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Full Length Research Paper

Evaluation and validation of limited sampling strategy for estimating individual exposure of mycophenolic acid in renal transplant children receiving concomitant tacrolimus

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Limited sampling strategies (LSS) for estimating the area under the concentration-time curve (AUC) of mycophenolic acid (MPA) in renal transplant children receiving concomitant tacrolimus have been developed, but they have not yet undergone full validation. The objectives of the present study were to evaluate the predictive performance of previously published LSS of MPA in an independent pediatric population and to validate a reliable and clinically applicable LSS for routine clinical practice. Published MPA LSS in renal transplant children were screened from the literature, and MPA predicted AUCs were calculated using these LSS. These predicted AUCs were then compared with the reference AUCs, which were calculated by the trapezoidal rule. External validation was prospectively performed in an independent validation group consisting of 44 renal transplant children. This group had a mean age of 12 years (range 3.45 - 20.57) and a mean weight of 35.8 kg (range 15.5 - 77.5). In this external validation dataset, the LSS2 equation: MPA-AUC0-12 = 12.6 + 7.78.C0 + 0.9.C1 + 1.3.C2 demonstrated good predictive performance. The correlation between the predicted and reference AUC using the LSS2 equation was r2= 0.88, with a percent error (PE) of 6.64, a root mean square error (RMSE) of 9.63, and a Bland-Altman bias of -1.36. The mean MPA-AUC0-12h values obtained from the LSS1 equation (MPA-AUC0-12 = 10.0 + 3.95×C0 + 3.24×C0.5 + 1.01×C2) and LSS2 were 65.23±33.84 µg.h/ml, 50.24±19.41 µg.h/ml, and 51.75±19.41 µg.h/ml, respectively. The correlation (r2) between full MPA-AUC0-12h and LSS2 (r2 = 0.88) was higher than that with LSS1 (r2 = 0.69). The linear regression value was superior with LSS2 (R2 = 0.77) compared to LSS1 (R2 = 0.472). Additionally, the RMSE was lower for the David Netto method (DNm; RMSE = 9.63) than for LSS1 (RMSE = 28.82). The Bland-Altman plot showed a bias of -1.36 for LSS2 and -14.99 for LSS1. These results indicate that a reliable and clinically applicable LSS was validated to predict the mycophenolic acid (mycophenolate mofetil [MFF]) AUC in renal transplant children receiving concomitant tacrolimus. This LSS can be routinely used to adapt individual doses of MMF.

Key words: Limited sampling strategy, mycophenolate mofetil, renal transplantation, children, tacrolimus.

INTRODUCTION

Mycophenolate mofetil (MMF), the ester prodrug of the active compound mycophenolic acid (MPA), is widely used for maintenance immunosuppressive therapy in pediatric organ transplant recipients (Zhao et al., 2010). It acts as a potent, reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), which is the key enzyme in de novo purine biosynthesis in proliferating T and B lymphocytes (Kant et al., 2022).

The pharmacokinetics of MMF demonstrates wide inter-individual variability, particularly in children (Zhao et al., 2010). Due to its narrow therapeutic index, therapeutic drug monitoring (TDM) has become mandatory in routine clinical practice to individualize dosing regimens of MMF (Ehren et al., 2021). An AUC0-12 target of 30 to 60 mg×h/L has been recommended to prevent rejection during both the early post-transplant period and maintenance therapy, while also decreasing the risk of adverse events (Resztak et al., 2021). However, implementing the full pharmacokinetic profile over 12 h with repeated blood sampling in pediatric clinical practice is not feasible due to ethical and practical concerns. Therefore, limited sampling strategies (LSS) have been proposed to address this issue. Several LSS have been developed and validated in adult organ transplant recipients, but few studies have been conducted in children, and crucially, external validation has not been performed in children receiving co-treatment of MMF-tacrolimus, which has become the most common combination in pediatric organ transplant recipients (Lancia et al., 2014). This issue has been emphasized in a recent review by van der Meer et al. (2011).

