Clinical Chemistry 48:9
1497–1504 (2002)

Drug Monitoring and Toxicology

Limited Sampling Strategy for the Estimation of Mycophenolic Acid Area under the Curve in Adult Renal Transplant Patients Treated with Concomitant Tacrolimus

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Background: **Significant relationships between the mycophenolic acid (MPA) area under the concentration–time curve (AUC_{0-12h}) and the risks for acute rejection and side effects have been reported. We developed a practical method for estimation of MPA AUCs. Regression equations were developed using repeated cross-validation for randomly chosen subsets, characterized statistically, and verified for acceptable performance.**

Methods: **Twenty-one renal transplant patients receiving 0.5 or 1.0 g of mycophenolate mofetil twice daily and concomitant tacrolimus provided a total of 50 pharmacokinetic profiles. MPA concentrations were measured by a validated HPLC method in 12 plasma samples collected at predose and at 30 and 60 min; 2, 3, 4, 6, 8, 9, 10, 11, and 12 h; 1 and 2 weeks; and 3 months after transplantation. Twenty-six 1-, 2-, or 3-sample estimation models were fit (r^2 = 0.341–0.862) to a randomly selected subset of the profiles using linear regression and were used to estimate AUC_{0-12h} for the profiles not included in the regression fit, comparing those estimates with the corresponding AUC_{0-12h} values, calculated with the linear trapezoidal rule, including all 12 timed MPA concentrations. The 3-sample models were constrained to include no samples past 2 h.**

Results: **The model using c_{0h}, $c_{0.5h}$, and c_{2h} was superior to all other models tested (r^2 = 0.862), minimizing prediction error for the AUC_{0-12h} values not included in the fit (i.e., the cross-validation error). The regression equation for AUC estimation that gave the best performance for this model was: $7.75 + 6.49c_{0h} + 0.76c_{0.5h} + 2.43c_{2h}$. When we applied this model to the full data set, 41 of the 50 (82%) estimated AUC values were within 15% of the value of AUC_{0-12h} calculated using all 12 concentrations.**

Conclusions: **This limited sampling strategy provides an effective approach for estimation of the full MPA AUC_{0-12h} in renal transplant patients receiving concomitant tacrolimus therapy.**

Mycophenolate mofetil (MMF),[4] an ester prodrug of the immunosuppressant mycophenolic acid (MPA), is widely used for the prevention of rejection in patients receiving renal, heart, or liver transplants *(1–3)* and is under evaluation for its anti-graft-vs-host-disease effect in recipients of hematopoietic stem cell transplants. MMF is administered to patients who have undergone transplantation at a dosage of 0.5–1.5 g given twice daily. After oral administration, MMF is rapidly and extensively absorbed and hydrolyzed to MPA *(4)*. The latter is metabolized by UDP-glucuronosyltransferase to the phenolic glucuronide of mycophenolic acid, which is pharmacologically inactive *(5, 6)*.

MPA is avidly and extensively bound to plasma albumin *(7)*. Several investigators have reported a significant relationship between the MPA dose interval area under the plasma concentration–time curve (AUC) and the risks

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Received April 3, 2002; accepted May 16, 2002.

[4] Nonstandard abbreviations: MMF, mycophenolate mofetil; MPA, mycophenolic acid; AUC, area under the plasma concentration–time curve; CsA, cyclosporine; LSS, limited sampling strategy; and CI, confidence interval.

for rejection *(4, 8–15)* and hematologic side effects *(14, 16)*. A >10-fold range of MPA AUC values has been observed in renal and heart transplant patients who received a fixed dose of 1 g of MMF twice daily *(12, 14, 17)*. Thus the interindividual variability of MPA pharmacokinetics is extensive.

Recent clinical investigations suggest that improved effectiveness and tolerability will result from the incorporation of MPA therapeutic drug monitoring into routine clinical practice, providing effective MMF dose individualization in renal and heart transplant patients *(2, 11–13, 15, 18)*. A target range of 30–60 mg · h/L for the MPA AUC has been proposed for guidance of MMF dosage to optimal values in renal and heart transplant patients receiving concomitant cyclosporine (CsA) and steroid immunosuppression *(12, 13, 15)*. However, the routine measurement of the full 12-h dose interval MPA AUC is very impractical and would be cost-prohibitive. Recent studies have therefore focused on the development and use of abbreviated sampling schemes for the reliable estimation of MPA AUC_{0-12h}. Results from three such studies have concluded that inclusion of a 6-h sample is critical for the reliable estimation of the MPA AUC_{0-12h} *(19–21)*. Inclusion of a 6-h timed sample is impractical, however, for routine practice in many centers because of patient inconvenience. In our experience this is a very important practical factor that limits the use of abbreviated sampling approaches in clinical practice. With these considerations in mind, we investigated the development of a limited sampling procedure using one, two, or three samples. For 1-sample regressions, each time point over the entire 12-h interval was tested. For the 2-sample regressions, the predose sample was tested with each time point over the 12-h interval. For the 3-sample regressions, the predose sample was tested with each combination of two samples from the first 2 h. To minimize the effect of unfavorable sampling on the linear regression modeling, we used repeated cross-validation, similar to the "bootstrap" approach, to identify the most robust models.

