Clinical toxicology

PREDICTIVE PERFORMANCES OF DIFFERENT THERAPEUTIC DRUG MONITORING APPROACHES TO ASSESS TACROLIMUS AND MYCOPHENOLIC ACID EXPOSURE

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Key words: therapeutic drug monitoring, pharmacokinetics, bayesian estimators, area under the curve

ABSTRACT

Objective: Mycophenolic acid (MPA) and Tacrolimus (TAC) are immunosuppressive drugs routinely used after solid organ transplantation. MPA and TAC have a rather narrow therapeutic window and their pharmacokinetics show considerable variability. In most centers, TDM is frequently performed based only on trough concentrations (C_0). The main objective of the present study was to compare performances of three different approaches of C_0 based TDM of these two drugs, in their ability to predict MPA and TAC area under the curve (AUC).

Methods: Linear regression models and Bayesian estimators were developed based on solely on C_0 levels. Kappa and McNemar concordance and divergence tests were used to see the ability of different C_0 TDM approaches to predict the same dosage recommendation as AUC TDM.

Results: 52% and 57% of the observed AUC values from patients receiving a fixed dose were outside the recommended therapeutic range for MPA and TAC, respectively. For both TAC and MPA, the Bayesian estimators performed much better than the two other approaches, followed by linear regression models.

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INTRODUCTION

Therapeutic drug monitoring (TDM) has been used for years for drugs with narrow therapeutic windows, to avoid toxicity and lack of efficacy. TDM has been successfully used for immunosuppressive drugs including Mycophenolic acid (MPA) and Tacrolimus (TAC), two immunosuppressors routinely used after solid organ transplantation. TDM is recognized as having played a major role in the progress recorded in transplantation these last years (1).

Nevertheless, the level of rejection remains unacceptably high, particularly in the long term after trans-

plantation, and progress is still needed to prolong graft survival rates and quality of life after renal transplantation.

Historically, MPA and TAC TDM has been performed based on trough levels (C_0), targeting levels of 2-5 mg/L and 5-15 µg/L, for MPA and TAC, respectively (2, 3). This approach is still used in most clinical centers where TAC and MPA TDM are performed. C_0 levels have been shown to be particularly well linked to the toxicity of both drugs. However, several studies have been published more recently showing a stronger relationship between the area under the concentration-time curve (AUC) and the outcome of the transplanted patients (4-6).

The aim of the present report is to compare the performances of three MPA and TAC TDM approaches, based on trough levels, to predict the same dosage recommendation as AUC based TDM:

1° TDM based solely on trough levels

2° TDM based on AUC values predicted from trough levels using a linear regression equation

3° TDM based on AUC values predicted from trough levels with Bayesian estimation.

PATIENTS AND METHOD

Patient Characteristics and Study Design

Data from 169 stable adult renal allograft recipients, transplanted in one of the three participating belgian university hospitals (Cliniques Universitaires Saint Luc, Universitair Ziekenhuis Brussels and University Ziekenhuis Antwerpen), were retrospectively analyzed in this report. Each of the trials was approved by the local ethic committees and all the patients signed an informed consent. Table I show details of the different studies characteristics for MPA and TAC. For the determination of the full pharmacokinetic profiles, blood samples were collected in EDTA tubes; blood and plasma were kept frozen at -20 °C until analysis. Sampling times included: before (0) and at 20, 30 and 40 minutes and at 1.25, 2, 3, 4, 6, 8 and 12 hours following drug administration.

Table I: Studies characteristics in kidney transplantation from the three university centers

Studies	Drug	Number of patients	Clinical center	Time after transplantation	Reference
1	MPA	40	- Universitair Ziekenhuis Antwerpen - Universitair Ziekenhuis Brussels	6, 9 and 17 months	10
2	MPA and TAC	96	Cliniques Universitaires Saint Luc	2 weeks	unreported
3	TAC	19	Cliniques Universitaires Saint Luc	Before transplantation	11
4	TAC	14	Cliniques Universitaires Saint Luc	Day 7 and 2 months	unreported

Table II: Predictive performances of LR model and Bayesian estimators

Model	Model equation	r^2	rRMSE (%)	MRPE (%)
LR on MPA	4.32+11.13 • C0	0.42	33	19
LR on TAC	10.69+7.90 • C0	0.72	24	8
MAP BE on MPA	-	0.73	27	11
MAP BE on TAC	-	0.70	23	5.2

LR: Linear regression
MAP BE: Maximum a posteriori Bayesian estimation

Analytical Method (Drug analysis)

Mycophenolic acid quantification

The patients samples were analysed by UPLC as described elsewhere (7). Briefly, MPA was extracted from plasma using Isolute C2 SPE-cartridges (100 mg, 3mL). UPLC separation was performed with a Waters BEH C18 column (50x2.1mm, 1.7µm) maintained at 65°C on a Waters Acquity instrument equipped with a PDA detector.

System pressure was around 7000 psi and the flow rate remained at 0.75 mL/min throughout the 3.5-minute run. The method was linear in the range of 0.1-40 µg/mL for MPA. Relative standard error and mean relative prediction error were <15% for all tested quality controls.

TAC quantification

The immunoassay used for all blood specimens was the Microparticle Enzyme ImmunoAssay (MEIA) performed on the IMx analyzer from Abbott Diagnostics (Wiesbaden, Germany). This method, linear from 3-30 ng/mL, was found to be precise on an inter-day basis: coefficient of variation (CV) <11% for all QC samples tested. By MEIA, Tacrolimus metabolites: 13-O-demethyl Tacrolimus (MI), 31-O-demethyl Tacrolimus (MII), 15-O-demethyl Tacrolimus (MIII) and 12-hydroxytacrolimus (MIV) are known to display cross-reactivity with the antibody of <1%, 109%, 90.5% and 8.8%, respectively (8).

The laboratory successfully participates in both the MPA and TAC International Proficiency Testing Schemes (Analytical Services International, UK, David Holt).

Linear regression models

Linear regression (LR) formulas were developed to predict MPA and TAC AUCs calculated based on the full PK profiles by MLR (JMP 6°/SAS, Cary; NC, USA) using trough levels. The predicted AUC model was compared to the observed as described below. The predictive performance of the t model was further internally evaluated in the model building group by repeated cross validation as described by Pawinski (9). Briefly, the data set was repeatedly and randomly divided into two equal groups: a training group and an evaluation group. This process was repeated 20 times. The training group records were used to determine the relationship (i.e. regression coefficients) between observed MPA AUC and the LR model. The linear regression equations obtained in precedent step was used to estimate the MPA AUC for the profiles in the corresponding evaluation set. "Residuals" were calculated for each of the MPA AUC values in the evaluation group by taking the difference between the logarithm of the reference MPA AUC and the logarithm of MPA AUC estimated by the regression equation. The distribution of the entire set of residuals was examined to assure that the selected limited sampling equation for prediction of TAC and MPA AUC generated a distribution of estimated TAC and MPA AUC values in the evaluation sets that met certain statistical criteria.

Bayesian estimation

Bayesian estimation on the validation group (by the POSTHOC and MAXEVAL=0 option of the NONMEM estimation subroutine) was performed by using the population PK final models developed on each specific patients group and reported elsewhere (10-11). When full PK profiles were available for one patient, the reference AUC was considered to be the AUC obtained by the trapezoidal rule, otherwise, AUC was computed from individual predicted concentrations obtained from the population modelling step. Reference AUC values were compared to AUC computed using C_0-based Bayesian estimators as described below.

Evaluation of predictive performance of AUC predictors using patient data

Linear regression was performed to evaluate the strength of the relationship between the AUC values predicted by the LR/Bayesian estimators and the reference AUC values. The Pearson coefficient of determination r^2 was one of the criteria to select the best limited sampling strategy (LSS). In addition, predictive performance of the various LSS and agreement between predicted and observed AUC were assessed as described by Sheiner and Beal (12) and Bland and Altman (13), respectively. Sheiner and Beal described two parameters: 1) the root mean square error (RMSE) to characterize the precision of the model, and the prediction error to estimate the bias on each difference between predicted and observed AUC. The lower the RMSE and PE values, the better the model. Bland and Altman used the 95% confidence interval around the mean relative prediction error (RPE) to assess the predictive performance of the LSS. Equations 1, 2 and 3

display expressions of estimation of relative root mean squared error (rRMSE), relative prediction error and mean relative prediction error (MRPE), respectively.

$$rRMSE = \frac{100}{N}\sqrt{\sum\left(\frac{AUC_{pred} - AUC_{ref}}{AUC_{ref}}\right)^2} \quad \text{Equation 1}$$

$$RPE = \left(\frac{AUC_{ref} - AUC_{pred}}{AUC_{ref}}\right)\times 100 \quad \text{Equation 2}$$

$$MRPE = \frac{1}{N}\sum\left(\frac{AUC_{ref} - AUC_{pred}}{AUC_{ref}}\times 100\right) \quad \text{Equation 3}$$

where AUC_{ref} represents the observed or the reference AUC and AUC_{pred} the AUC predicted by the model.

In a second time, Kappa and McNemar (14) concordance and divergence tests were used to see the ability of different C_0 levels to predict the same outcome as AUC TDM i.e. to compare actions suggested by these different approaches in term of dosage adjustment.

RESULTS

Figure 1 shows individual MPA plasma and TAC blood concentration-time profiles. A very high variability was observed for both drugs: 52 and 57% of the AUC values from patients receiving fixed doses, were outside the therapeutic range for MPA and TAC, respectively, suggesting a need for TDM. LR analysis was used to predict AUC_{ref} from MPA and TAC trough levels. LR model equations are shown in Table III together with a measure of correlation (Pearson r^2), accuracy (MRPE) and precision (RMSE). Bayesian estimators have also been developed based only using the trough levels and the final population PK model. Results on their predictive performances are also shown in Table III.

A Kappa test has been performed to assess the predictive performances of the three TDM approaches, and results are shown in figure 2. A good concordance has been observed between results from AUC_{ref} and AUC computed from MLR and from the Bayesian estimation even though the Bayesian estimators performed significantly better than the MLR equations. Interestingly, the poorer relationship with AUC_{ref} was observed from TDM based only on C_0.

DISCUSSION

These last years a lot of information has been available, not only on these drugs PK characteristics, but also on different tools to perform efficient TDM and dosage individualization (15-19). The decision of performing TDM for a particular drug in a particular group of patients should be driven by the safety and effectiveness of the drug in this particular group of patient: TDM should be justified only in groups of patients in which the drug of interest has an unpredictable between-subject variability (BSV), higher than the safe and effective variability (SEV), together with an unpredictable within-subject variability (WSV), lower than the safe and effective variability. Otherwise, a covariate dosing should be used (when BSV<SEV) or the

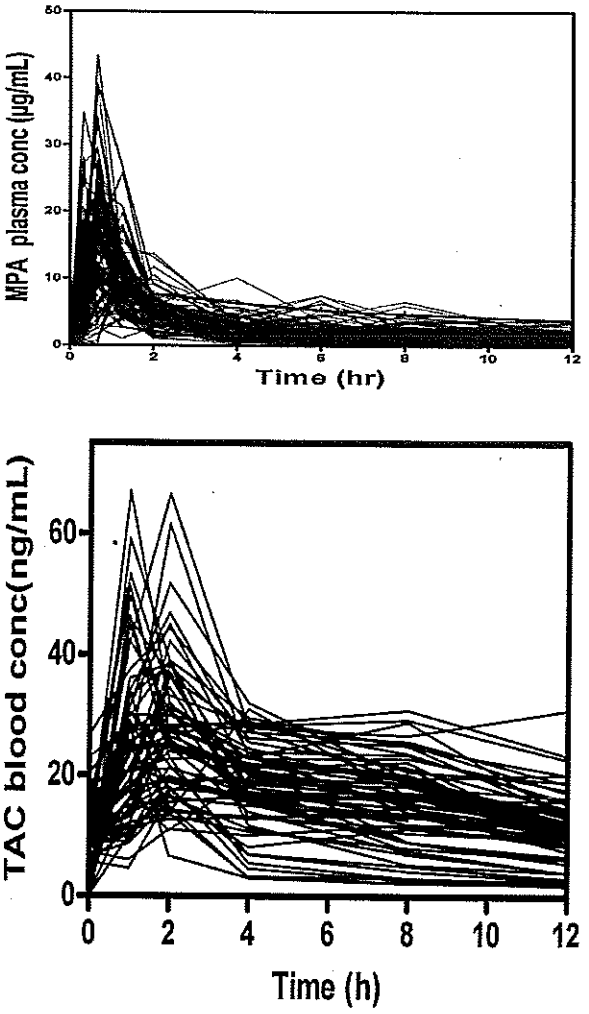
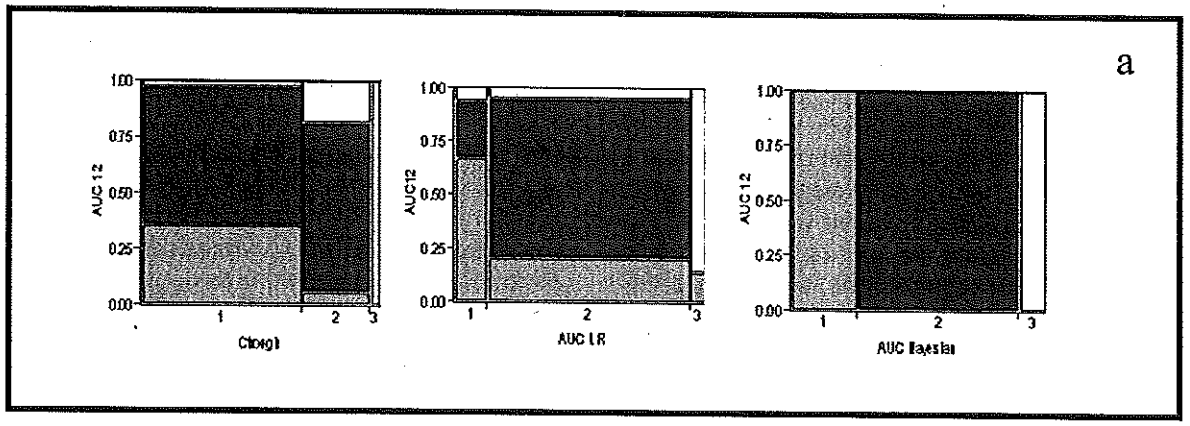