Indeed, validation in an independent dataset is essential for the clinical applicability of LSS. The aims of the present study were to review published LSS of MMF in renal transplant children, evaluate the predictive performance of these LSS in an independent pediatric population, and validate a reliable and clinically applicable LSS for routine clinical practice.

MATERIALS AND METHODS

Study population

Renal transplant children from the Department of Pediatric Nephrology at Robert Debré Hospital, Paris (France), were enrolled in this study. This observational study was designed in accordance with legal requirements and the Declaration of Helsinki, presented to the National Committee for Informatics and Liberties (CNIL), and approved by the local research ethics committee. The triple immunosuppressive therapy consisted of MMF, tacrolimus, and prednisone. Pharmacokinetic data were obtained according to local clinical practice. A full concentration-time profile was determined during hospitalization and/or routine follow-up visits when a steady-state condition was achieved. A limited number of samples were obtained before, 0.5, 1, 2, 3, 8, and 12 h after drug intake. MMF dose was adjusted to maintain AUC between 30 and 60 mg/L×h.

MPA assay

The plasma MPA concentration was measured using the Roche Mycophenolic Acid TM assay, which relies on the enzymatic activity of recombinant Inosine Monophosphate Dehydrogenase 2. This method exhibited excellent correlation with high-performance liquid chromatography (Brandhorst et al., 2008). The assay was conducted on a Cobas Integra system (Roche Diagnostics, Meylan, France), following the manufacturer's guidelines. Both intraday and interday coefficients of variation were less than 2%. The lower limit of quantification was 0.3 µg/ml.

Pharmacokinetic analysis and validation procedure

The predicted AUC0-12 was calculated using the identified LSS. The reference AUC0-12 was calculated using the non-compartmental method and the linear up/log-down trapezoidal procedure within WinNonlin version 5.2 (Pharsight Corporation; Mountain View, CA, USA). The predictive performance was evaluated by calculating the prediction error (PE) and root mean square error (RMSE). These metrics were calculated using the following equations:

$$PE = 1/n \sum (Observed - Real) / (Observed)$$

$$RMSE = 1/n \sum (Observed - Real)^2$$

The Bland-Altman test was used to evaluate the agreement between the two methods of AUC calculation (Giavarina, 2015).

RESULTS

The external validation dataset comprised 44 full pharmacokinetic profiles of MMF obtained from 44 renal transplant children, including 21 females. The mean (SD) age was 12 (5.3 years) ranging 3.45 to 20.57 years, and the mean (SD) bodyweight was 35.8 (18.8 kg) ranging 15.5 to 77.5 kg. The time post-transplant varied from 18 days to 9 years, covering both the immediate post-transplant and long-term follow-up periods. The characteristics of the patients are summarized in Table 1. The concentration-time profiles of MPA are demonstrated in Figure 1. Figure 2 shows the whole concentration points. The mean (SD) reference AUC was 50.2 (10.4 mg×h/L) ranging 19.9 to 95.6 mg×h/L. Two LSS of MMF, developed in renal transplant children receiving concomitant tacrolimus, were identified through a literature review (Ehren et al., 2021; Resztak et al., 2021;

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Table 1. Baseline characteristics of 44 children.

Demographic data	Mean±SD	Range
Gender (male/female)	23/21	-
MMF dose range (mg/dose)	472.5 ± 156.7	250 - 1000
Age (years)	12.0 ± 5.3	3.45 - 20.57
Weight (kg)	35.8 ± 18.8	15.5 - 77.5
Time kidney transplantation to enrolment (years)	1.8 ± 2.6	0.05 - 9.32

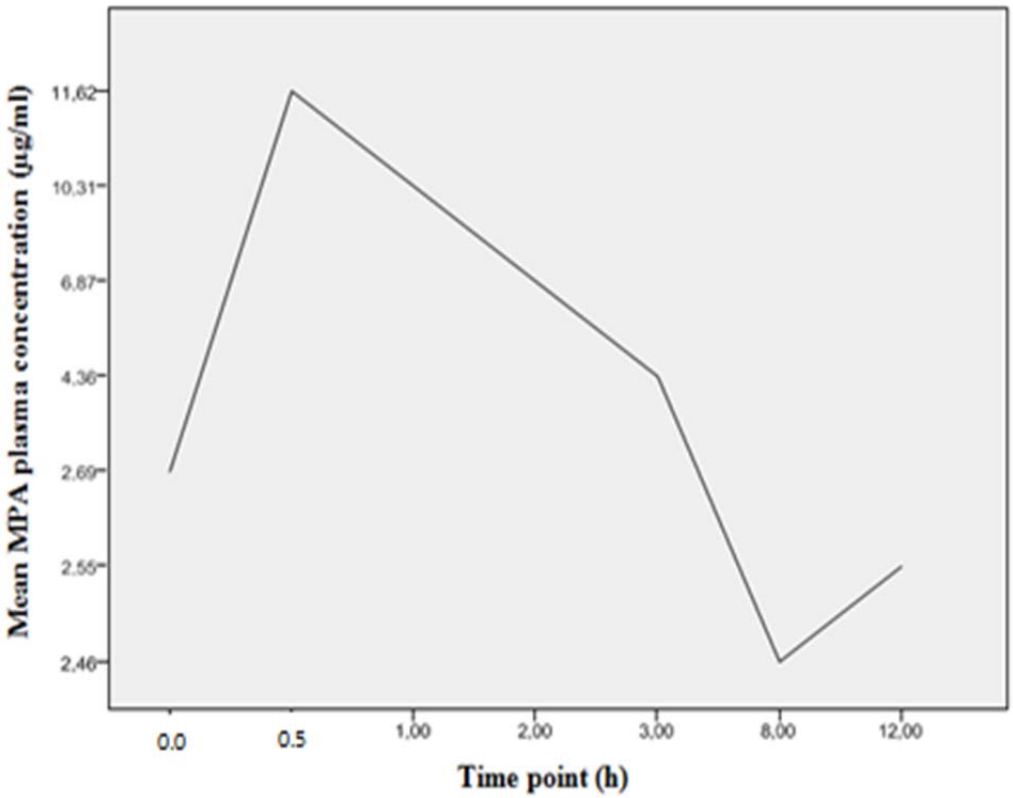

Figure 1. Mean MPA plasma concentration.

Lancia et al., 2014). The LSS equations were expressed as follows: LSS1: AUC0-12 = 10.0 + 3.95 × C0 + 3.24×C0.5 + 1.01×C2; LSS2: AUC0-12 = 12.6 + 7.78 × C0 + 0.9 × C1 + 1.3 × C2. Using LSS1, the mean (SD) predicted AUC was 65.2 (33.8 mg×h/L) ranging 23.4 to 162.1 mg×h/L. The mean PE and RMSE were 31.8 and 28.82 mg×h/L, respectively. The correlation between reference and predicted AUCs using LSS1 was significant (r = 0.69, p < 0.001, Pearson correlations test) (Table 2).

The Bland-Altman analysis is as shown in Figures 3 and 4. The mean difference between the reference and LSS1-predicted AUC was -14.99 µg.h/ml (95% CI: -64.23, 34.24). Using LSS2, the mean (SD) predicted AUC was 51.8 (19.4 mg×h/L) ranging 25.7 to 103.7 m×h/L. The mean PE and RMSE were 6.84 and 9.63 mg×h/L, respectively. The correlation between reference and predicted AUCs using LSS2 was significant (r = 0.88, p < 0.001, Pearson correlations test) (Table 2). The Bland-Altman analysis is as shown in Figures 5 and 6. The mean difference between the reference and LSS2-predicted AUC was -1.36 mg×h/L (95% CI: -21.09, 18.36).

DISCUSSION

The studies on therapeutic drug monitoring of MPA exposure are primarily reported in renal transplantation (Le Meur et al., 2011; Smits et al., 2014; Tett et al., 2011;

Table 2. Pearson correlation.

Parameter	MPA-AUC Filler	MPA-AUC David
MPA-AUC0-12 h	0.69	0.88

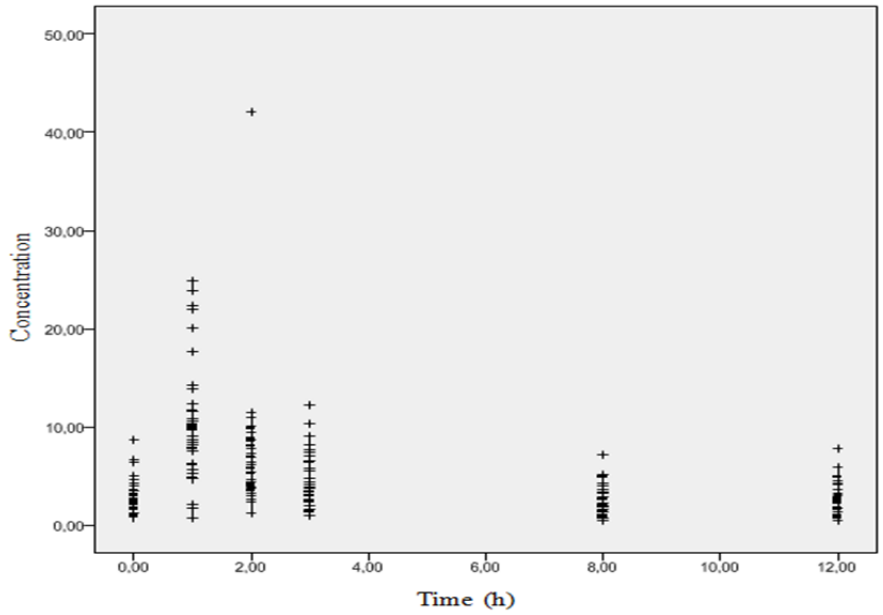

Figure 2. The whole concentrations points.

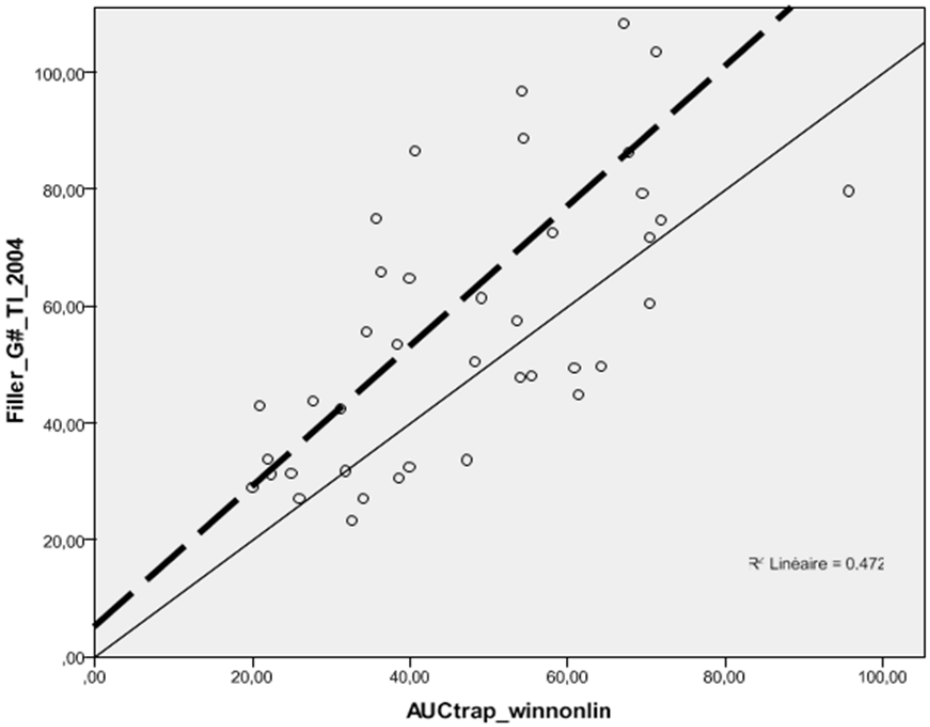

Figure 3. Relationship between the abbreviated pharmacokinetic profile derived from Filler equation and the corresponding regression line.