Patients and Methods

PATIENTS

A total of 50 MPA AUC_{0-12h} values were measured in 21 recipients of a kidney transplant. Patients received 0.5 g (n = 11) or 1 g (n = 10) of MMF twice daily by the oral route for the duration of the study. Each patient received concomitant tacrolimus, initially at an oral dose of 0.2 $mg \cdot kg^{-1} \cdot day^{-1}$ and then dose-adjusted to achieve a steady-state blood concentration of 15 μg/L. MPA pharmacokinetic profiles were determined 1 and 2 weeks and 3 months after transplantation. Plasma samples (EDTA) were collected at the following 12 times after an overnight fast: predose and 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 11, and 12 h after the morning dose of MMF. Only full 12-sample profiles were included in this investigation. Thus, profiles were excluded from this study because samples were missing (12-h samples were not obtained for two profiles at 3 months) or because MPA concentrations were below the lower limit of quantification (<0.2 mg/L in six samples for 1 profile at 2 weeks and in one or more samples for 10 profiles obtained during week 1), which precluded the use of these profiles in this investigation. There were no restrictions on the type of food consumed starting no sooner than 1 h after the MMF dose. Plasma samples were stored at −20 °C until analysis. The study protocol received Institutional Review Board approval. Written informed consent was obtained from each study patient.

ANALYTICAL METHODS

Plasma MPA concentrations were measured by a validated HPLC method *(22)*. Full 12-h AUC values were calculated using the linear trapezoidal rule.

STATISTICAL PROCEDURE

Limited sampling strategy (LSS) evaluation. Repeated cross-validation was used to evaluate each LSS, similar to a bootstrap procedure. These are important general techniques for the evaluation of bias and for estimating the precision of a study parameter *(23, 24)*. Below, we present an outline of the method:

Step 1. The full MPA AUC_{0-12h} was calculated for each of the 50 MPA concentration profiles, using each set of 12 MPA concentrations. A data set containing 50 records (one per profile) was then constructed that included the variables DOSE, PATIENT, SAMPLING DAY, MPA AUC_{0-12h}, and the following 12 MPA concentrations: c_{0h}, $c_{0.5h}$, c_{1h}, c_{2h}, c_{3h}, c_{4h}, c_{6h}, c_{8h}, c_{9h}, c_{10h}, c_{11h}, and c_{12h}.

Step 2. In this step the data set was repeatedly randomly divided into two groups of 25 each: a training group and an evaluation (or testing) group. The training group records were used to determine the relationship (i.e., regression coefficients) between MPA AUC_{0-12h} and each of the 26 previously described linear regression models. This process of randomly dividing the data sets into two equal groups, a training and an evaluation group, was repeated a total of 50 times. Each time this was done the 26 linear regression models were fit to the MPA AUC_{0-12h} by use of the MPA concentrations at the selected sampling times for the 25 records in the training group, using multiple linear regression analysis (SPSS-GP, Ver. 10 for Windows). This produced equations of the form: $AUC = \beta_1 c_1 \ldots + \beta_n c_n + I$, where β_1–β_n are regression coefficients, I is the *y*-intercept, n is the nominal sample collection time, and c_1–c_n are MPA concentration values measured at times 1 through n. The distributions of the *y*-intercept and regression coefficients for each of the 26 models were then examined.

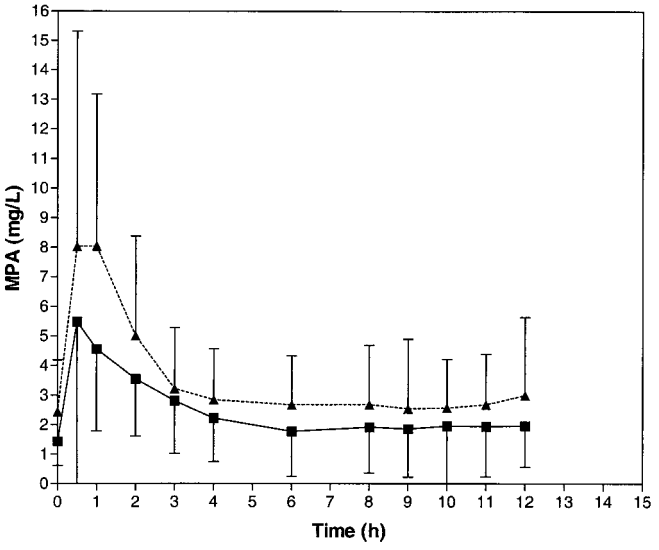

Fig. 1. MPA concentrations as a function of time plotted for the 50 full pharmacokinetic profiles.

