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Limited Sampling Strategies for Monitoring Tacrolimus in Paediatric Liver Transplant Recipients

THESE

préparée sous la direction du Professeur Manuel Pascual
avec la co-direction du Docteur Catherine Litalien

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

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par

Jean-Romain DELALOYE

Médecin diplômé de la Confédération Suisse
Originaire d' Ardon (Valais)

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Rapport de Synthèse

Limited Sampling Strategies for Monitoring Tacrolimus in Pediatric Liver Transplant Recipients

Ce travail de recherche a été réalisé dans le laboratoire de pharmacologie clinique, au Centre Hospitalier Universitaire Sainte-Justine, à Montréal.
C'est une étude rétrospective basée sur le suivi thérapeutique du Tacrolimus prescrit chez les enfants après transplantation hépatique. Ce suivi est nécessaire car le Tacrolimus possède une importante variabilité pharmacocinétique inter et intra-individuelle ainsi qu'un index thérapeutique très étroit. Actuellement, l'individualisation des doses prescrites est basée sur la mesure de la concentration de base du médicament dans le sang (C_0), mais des études récentes montrent que cette mesure ne reflète pas précisément l'exposition du Tacrolimus dans l'organisme chez les enfants. Le meilleur reflet de cette exposition est la mesure de l'aire sous la courbe (AUC). Cependant, cette dernière implique la mesure de multiples concentrations tout au long de l'intervalle entre 2 doses de médicament (Tacrolimus: 12 heures) ce qui est long, cher et impraticable en ambulatoire. De nouvelles méthodes utilisant un nombre limité de prélèvements ont donc été développées pour prédire au mieux cette AUC. Ce sont les "Limited sampling strategies" ou LSS. La plupart de ces LSS pour le Tacrolimus ont été développées et validées chez des patients transplantés adultes et leur application directe chez les transplantés pédiatriques n'est pas possible en raison de différences importantes au niveau des paramètres pharmacocinétiques du médicament entre ces deux populations.
Aussi, le but de ce travail était de développer et valider, pour la première fois, des LSS chez les enfants transplantés hépatiques. Pour cela, une analyse de 36 profils pharmacocinétiques de 28 patients transplantés hépatiques âgés de 0.4-18.5 ans a été effectuée. Tous les profils ont été réalisés au Centre Hospitalier Universitaire Sainte-Justine entre janvier 2007 et janvier 2009.
Les LSS comportant au maximum 4 mesures de concentration ont été développées en utilisant une analyse de régression multiple. Parmi tous les modèles obtenus, cinq ont été sélectionnés sur la base de critères précis puis validés selon la méthode décrite par Sheiner et Beal.
Les résultats montrent que ces cinq modèles peuvent prédire l'AUC du Tacrolimus avec une précision cliniquement acceptable de ± 15% alors que la C_0 présente la plus faible corrélation avec l'AUC.
En conclusion, cette étude confirme que la C_0 ne permet pas de prédire de manière efficace l'exposition du Tacrolimus dans l'organisme dans notre population de patients pédiatriques contrairement aux LSS analysées qui offrent une méthode pratique et fiable. Par ailleurs, en permettant d'obtenir une estimation précise et simplifiée de l'AUC complète du Tacrolimus chez les patients, ces LSS ouvrent la porte à de futures études prospectives visant à mieux définir l'AUC cible du médicament et à déterminer si le suivi basé sur la mesure de l'AUC est plus efficace et plus sûr que celui basé sur la mesure de la C_0.

Limited Sampling Strategies for Monitoring Tacrolimus in Pediatric Liver Transplant Recipients

Jean-Romain Delaloye, MD,† Nastya Kassir, PharmD,‡† Anne-Laure Lapeyraque, MD,†§ Fernando Alvarez, MD, PhD,¶ Michel Lallier, MD,* Mona Beaunoyer, MD,* Line Labbé, PhD,‡ Yves Théorêt, PhD,†‖** and Catherine Litalien, MD†**††*

Objective: To develop and validate limited sampling strategies (LSSs) for tacrolimus in pediatric liver transplant recipients.

Methods: Thirty-six 12-hour pharmacokinetic profiles from 28 pediatric liver transplant recipients (0.4–18.5 years) were collected. Tacrolimus concentrations were measured by immunoassay and area under the curve (AUC_{0-12}) was determined by trapezoidal rule. LSSs consisting of 1, 2, 3, or 4 concentration–time points were developed using multiple regression analysis. Eight promising models (2 per category) were selected based on the following criteria: $r^2 \geq 0.90$, inclusion of trough concentration (C_0), and time points within 4 hours postdose. The predictive performance of these LSSs was evaluated in an independent set of data by measuring the mean prediction error and the root mean squared prediction error .