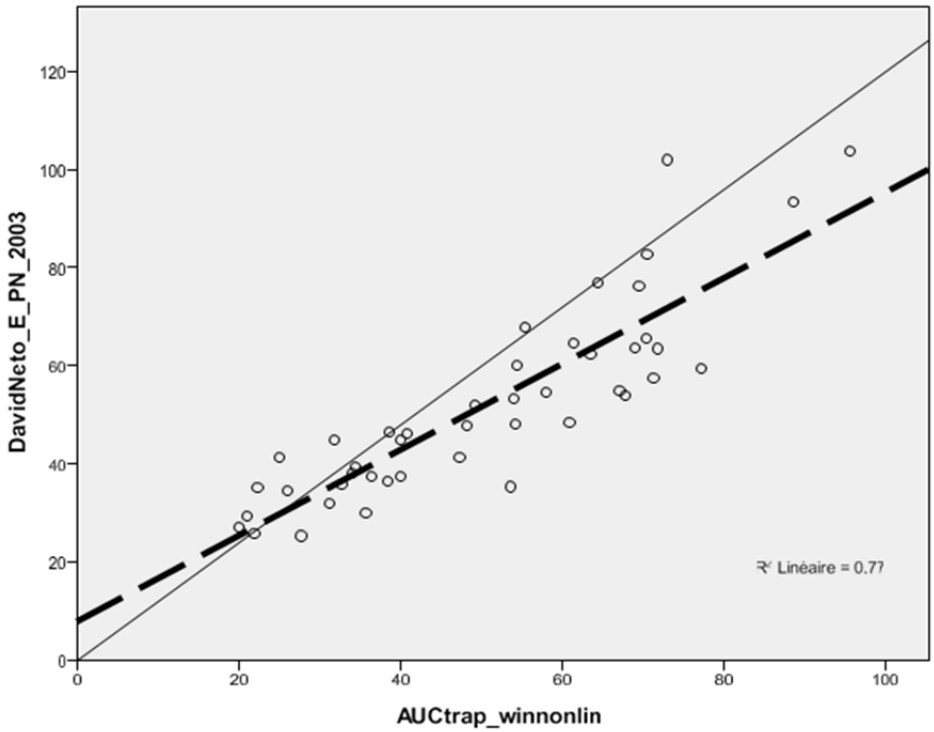

Figure 4. Relationship between the abbreviated pharmacokinetic profile derived from David Neto equation and the corresponding regression line.