Concentrations are the mean ± SD (*error bars*). ■, mean MPA concentrations for patients receiving 0.5 g of MMF twice daily; ▲, mean MPA concentration values for patients receiving 1 g of MMF twice daily.

Step 3. Each of the linear regression equations (26 models) obtained in step 2 was used to estimate the MPA AUC for the 25 profiles in the corresponding evaluation set. This step was repeated for each of the 50 times the data set was randomly divided.

Step 4. "Residuals" were calculated for each of the 25 MPA AUC_{0-12h} values in the evaluation group by taking the difference between the natural log (ln) of the full MPA AUC_{0-12h} and the natural log (ln) of the MPA AUC estimated by the regression equation. This procedure produced a total of 1250 (i.e., 25 × 50) prediction residuals. Note that these are not the usual regression residuals, as the regression comes from the training set, whereas the residuals come from the application of the derived regression equation to the evaluation set. The distribution of the entire set of residuals was examined (mean, median, SD, and symmetry) to assure that the selected limited sample equation for prediction of MPA AUC produced a distribution of estimated MPA AUC values in the evaluation sets that met certain statistical criteria (mean value for the entire set of residuals close to 0 and with a very small SD). The model (of the 26) that yielded the most favorable distribution (mean near zero, smallest range encompassing most of the residuals) of residuals was selected as providing the best performance. Once the general model (of the 26) was selected, the proposed regression coefficients were taken as the median of the distribution of regression coefficient values described in step 2. These final LSS models were used to calculate prediction error for each patient, using the equation (estimated AUC − measured AUC)/measured AUC) × 100 and expressed as a percentage. Mean estimation error [with 95% confidence intervals (CIs)] was calculated as the arithmetic mean of the prediction errors for the 50 patient profiles for each individual model.

ROLE OF THE SPONSORS

GlaxoSmithKline and Fujisawa Healthcare, Inc. participated in the data analysis and manuscript preparation.

Results

Twenty-six models were developed and analyzed for their ability to estimate MPA AUC_{0-12h} based on a limited number of samples. A total of 50 full MPA pharmacokinetic profiles were used to test the performance of these models. The MPA AUC_{0-12h} values ranged from 9.5 to 90.8 mg · h/L (median value, 33.3 mg · h/L; mean ± SD, 35.6 ± 17.8 mg · h/L). The medians, means ± SD, and (ranges) for c_{max}, t_{max}, and c_{0h} were, respectively: 7.6 mg/L, 9.5 ± 6.2 mg/L (1.6–31.2 mg/L); 1 h, 2.1 ± 2.7 h (0.5–11 h); and 1.73 mg/L, 1.94 ± 1.43 mg/L (0.2–5.2 mg/L). The mean (± SD) MPA concentrations at the studied time points are displayed in Fig. 1.

When we used the repeated cross-validation procedure described above, the best model for predicting the full MPA AUC_{0-12h} was 3-time point model 10 (c_{0h}, $c_{0.5h}$, c_{2h};

Table 1. Multiple regression analysis to correlate abbreviated MPA AUC values with AUC values calculated using the full set of 12 timed MPA concentrations.