Results: Five models including 2–4 time points predicted AUC_{0-12} with a ±15% error limit. Bias (mean prediction error) and precision (root mean squared prediction error) of LSS involving C_0, C_1, and C_4 ($AUC_{predicted} = 9.30 + 3.69 \times C_0 + 2.19 \times C_1 + 4.69 \times C_4$) were −4.98% and 8.29%, respectively. Among single time point LSSs, the model using C_0 had a poor correlation with AUC_{0-12} ($r^2 = 0.53$), whereas the one with C_4 had the highest correlation with tacrolimus exposure ($r^2 = 0.84$).

Conclusions: Trough concentration is a poor predictor of tacrolimus AUC_{0-12} in pediatric liver transplant recipients. However, LSSs using 2–4 concentration–time points obtained within 4 hours postdose provide a reliable and convenient method to predict tacrolimus exposure in this population. The proposed LSSs represent an important step that will allow the undertaking of prospective trials aiming to better define tacrolimus target AUC in pediatric liver transplant recipients and to determine whether AUC-guided monitoring is superior to C_0-based monitoring in terms of efficacy and safety.

Key Words: tacrolimus, therapeutic drug monitoring, pediatrics, liver transplantation, limited sampling strategies

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INTRODUCTION

Tacrolimus (Prograf, Fujisawa Healthcare Inc) is the most prescribed immunosuppressive agent in pediatric solid organ transplant recipients; the proportion of pediatric patients receiving tacrolimus increased from 14.5% in 1997 to 63.2% in 2006.[1] As with cyclosporine, tacrolimus inhibits calcineurin and blocks the transcription of cytokines that drive the proliferative T-cell response, particularly interleukin-2.

Therapeutic drug monitoring has become a standard of care for tacrolimus dosing optimization because of its significant interindividual and intraindividual pharmacokinetic (PK) variability and its narrow therapeutic index. Although whole blood trough concentration (C_0) is the current method used to guide dose individualization, measurement of C_0 often fails to reflect total drug exposure for drugs with variable bioavailability and unpredictable elimination characteristics such as tacrolimus. In such instances, systemic drug exposure may be best reflected by the area under the concentration–time curve (AUC). Many pediatric studies have shown that the relationship between tacrolimus C_0 and AUC is highly variable, with correlation (r^2) ranging from 0.30 to 0.88.[2–13] Additionally, controversies remain about the relationship between C_0 and clinical outcomes.[14,15] In liver transplantation, although a number of studies have shown a significant correlation between C_0 and tacrolimus nephrotoxicity and neurotoxicity, reports failed to demonstrate a linear relationship between tacrolimus C_0 and the development of graft rejection.[16–18]

In adult kidney recipients, Undre et al[19] reported a significant correlation between low systemic exposure of tacrolimus and acute rejection, whereas Mahalati et al[20] demonstrated that AUC was a better predictor of clinical outcomes than C_0 for the

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From the *Division of General Pediatric Surgery, Department of Surgery; †Clinical Pharmacology Unit, Centre Hospitalier Universitaire Sainte-Justine; ‡Faculty of Pharmacy, Université de Montréal; §Division of Nephrology; ¶Division of Gastroenterology, Department of Paediatrics; ‖Department of Biochemistry, Centre Hospitalier Universitaire Sainte-Justine; **Department of Pharmacology, Université de Montréal; and ††Division of Paediatric Critical Care, Department of Paediatrics, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada.

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J.-R. Delaloye and N. Kassir contributed equally to this work.

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Correspondence: Dr Catherine Litalien, MD, Division of Paediatric Critical Care, Department of Paediatrics, Director, Clinical Pharmacology Unit, Centre Hospitalier Universitaire Sainte-Justine, 3175 Chemin de la Côte Sainte-Catherine, Montréal (Québec), Canada H3T 1C5 (e-mail: catherine.litalien.hsj@ssss.gouv.qc.ca).

related calcineurin inhibitor cyclosporine. However, AUC-based monitoring, which implies the measurement of multiple concentration–time points over the entire dosing interval, is time consuming, expensive, and often impractical for routine clinical practice, especially in the pediatric population.[21] Alternatively, AUC can be predicted using the limited sampling strategy (LSS) approach, which has been developed so that a restricted number of sampling times can be used. Most LSSs for tacrolimus have been derived from adult transplant recipients,[22–26] and their direct application in children may not be feasible because of different tacrolimus PKs.[27] In the pediatric population, 3 studies have proposed LSSs to estimate tacrolimus exposure[2,3,28]; none of these LSSs have been validated in an independent set of data.[21,29,30] Furthermore, 2 of these trials[2,3] involved stable renal transplant patients and, as such, their use in other types of transplantation is not recommended because LSSs should only be applied to the patient population for which they have been validated.[21] The third trial dealt with children who underwent liver transplantation but provided only limited information with regard to the population studied and the methodological approach used.[28]

The aim of this study was to develop and validate LSSs for tacrolimus in pediatric liver transplant recipients.

MATERIALS AND METHODS

Patients and Study Design

This is a retrospective study analyzing 12-hour PK profiles performed in the pediatric liver transplant population of the Centre Hospitalier Universitaire Sainte-Justine (Montreal, Canada) between January 2007 and January 2010. The study was approved by the Institutional Research Ethics Committee. All liver transplant recipients ≤18 years of age who underwent full tacrolimus PK profile as part of their clinical care were considered to be eligible for inclusion. Exclusion criteria included patients not receiving oral tacrolimus twice daily and those for whom a full PK profile was not obtained at least 3 days after receiving the same dose of tacrolimus.

Tacrolimus was administered as a capsule or suspension (5 mg/mL) without any food intake recommendation. The dosage was adjusted by the liver transplant team to keep tacrolimus C_0 within a suggested target range of 5–15 ng/mL according to time posttransplantation and concomitant immunosuppression. However, the measurement of AUC_{0-12} was requested in some specific situations including at or around patient discharge; when nephrotoxicity occurred despite C_0 levels within the target range; when important intraindividual C_0 variability occurred in the absence of dose modification; and at mycophenolate mofetil initiation.

Whenever available in the medical chart, the following data were recorded for each patient: demographic parameters, type of liver transplantation, underlying diagnosis, time posttransplantation at which the PK profile was performed, tacrolimus dosing regimen and formulation, concomitant immunosuppression, blood chemistry (liver and renal function tests, hemoglobin, hematocrit, and albumin), and presence of clinically relevant CYP3A4 and/or P-glycoprotein inducer or inhibitor.

Sample Collection and Analytical Methods

Serial blood samples were collected in ethylenediaminetetraacetic acid–containing vacutainers immediately before tacrolimus administration (C_0), and after 0.5 ($C_{0.5}$), 1 (C_1), 1.5 ($C_{1.5}$), 2 (C_2), 3 (C_3), 4 (C_4), 8 (C_8), and 12 (C_{12}) hours. Tacrolimus whole-blood concentrations were determined using the microparticle enzyme immunoassay IMx (Abbott Laboratories, Abbott Park, IL). The lower and upper limits of detection were 1.5 and 30 ng/mL, respectively. The between-run coefficients of variation were 14.10% at 5 ng/mL, 11.15% at 11 ng/mL, and 10.21% at 22 ng/mL.

Pharmacokinetic and Statistical Analyses

PK parameters were estimated using noncompartmental methods. Tacrolimus peak concentration (C_{max}), time to reach C_{max} (T_{max}), and C_0, were determined for each patient from direct data observation. Observed tacrolimus AUC (AUC_{0-12}) was calculated using the linear trapezoidal rule (S-plus 8.1, Insightful Corporation, Seattle, WA).

PK data were randomly split into 2 equal groups: a training group and a validation group. In the training group, a multiple regression analysis was used to determine the relationship between observed tacrolimus AUC_{0-12} (dependent variable) and the concentrations at various time points (independent variable or predictor). This was carried out using the best-subset regression in conjunction with the stepwise forward selection technique. This method consists of starting with an equation with no predictor, trying them out one by one sequentially, and calculating the coefficient of determination for each equation (r^2). Multiple linear regression models were developed in which 1, 2, 3, or 4 concentration–time points were used as predictors. All possible equations were derived and the 2 best regression models using 1, 2, 3, and 4 predictors were identified based on the following criteria: $r^2 \geq 0.90$, inclusion of C_0, and time points within 4 hours posttacrolimus administration.

Equations were then validated in an independent set of patients, the validation group. The latter was used to estimate the predictive performance of the regression models developed in the training group. As suggested by Sheiner and Beal,[30] 2 error indices, the mean prediction error (ME) and the root mean squared prediction error (RMSE), were calculated to evaluate bias and precision, respectively. Their relative values, expressed as percentages, were also calculated. The following equations were used:

$$\mathrm{ME} = \frac{1}{N}\sum_{i=1}^{N}(\mathrm{Pred} - \mathrm{Obs}),$$

$$\mathrm{MSE} = \frac{1}{N}\sum_{i=1}^{N}(\mathrm{Pred} - \mathrm{Obs})^2,$$

$$\mathrm{RMSE} = \sqrt{\mathrm{MSE}},$$

$$\mathrm{ME}(\%) = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{\mathrm{Pred} - \mathrm{Obs}}{\mathrm{Obs}}\right)\times 100,$$

$$\text{RMSE}(\%) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{\text{Pred} - \text{Obs}}{\text{Obs}}\right)^2} \times 100.$$

where Pred is the predicted value of AUC_{0-12}, Obs is the AUC_{0-12} observed value, MSE is the mean squared prediction error, and N is the number of patients. A 95% confidence interval (CI) was calculated for each parameter. In addition, the predictive performance of the LSSs was tested by the method of Bland and Altman.[31] Finally, an approach based on CIs was used to compare the predictive performance of the different regression models.[30] This consisted of computing the difference in MSEs (ΔMSE) and MEs (ΔME) between the 2 models compared and calculating their CIs. If the CI did not include zero, the model with the smaller MSE or ME was considered significantly more precise or less biased, respectively. On the other hand, when the CI included zero, the MSE or ME difference was considered not significant. Clinical and PK data are presented as mean ± SD or median (range).

Comparisons between patient characteristics and PK parameters of the training and validation groups were performed using the unpaired *t*-test for normally distributed data and the Wilcoxon Rank-Sum test for skewed data. Statistical significance was defined at P value ≤ 0.05. All statistical analyses were performed using S-plus 8.1, SAS 9.2 (SAS Institute Inc, Cary, NC), and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

RESULTS

A total of 42 full PK profiles obtained from 31 liver transplant recipients were available for this study. Thirty-six profiles from 28 patients (15M/13F) aged between 0.4 and 18.5 years were included. Six profiles from 3 patients were excluded because either they were not obtained under steady-state conditions (n = 3), or they were obtained while patients were receiving tacrolimus 3 times a day. The indications for whole liver (10 patients) or cut-down liver (18 patients) transplantation were biliary atresia (n = 11), tyrosinemia (n = 7), North American Indian childhood cirrhosis (n = 2), fulminant hepatitis (n = 2), Alagille syndrome (n = 2), histiocytosis (n = 2), sclerosing cholangitis (n = 1), and autoimmune hepatitis (n = 1).

Patient characteristics are summarized in Table 1. As shown, the 36 PK profiles were equally divided in the training and validation groups. One patient with 3 full measurements of AUC_{0-12} was included in both groups, with 2 profiles in the training group and 1 in the validation group. The patient characteristics were not statistically different between the 2 groups, except for 2 liver parameters (alanine aminotransferase and γ-glutamyltranspeptidase).

Concomitant use of corticosteroids, which may induce CYP3A isoenzyme, was present for 22 PK profiles (61.1%). In about half of the profiles (52.8%), the patients were receiving 1 or 2 drugs known as potential CYP3A4 and/or P-glycoprotein inhibitors: amlodipine and lansoprazole (n = 6), amlodipine and enalapril (n = 1), amlodipine (n = 9), lansoprazole (n = 2), and diltiazem (n = 1).

Tacrolimus concentration–time profiles and PK parameters (Fig. 1 and Table 2, respectively) exhibited a high degree of between-individual variability. When PK parameters were normalized for a tacrolimus dose of 0.1 mg/kg, no significant difference was observed between the training and validation groups (P = 0.73, 0.87, and 0.44 for AUC_{0-12}, C_0, and C_{max}, respectively).

LIMITED SAMPLING STRATEGY DEVELOPMENT

Two hundred fifty-five regression equations were developed to predict tacrolimus AUC_{0-12} using a maximum of 4 different concentration–time points. Among these equations, 5 were identified as best regression models based on the predefined selection criteria (models 1–5; Table 3). For comparison purposes, 4 equations that did not meet these criteria (models 6–9) are also presented. Among the 3 single concentration–time point equations shown, those involving C_0 and C_4 had the poorest (r^2 = 0.53) and the highest (r^2 = 0.84) correlation with AUC_{0-12}, respectively.

LIMITED SAMPLING STRATEGY VALIDATION

The predictive performance of the selected LSSs is summarized in Table 4. The %RMSE (precision) of the 5 regression equations and their CIs were <15%. The %ME (bias) was also <15%, although all the models tended to underestimate tacrolimus AUC_{0-12}. The relative performance of the different LSSs is shown in Table 5. For 3 out of the 4 regression models using 3 or 4 concentration–time points (models 1–3), precision and bias were significantly better than for the model using 2 point equation (model 5). The fourth model using 3 time points (model 4) was significantly more precise than model 5, but there was no difference in bias between the 2 models. Finally, no significant difference was observed between the predictive capacity of LSSs involving 3 and 4 concentration–time points.

Figures 2A and B depict the linear correlation of observed AUC_{0-12} and AUC_{0-12} predicted by model 3 (C_0–C_1–C_4) and model 5 (C_0–C_4), respectively. Figures 2C and D show the Bland and Altman analysis of the paired samples for both models, with the average of predicted and observed AUC_{0-12} ranging from 83.6 to 305.0 ng·h/mL. In accordance with %ME, the predicted AUC_{0-12} was lower than the observed AUC_{0-12}, with a bias of −5.4% for model 3 (Fig. 2C) and −10.5% for model 5 (Fig. 2D).