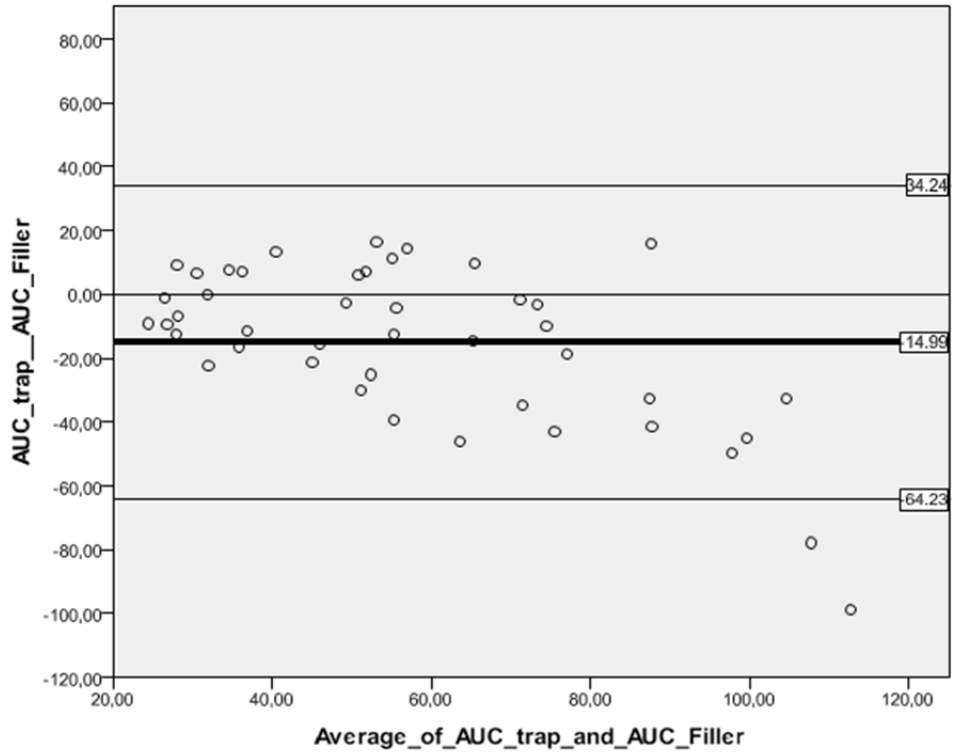

Figure 5. Bland and Altman analysis of the difference between LLS of Filler and ful MPA-AUC0-12 h.

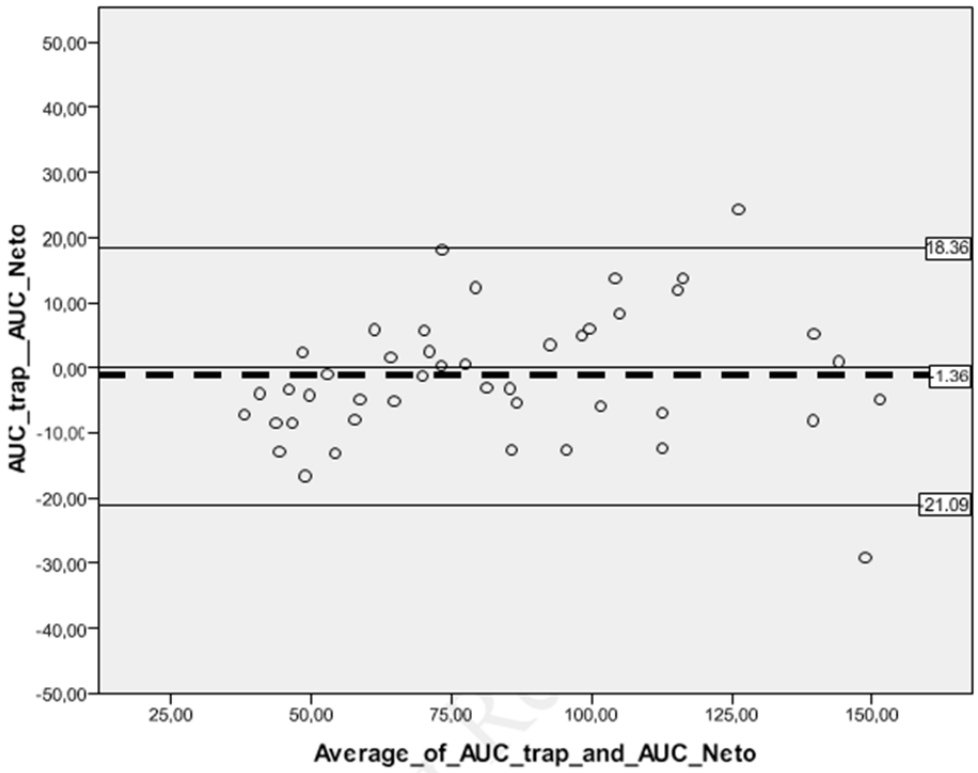

Figure 6. Bland and Altman analysis of the difference between LLS of David Neto and full MPA-AUC0-12 h.