Model	Sampling times, h	Model equation	r^2
1	0	$8.32c_{0h} + 21.53$	0.434
2	0.5	$1.85c_{0.5h} + 23.36$	0.341
3	1	$2.77c_{1h} + 20.04$	0.341
4	2	$4.83c_{2h} + 14.58$	0.370
5	0, 0.5	$14.95 + 8.29c_{0h} + 0.53c_{0.5h}$	0.635
6	0, 1	$15.41 + 7.06c_{0h} + 0.83c_{1h}$	0.626
7	0, 2	$20.61 - 0.83c_{0h} + 3.28c_{2h}$	0.793
8	0, 0.5, 1	$10.88 + 8.18c_{0h} + 0.66c_{0.5h} + 0.76c_{1h}$	0.810
9	0, 1, 2	$10.32 + 6.72c_{0h} + 0.81c_{1h} + 1.87c_{2h}$	0.772
10	0, 0.5, 2	$7.75 + 6.49c_{0h} + 0.76c_{0.5h} + 2.43c_{2h}$	0.862
11	3	$21.10 + 4.68c_{3h}$	0.248
12	4	$21.08 + 4.82c_{4h}$	0.293
13	6	$14.10 + 9.9c_{6h}$	0.515
14	8	$15.79 + 8.28c_{8h}$	0.686
15	9	$23.69 + 5.57c_{9h}$	0.510
16	10	$17.87 + 7.79c_{10h}$	0.620
17	11	$12.39 + 10.24c_{11h}$	0.673
18	12	$19.41 + 4.25c_{12h}$	0.527
19	0, 3	$10.94 + 4.42c_{0h} + 3.90c_{3h}$	0.587
20	0, 4	$8.55 + 5.68c_{0h} + 4.81c_{4h}$	0.720
21	0, 6	$10.18 + 3.64c_{0h} + 6.81c_{6h}$	0.792
22	0, 8	$14.52 + 4.87c_{0h} + 4.59c_{8h}$	0.705
23	0, 9	$17.68 + 6.42c_{0h} + 2.24c_{9h}$	0.592
24	0, 10	$15.12 + 7.66c_{0h} + 3.40c_{10h}$	0.710
25	0, 11	$10.99 + 7.43c_{0h} + 5.19c_{11h}$	0.782
26	0, 12	$15.12 + 7.51c_{0h} + 3.04c_{12h}$	0.642

Table 2. Distribution of intercepts, coefficients, and residuals and summary statistics for repeated cross-validation for MPA AUC estimation models 1, 7, and 10.

	Model				Model		
Quantile, %	1	7	10	Quantile, %	1	7	10
Intercept (I; n = 50)				Coefficient β_3 (n = 50)			
100.0	25.834	25.854	12.359	100.0			3.334
99.5	24.367	24.967	12.211	99.5			3.228
97.5	24.042	23.078	11.432	97.5			3.097
90.0	23.547	22.256	10.512	90.0			2.773
75.0	22.338	21.234	8.393	75.0			2.521
50.0	21.534	20.612	7.745	50.0			2.433
25.0	20.322	19.032	7.072	25.0			2.106
10.0	18.354	18.367	5.143	10.0			1.858
2.5	17.432	17.101	3.842	2.5			1.312
0.5	16.571	16.783	3.251	0.5			1.234
0.0	15.392	16.502	3.086	0.0			1.156
Coefficient β_1 (n = 50)				Residual			
100.0	13.522	−1.234	8.498	100.0	0.89	0.75	0.76
99.5	12.876	−1.155	8.321	99.5	0.54	0.68	0.53
97.5	11.253	−1.087	7.862	97.5	0.53	0.66	0.47
90.0	10.034	−0.967	7.146	90.0	0.27	0.48	0.23
75.0	9.674	−0.912	6.733	75.0	0.13	0.29	0.08
50.0	8.321	−0.827	6.490	50.0	−0.08	−0.03	−0.03
25.0	7.483	−0.792	6.115	25.0	−0.38	−0.28	−0.22
10.0	7.102	−0.730	4.841	10.0	−0.63	−0.56	−0.35
2.5	6.875	−0.675	3.375	2.5	−0.79	−0.67	−0.57
0.5	5.212	−0.623	3.259	0.0	−1.06	−1.06	−0.65
0.0	4.763	−0.576	3.101	Summary statistics for residuals			
Coefficient β_2 (n = 50)							
100.0		4.085	1.310	Mean	−0.1406	−0.0239	−0.0519
99.5		3.903	1.260	SD	0.3928	0.4174	0.0391
97.5		3.823	1.152	Median	−0.08	−0.03	−0.03
90.0		3.765	0.981	Mode	0	−0.28	−0.07
75.0		3.346	0.879	Range	1.95	1.81	1.41
50.0		3.280	0.760	Minimum	−1.06	−1.06	−0.65
25.0		3.045	0.634	Maximum	0.89	0.75	0.76
10.0		2.856	0.476	n	1250	1250	1250
2.5		2.589	0.328				
0.5		2.254	0.278				
0.0		1.924	0.245				

r^2 = 0.862). Not only did this model have the highest r^2 value, but the SD of the prediction residuals (0.0391) was much better than that obtained for all of the other models tested (Tables 1 and 2; Fig. 2). The 2-sample model that had the best r^2 value (0.793) was model 7 (c_{0h}, c_{2h}). The SD of the prediction residuals (0.4174) for model 7 was more than 10-fold larger than that for model 10, and the mean prediction error of 11.9% ± 50.6% was almost double that for model 10 (6.1% ± 19%). There was poor correlation between the full MPA AUC_{0-12h} and each of the single MPA concentrations obtained at times up to the first 2 h (r^2 = 0.341–0.434; