed using the Cockcroft and Gault equation [3], using lean body weight instead of total body weight for obese patients (BMI ≥ 30) [4]

Table 1: Patient characteristics. Demographic covariates were collected at baseline, while biochemical covariates were measured on both occasions. AST, ALT, ALP, GGT stand for aspartate amino transferase, alanine amino transferase, alkaline phosphatase and gamma glutamyl transferase respectively.

PK data

- PK data collected in 27 patients
 - 20 subjects with the two occasions, 2 with only PK1 and 4 with only PK2
 - two outlier profiles excluded from model building
 - missing values for biochemical variables imputed to the mean of the population at each occasion
- PK data used in the analysis (figure 1)
 - large variability both between and within subject, also in the exposure to MPA and MPAG at both occasions
 - (generally) higher dose of MMF received at PK1 (dose at PK1: 1500 mg for N=18, 1000 mg for N=2; dose at PK2: 1000 mg for N=23, 500 mg for N=2)
 - increase in concentrations around 12 h $\Rightarrow$ EHR

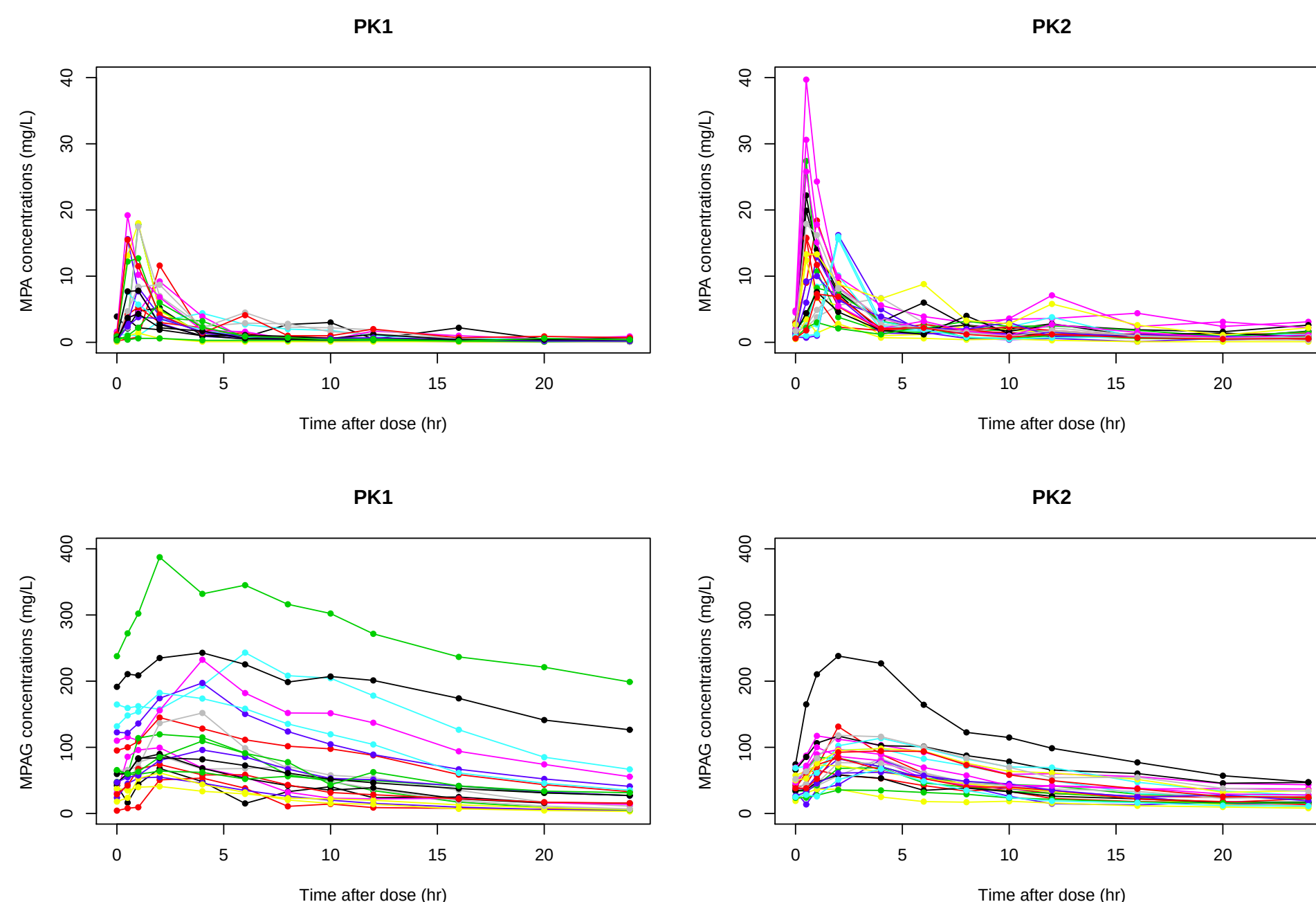


Figure 1: Data collected in the study, split by occasion (top: MPA, bottom: MPAG). Each subject is represented by a different colour.

Structural model selection

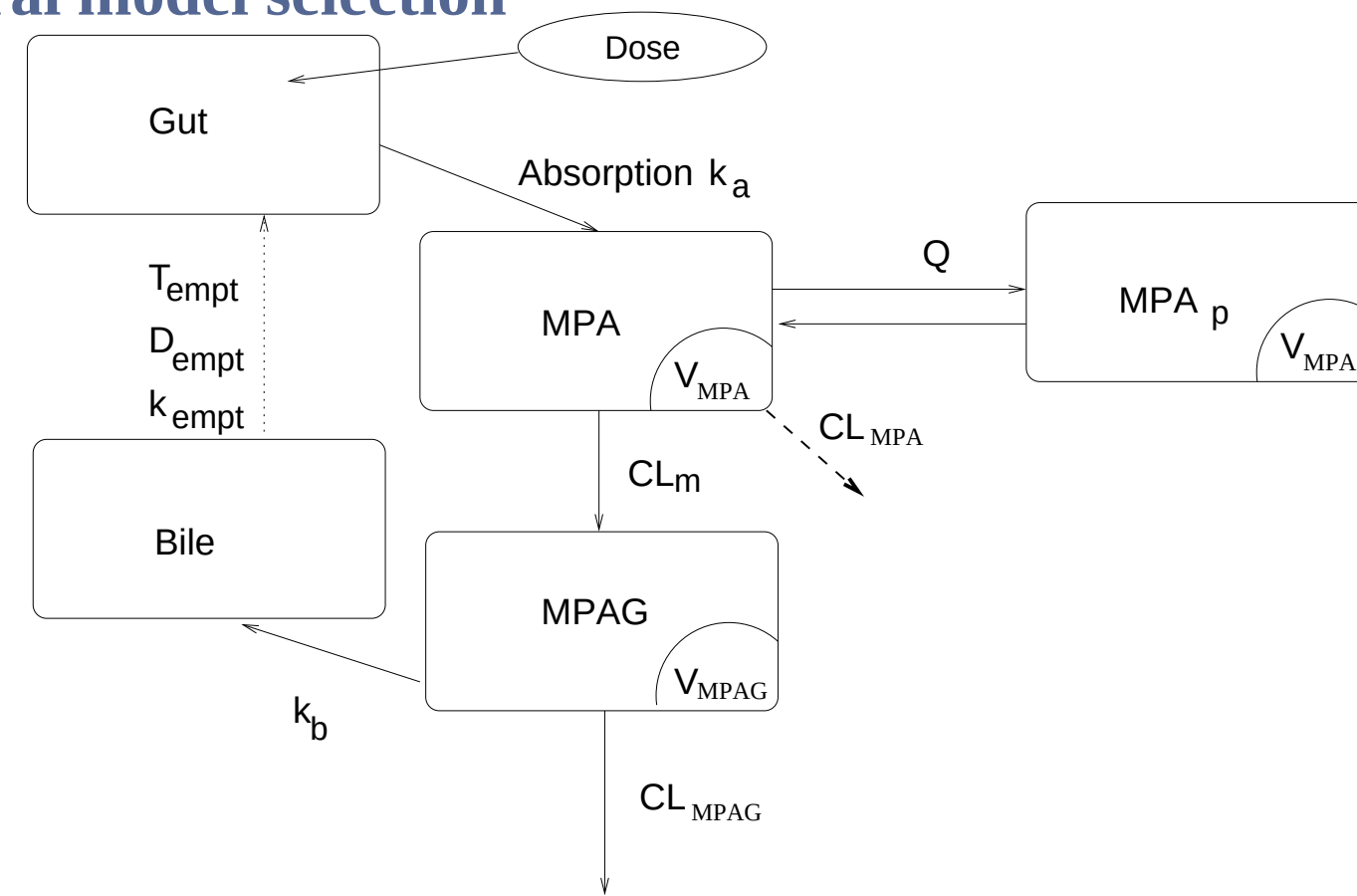


Figure 2: Model describing the PK of MPA and MPAG after daily doses of MMF.

- Effect of Kehr drain: modify the amount of recycling (EHCP)
- Model identifiability
 - elimination of MPA (CL_{MPA}) small and highly variable from run to run: removed from the final model
 - parameters of the enterohepatic cycle (T_{empt} , k_{empt} , D_{empt} , $EHCP = \frac{k_b}{k_b + CL_{MPAG}/V_{MPAG}}$) estimated one by one through a grid approach and fixed in the final estimation step
- Fixed parameter estimates reported in table 2 for the final model
 - occasion effect modelled as relative to PK2
 - * patient status stabilised after surgery
 - * Kher drain clamped
 - free-flowing drain during PK1: reduction of EHCP by $\sim 40\%$
 - effect of demographic and biological covariates:
 - * allometric model on volumes and clearances
 - * CL_{MPAG}: increases with creatinin clearance
 - * CL_m: decreases with albumin
 - * decrease of both V_{MPAG} and CL_m between PK1 and PK2 (respectively by 40 and 60%)

Parameter	Estimate (RSE%)	IIV % (RSE%)	IOV % (RSE%)
k_a (h ⁻¹)	0.87 (11)	57 (1)	-
V _{MPA} (L)	18.5 (19)	-	122 (12)
Q (L.hr ⁻¹)	16.1 (12)	54 (9)	-
V _{MPA,p} (L)	311 (17)	76 (6)	-
CL _m (L.hr ⁻¹)	18.8 (6)	-	28 (12)
CL _{MPAG} (L.hr ⁻¹)	2.0 (8)	35 (18)	-
V _{MPAG} (L)	7.3 (11)	38 (25)	34 (21)
$\sigma_{MPA,b}$ (%)	39.0 (4)		
$\sigma_{MPAG,a}$ (-)	2.9 (11)		
$\sigma_{MPAG,b}$ (%)	6.9 (10)		

Table 2: Fixed effect estimates for the final model. The parameters of the EHR were fixed to: $T_{empt}=10$ hr, $k_{empt}=10$ hr⁻¹, $D_{empt}=0.35$ hr, $EHCP=0.1$.

Model evaluation

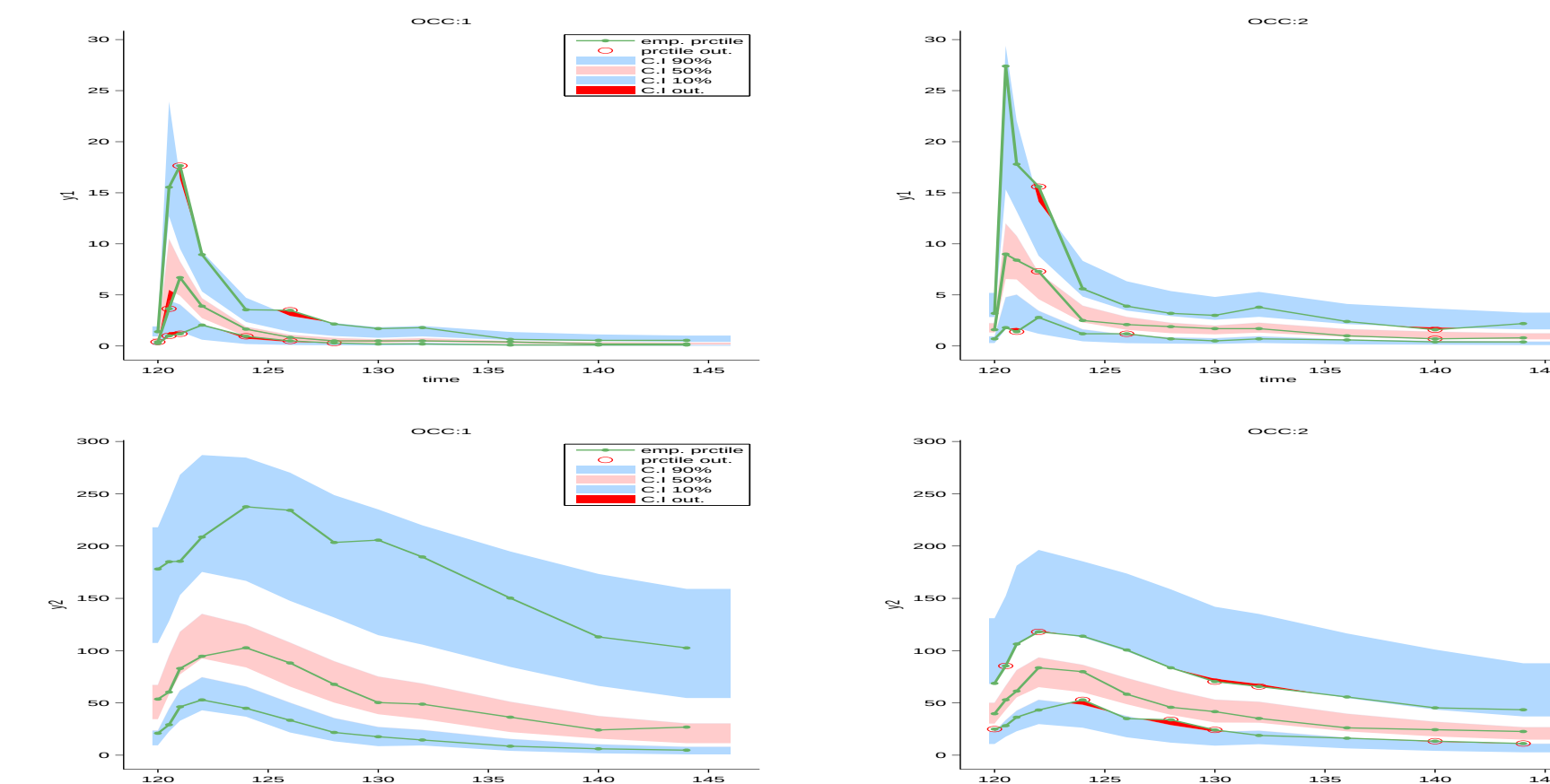


Figure 3: Visual Predictive Check (VPC), split by occasion (top: MPA, bottom: MPAG) for the final model.

- Variability somewhat overestimated for MPAG

CONCLUSION

- Model selection
 - limited enterohepatic recycling ($\sim 10\%$)
 - significant interoccasion variability
 - improvement in hepatic function at PK2: confounding effect of changes in patient status between the two occasions
- Large variability both between and within patient of the PK of MPA and its metabolite
 - limited improvement in variability when taking into account covariates in this study
 - need for large scale studies to identify the determinants of MPA and MPAG PK
 - additional samples needed to estimate the parameters governing enterohepatic recycling
- Perspectives
 - explore the effect of comedications
 - sensitivity analysis to evaluate the robustness
 - estimation of patient exposure

REFERENCES

[1] K Boudjema, C Camus, F Saliba, Y Calmus, E Salame, G Pageaux, C Ducerf, C Duvoux, C Mouchel, A Renault, P Compagnon, R Lorthol, and E Bellissant. Reduced-dose tacrolimus with mycophenolate mofetil vs. standard-dose

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[3] DW Cockcroft and MH Gault. Prediction of creatinine clearance from serum creatinine. *Nephron*, 16:31–41, 1976.

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