Figure 1: MPA (a) AND TAC (b) Pharmacokinetic profiles



1: Patients with C_0 <2 µg/mL

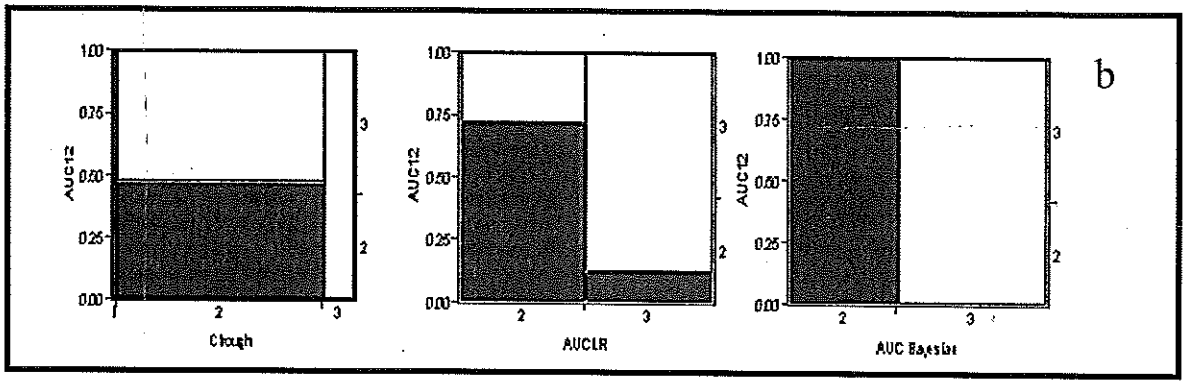
2: Patients with 2 µg/mL <C_0 <5 µg/mL

3: Patients with C_0 >5 µg/mL

Patients with AUC <30 µg/mL

Patients with 30 µg.h/mL <AUC <30 µg.h/mL

Patients with AUC >60 µg/mL



1: Patients with C_0 <5 µg/L

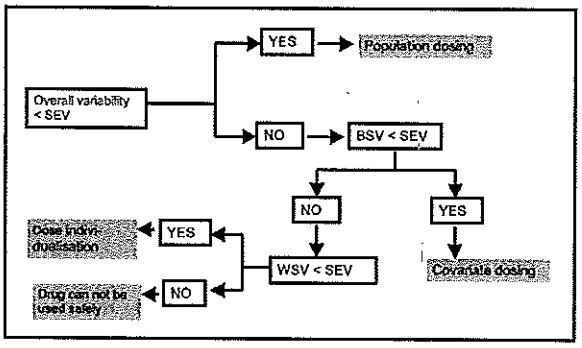
2: Patients with 5 µg/mL <C_0 <15 µg/L

3: Patients with C_0 > 15 µg/L

Patients with 90 µg.h/L <AUC <130 µg.h/L

Patients with AUC >130 µg/L

Figure 2: Kappa and McNemar tests results for MPA (a) and TAC (b)



SEV: Save and efficient variability
BSV: Between subject variability
WSV: Within subject variability

Figure 3: Schema for decision making about therapeutic drug monitoring

drug cannot be safely used (when WSV>SEV). Figure 3 shows a tree for decision making about TDM of drugs. In the case of MPA, with a C_0 therapeutic window of 2-5 µg/mL and an AUC therapeutic window of 30-60 mg.h/L, the SEV can be considered to be around 20%, whereas for TAC, with a C_0 therapeutic window of 5-15 µg/L and an AUC therapeutic window of 90-130 µg.h/L, the SEV can be considered to be around 25%. These values have been computed based on the definition of the SEV as presented by Holford (20). For both drugs, a TDM is justified because the inter-individual variability is > 30%, and is particularly high in the early period post transplantation. In addition, PK modeling of these two drugs allows having a precise idea of the remaining overall unexplained residual variability which is < 15%.

Once the need of TDM has been established for these drugs, the following question should be: which parameter to monitor? C_0 has been historically used, and this can be justified when the aim of the TDM is solely to avoid side effects and detect drug interactions (21-22). Several reports, however, pointed out the fact that AUC should be a better marker of pharmacological efficacy (4-6). If C_0-based TDM has the advantage of being easy to implement in routine practice, AUC based TDM should raise ethical and practical issues if full AUC is computed by the trapezoidal rule. An alternative approach could be the use of LSS based on C_0 to predict AUC and therefore to avoid the ethical and practical issues. This was the object of this study and two strategies have been explored: first a simple linear regression model and secondly a maximum a posteriori Bayesian (MAP) estimator.

The PK of MPA and TAC were determined in kidney transplant recipients. MPA plasma concentrations and TAC blood concentrations were measured at different occasions. Linear regression models and Bayesian estimators were developed to predict individual AUC values only using C_0 levels. These predictors were compared to the simple C_0 TDM, in term of the actions suggested regarding the dose adjustment (maintain or change the dose). Kappa and McNemar tests were used, and results obtained in this study showed that the TDM based solely on C_0, as presently performed in most centers was the worst predictor of AUC, out of the three explored approaches for both drugs (MPA and TAC) in term of the actions suggested regarding the dose adjustment.

This can be explained by the fact that the PK of these drugs is better described by two compartments models: AUC is only influenced by the drug's clearance, whereas C_0 is influenced by the distribution and the clearance of the drugs. Factors only influencing the distribution of the drugs will therefore influence C_0 but not AUC.

The difference between these two indicators of drug exposure (C_0 and AUC) is less apparent in case of maximum a posteriori Bayesian estimation, surely because unlike LR, in the case of Bayesian estimation, C_0 is not the only predictor of the individual AUC: there is also prior information about the population parameters (including AUC) "enclosed" in the estimator which is indeed called a "posterior". In the context of this study, the Bayesian estimator performed much better than the linear regression equation.

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