de Winter et al., 2011). It has been demonstrated that the most effective methodology relies on abbreviated pharmacokinetic parameters derived from limited sampling strategies (Weber et al., 2006). Furthermore, MPA-AUC0-12 h has shown a strong correlation with acute rejection compared to predose plasma concentration, making it the preferred pharmacokinetic parameter for drug monitoring according to most investigators (Höcker et al., 2011; de Winter et al., 2011; Le Meur et al., 2007). As a result, several algorithms based on a limited number of pharmacokinetic sampling time points have been developed (Zhang et al., 2018; Sánchez Fructuoso et al., 2012; Berger et al., 2019).

Indeed, LSS for MMF have been extensively developed and validated in conjunction with tacrolimus in the adult population (Pawinski et al., 2013; Cai et al., 2015; Yamaguchi et al., 2013), but there is been limited research in the pediatric population. In pediatric transplant recipients receiving MMF and tacrolimus, we identified two LSS, neither of which has been validated in another population group.

These LSS equations for MMF utilized the MPA concentration at three-time points to estimate the AUC0-12 h. Sobiak et al. (2023) found, after a repeated cross-validation procedure of several models, that the three-point model within 2 h was superior. However, Xiang et al. (2024) demonstrated that the four-point approach within 6 h was better than the three-point approach; nevertheless, the three-point method yielded acceptable results.

In this study, the mean MPA-AUC0-12 h value (50.24±19.41 µg.h/ml) was lower than those obtained by Filler (57.56 ± 28.8 µg.h/ml) and by Neto (63 ± 22 µg.h/ml) in their populations. This difference may be explained by the considerable interpatient variability in exposure (Woillard et al., 2014) and the different characteristics of the three populations. High doses of MMF are typically needed immediately after renal transplantation to prevent acute rejection of the graft (Fukuda et al., 2011).

When using C0 as a parameter, the Pearson's correlation value was higher with David Neto's method (DNm; r2 = 0.87) than with MPA-AUC0-12 h (r2 = 0.7). This result is consistent with previously published studies (Radzevičienė et al., 2020; Pan et al., 2014), where authors demonstrated that C0 was a poor parameter in clinical outcome criteria. We also measured, via Pearson's correlation, the strength between MPA-AUC0-12 h and the two abbreviated models. Our results showed that DNm had the highest correlation (r2 = 0.88), compared to 0.69 for FGm. However, during the validation of these two models in their respective populations, the Pearson's correlation for FGm was r2 = 0.99, superior to DNm (r2 = 0.899). These differences are

likely due to the baseline characteristics of our population.

The linear regression value between MPA-AUC0-12 h and DNm (R2 = 0.77) was higher than that obtained with FGm (R2 = 0.472). Furthermore, the root mean square error (RMSE) was three times higher for FGm (28.82) compared to DNm (9.63). Bland-Altman analysis revealed good agreement with David Neto's equation compared to Filler's equation; the mean difference was -1.36 for DNm compared to -14.99 for FGm (Filler et al., 2017). Additionally, the limit of agreement with FGm was twice that of DNm. Therefore, the most accurate estimate of MPA-AUC0-12 h was achieved with DNm.

Conclusion

This study has demonstrated significant differences in MPA parameters among pediatric renal transplant recipients receiving MMF and tacrolimus therapy. The David Neto abbreviated equation was found to be more accurate than the Filler G equation for predicting MPA-AUC0-12 h in our population (David-Neto et al., 2003).

ABREVIATIONS

AUC, Area under curve; **CNIL,** Commission Nationale Informatique et Libertés; **DNm,** David Neto model; **FGm,** Filler Guido model; **IMPDH,** inhibitor of inosine monophosphate dehydrogenases; **LSS,** limited sampling strategies; **MPA,** mycophenolic acid; **MMF,** mycophenolate mofetil; **TDM,** therapeutic drug monitoring; **PE,** prediction error; **RMSE,** root mean square error.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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