DISCUSSION

This is the first study on the development and validation of LSSs for the prediction of tacrolimus AUC_{0-12} in pediatric liver transplant recipients. Five LSSs using 2–4 concentration–time points obtained within 4 hours of tacrolimus dosing and including C_0 have been developed and provide an accurate and convenient method to predict tacrolimus AUC_{0-12} in this population. All the 5 models predicted tacrolimus AUC_{0-12} with a ±15% prediction error limit; this is a clinically acceptable range because it represents the deviation from the observed AUC that usually initiates dosage adjustment.[29,32,33]

TABLE 1. Summary of Patient Characteristics on the Day of the PK Profile [Results Expressed as Number or Mean ± SD or Median (Range)]

Variable	Training Group (18 Profiles)	Validation Group (18 Profiles)	*P*
Age (yrs)	12.0 (0.4–16.9)	2.9 (0.6–18.5)	0.20
Weight (kg)	34.7 (4.5–61.3)	17.3 (6.2–59.1)	0.58
Time after transplantation (mos)	24.1 (0.5–166.2)	4.2 (0.5–185.5)	0.46
Tacrolimus dose ($mg \cdot kg^{-1} \cdot d^{-1}$)*	0.11 (0.05–0.54)	0.19 (0.05–0.42)	0.44
Tacrolimus formulations†			
Suspension (5 mg/mL)	7	11	
Capsule	11	6	
Concomitant immunosuppressive agents			
None	1	0	
Corticosteroids	7	9	
Corticosteroids + MMF	0	6	
MMF	10	3	
AST (U/L)	31 (16–304)	47 (24–148)	0.06
ALT (U/L)	45 ± 27 (17–125)	93 ± 69 (14–249)	0.01
GGT (U/L)	51 (8–261)‡	98 (13–886)	0.02
AP (U/L)	168 (49–448)	130 (66–567)‡	0.53
Bilirubine (μmol/L)	10 (4–25)‡	11 (5–110)	0.84
Hb (g/dL)	120 ± 21 (83–151)	120 ± 16 (84–143)	0.92
Hct (%)	0.34 ± 0.05 (0.25–0.44)§	0.36 ± 0.04 (0.26–0.43)‡	0.48
Serum albumin (g/L)	35 ± 3 (32–40)§	36 ± 4 (27–43)‡	0.66
Serum creatinine (mg/dL)	0.5 ± 0.2 (0.3–0.9)	0.4 ± 0.3 (0.1–0.9)	0.23

*Given twice a day.
†Data available for 17 profiles in the validation group.
‡Data available for 17 profiles.
§Data available for 13 profiles.
ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; Hb, hemoglobin; Hct, hematocrit; MMF, mycophenolate mofetil.

Among single concentration–time point LSSs, regression model using C_0 had a poor correlation with AUC_{0-12} (r^2 = 0.53), whereas the one with C_4 had the highest correlation with tacrolimus exposure (r^2 = 0.84). This is in accordance with other adult and pediatric studies that have shown C_0 to be a poor predictor of tacrolimus exposure in solid organ transplantation.[2,3,6,8,14,34,35]

LSSs developed in this study involved a limited number of blood samples within a short period of time after drug administration, originally suggested by Ting et al.[21] This method is convenient for both the patient and the clinician and is financially acceptable for the institution. The inclusion of predose measurement in the equations was a deliberate choice. Indeed, C_0 allows checking for compliance, helps identify patients with high tacrolimus clearance, and is a routinely used marker by clinicians.[29,36]

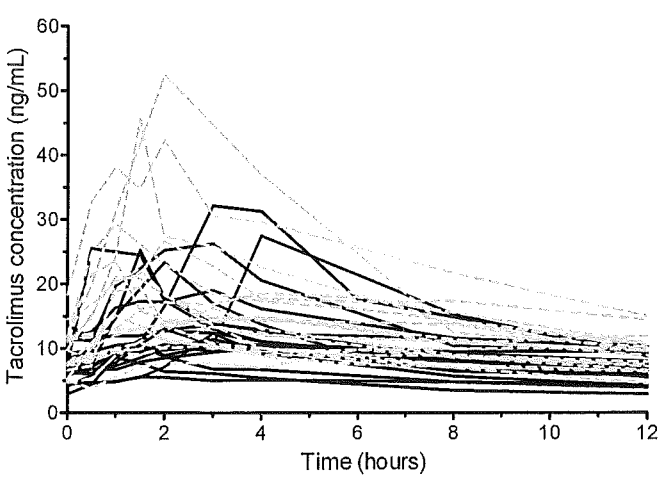

FIGURE 1. Tacrolimus concentrations as a function of time plotted for the 36 PK profiles (training group, solid lines; validation group, dashed gray lines).

TABLE 2. PK Parameters of Tacrolimus After Oral Administration in Pediatric Liver Transplant Recipients [Results Expressed as Mean ± SD or Median (Range)]

Parameter	Training Group (n = 18)	Validation Group (n = 18)	*P*
C_0 (ng/mL)	7.3 ± 2.8 (2.9–13.6)	9.7 ± 3.5 (4.7–18.3)	0.87*
C_{max} (ng/mL)	12.1 (5.6–32.1)	17.9 (9.8–52.5)	0.58*
T_{max} (h)	2.5 (0.7–4.0)	1.5 (1.0–6.0)	0.11
AUC_{0-12} (ng·h/mL)	114.0 (51.4–245.0)	141.2 (94.0–310.7)	0.70*

*For PK parameter normalized for a tacrolimus dose of 0.1 mg/kg.
AUC_{0-12}, area under the concentration–time curve from 0 to 12 hours postdose; C_0, trough concentration; C_{max}, peak concentration; n, number of PK profiles; T_{max}, time to reach C_{max}.

TABLE 3. Regression Equations for Predicting Tacrolimus AUC_{0-12} in Pediatric Liver Transplant Recipients With the Associated Coefficient of Determination (r^2)

Model	Time points	Regression Equation	r^2
1	C_0, C_1, C_2, C_4	$AUC_p = 9.15 + 3.65 \times C_0 + 1.81 \times C_1 + 0.51 \times C_2 + 4.55 \times C_4$	0.99
2	C_0, $C_{0.5}$, C_2, C_4	$AUC_p = 9.51 + 3.52 \times C_0 + 1.27 \times C_{0.5} + 1.52^*C_2 + 4.29 \times C_4$	0.99
3	C_0, C_1, C_4	$AUC_p = 9.30 + 3.69 \times C_0 + 2.19 \times C_1 + 4.69 \times C_4$	0.99
4	C_0, $C_{1.5}$, C_4	$AUC_p = 9.14 + 4.11 \times C_0 + 1.69 \times C_{1.5} + 4.67 \times C_4$	0.98
5	C_0, C_4	$AUC_p = 17.93 + 5.79 \times C_0 + 4.71 \times C_4$	0.94
6	C_0, C_2	$AUC_p = 18.56 + 6.97 \times C_0 + 4.05 \times C_2$	0.71
7	C_4	$AUC_p = 45.19 + 5.87 \times C_4$	0.84
8	C_2	$AUC_p = 44.33 + 6.01 \times C_2$	0.58
9	C_0	$AUC_p = 36.24 + 11.68 \times C_0$	0.53

AUC_p, predicted area under the concentration–time curve.

In pediatric organ transplantation, data published regarding LSSs are scarce. One report involving 14 liver recipients has suggested a 3 concentration–time points LSS (C_1–C_4–C_8), but without evaluation of its predictive performance.[28] In renal transplant recipients, 2 studies[2,3] have proposed LSSs, although with inconvenient sampling times for an outpatient setting (C_6) and no validation with an independent set of data.[21,29,30] Both studies showed a weak correlation between observed AUC and C_0 ($r^2 = 0.36$ and $r^2 = 0.56$) and among single concentration–time point, the best correlation was observed with C_4. These observations are in agreement with our results.

As in children, limited data are available in adult liver transplant recipients. Dansirikul et al[34] developed LSSs based on single time point. In their study, AUC_{0-6} was assumed to be a surrogate of the full tacrolimus AUC (AUC_{0-12}) and was used for LSS analysis. The authors showed that regression equations with sampling time at 2, 4, or 5 hours postdose rather than C_0 were superior in predicting tacrolimus exposure (AUC_{0-6}). However, only the LSS model involving C_5 was properly validated with a jackknife technique. In renal transplant patients, numerous LSSs have been published over the last decade although proper validation was often lacking.[23,33,37–39] More recently, Miura et al[40] developed and validated LSSs for the simultaneous estimation of AUC of tacrolimus and mycophenolic acid in this population. Similar to our findings, they reported that combinations of 2 or 3 concentration–time points including C_0 and C_4 (C_0–C_4 and C_0–C_2–C_4) provided reliable estimation of tacrolimus AUC_{0-12}; however, the best LSS for simultaneous prediction of the 2 immunosuppressive agents included C_2, C_4, and C_9. These results are not surprising considering the enterohepatic circulation of mycophenolic acid glucuronides, which causes a secondary plasma peak of mycophenolic acid.

The LSSs developed in this study showed good predictive performance in an independent validation group, despite high heterogeneity of the studied population in terms of demographic and clinical characteristics, time posttransplantation, and shape of PK profiles. This increases the likelihood that these LSSs will be used in various clinical settings. Additionally, in an "intention" to be highly representative of clinical reality, outlier PK profiles were not removed even if this was associated with the potential risk of underestimating the predictive performance of the LSSs. Another strength of these LSSs is the ease with which they could be applied by health care professionals; this is in contrast with LSSs derived from Bayesian analysis where users require more extensive training and specialized software programs.

This study has some limitations that deserve further comments. Ting et al[21] recently suggested that LSS should be applied only on transplant patient populations that are comparable with the population used to develop the LSS. As such, the predictive power of the LSSs reported in this study cannot be guaranteed for patient populations other than pediatric liver transplant recipients. By opposition to Bayesian-derived LSSs, LSSs developed with a multiple regression approach are less flexible in the sampling time wherein collection of samples at exact times is necessary.[41] Immunoassays may overestimate tacrolimus concentrations compared with those estimated by high-performance liquid chromatography methods, due to crossreactivity with tacrolimus metabolites.[15] Thus, caution should be exercised in the application of the proposed LLSs if tacrolimus concentrations are measured with analytic techniques different from the specific immunoassay used in this study.

TABLE 4. Evaluation of the Predictive Performance of LSSs to Estimate Tacrolimus AUC_{0-12} in Pediatric Liver Transplant Recipients, Mean (95% CI)

Models	Time Points	RMSE (ng/mL)	ME (ng/mL)	RMSE (%)	ME (%)
1	C_0, C_1, C_2, C_4	11.71 (6.97–15.02)	−7.77 (−12.25 to −3.28)	8.15 (3.22–11.07)	−5.07 (−8.34 to −1.81)
2	C_0, $C_{0.5}$, C_2, C_4	12.58 (8.50–15.63)	−8.74 (−13.37 to −4.11)	8.48 (4.25–11.22)	−6.15 (−9.14 to −3.16)
3	C_0, C_1, C_4	12.03 (6.59–15.68)	−7.91 (−12.55 to −3.28)	8.29 (3.29–11.28)	−4.98 (−8.37 to −1.59)
4	C_0, $C_{1.5}$, C_4	15.54 (9.08–20.01)	−10.68 (−16.46 to −4.91)	9.35 (5.60–11.98)	−6.75 (−10.06 to −3.44)
5	C_0, C_4	23.89 (14.25–30.65)	−18.06 (−26.06 to −10.05)	11.77 (8.65–14.22)	−9.80 (−13.14 to −6.46)

TABLE 5. Relative Performance of the 5 Different Models, Mean (95% CI)

Models*	Δ MSE (ng^2/mL^2)†	Δ ME (ng/mL)‡
1 versus 2	−21.17 (−70.02 to 27.69)	0.97 (−1.90 to 3.85)
1 versus 3	−7.54 (−27.82 to 12.74)	0.14 (−1.21 to 1.49)
1 versus 4	−104.25 (−236.95 to 28.44)	2.91 (−1.84 to 7.67)
1 versus 5	−433.76 (−785.75 to −81.77)	10.29 (2.54 to 18.03)
2 versus 3	13.63 (−48.76 to 76.02)	−0.83 (−4.80 to 3.14)
2 versus 4	−83.09 (−237.42 to 71.24)	1.94 (−3.87 to 7.75)
2 versus 5	−412.60 (−751.08 to −74.11)	9.31 (0.82 to 17.81)
3 versus 4	−96.71 (−233.49 to 40.07)	2.77 (−2.38 to 7.92)
3 versus 5	−426.22 (−776.36 to −76.09)	10.15 (2.87 to 17.43)
4 versus 5	−329.51 (−645.32 to −13.70)	7.37 (−0.63 to 15.38)

*Model 1: C_0–C_1–C_2–C_4; model 2: C_0–$C_{0.5}$–C_2–C_4; model 3: C_0–C_1–C_4; model 4: C_0–$C_{1.5}$–C_4; model 5: C_0–C_4.

†MSE for each model was as follows: model 1, 137.13; model 2, 158.29; model 3, 144.66; model 4, 241.38; model 5, 570.89.

‡ME for each model is reported in Table 4.

Even though the evaluation of the relative performance of the 5 LSSs has shown that 3 and 4 concentration–time point LSSs were statistically more precise and less biased than the one using 2 time points, all the equations can be used with confidence. Therefore, it is the ultimate choice of clinicians to decide which model to use according to the desired level of precision, balanced with the costs and feasibility in clinical practice.

CONCLUSIONS

Trough concentration is a poor predictor of tacrolimus AUC_{0-12} in pediatric liver transplant recipients. However, LSSs using 2–4 concentration–time points obtained within 4 hours of tacrolimus dosing have been developed and provide a reliable and convenient method to predict tacrolimus exposure in this population. By facilitating the accurate measurement of tacrolimus full AUC, the proposed LSSs represent an important step that will allow the undertaking of

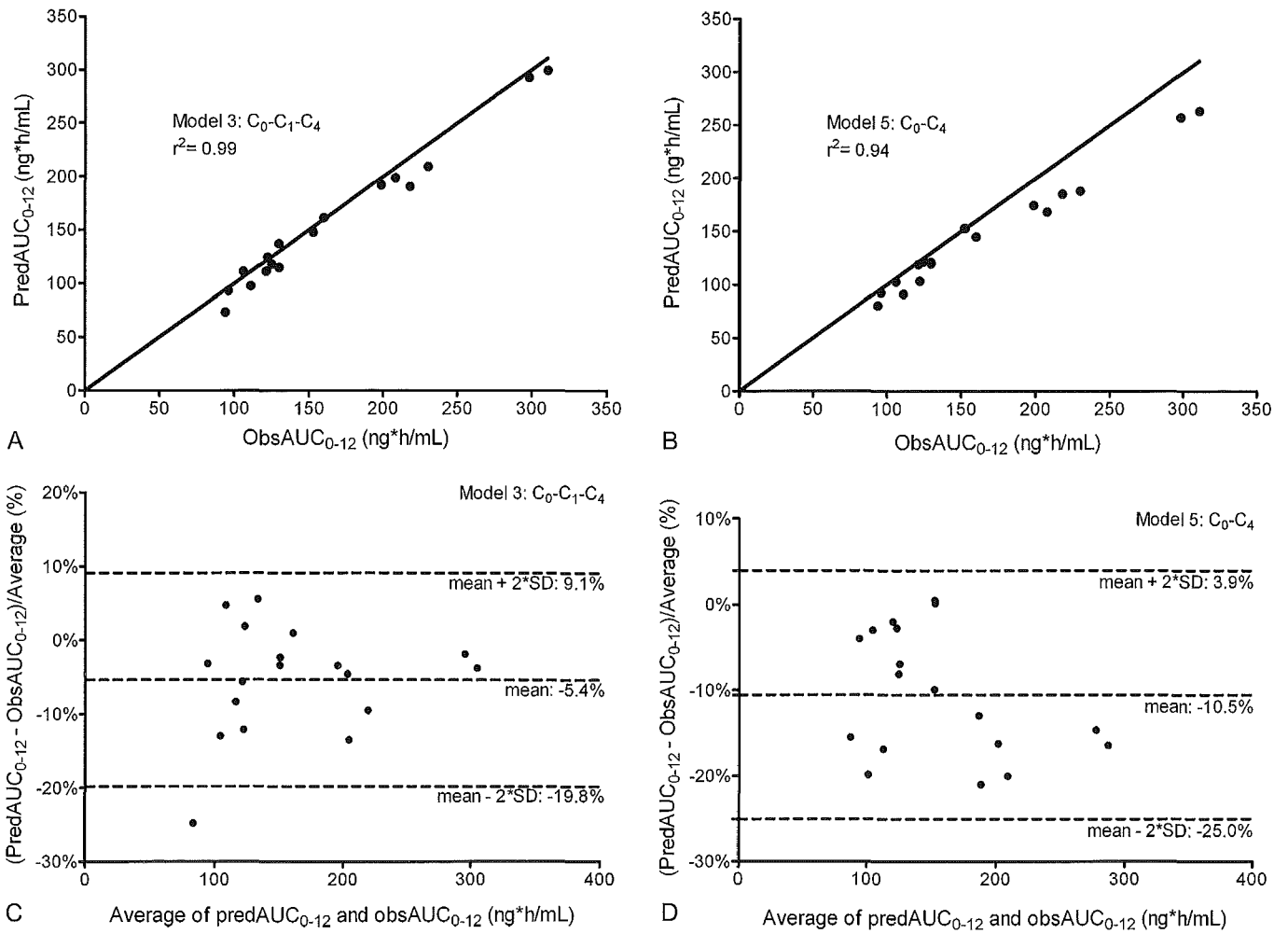

FIGURE 2. Correlation between the observed AUC_{0-12} ($ObsAUC_{0-12}$) and the predicted AUC_{0-12} ($PredAUC_{0-12}$) of tacrolimus calculated with the equations of model 3 (A) involving 3 concentration–time points (C_0–C_1–C_4) and model 5 (B) involving 2 concentration–time points (C_0–C_4), respectively. Bland and Altman analysis testing agreement between tacrolimus $ObsAUC_{0-12}$ and $PredAUC_{0-12}$ calculated with the equations of model 3 (C) involving 3 concentration–time points (C_0–C_1–C_4) and model 5 (D) involving 2 concentration–time points (C_0–C_4), respectively.

prospective trials aiming to better define tacrolimus target AUC in pediatric liver transplant recipients and to determine whether AUC-guided monitoring is superior to C_0-based monitoring in terms of efficacy and safety.

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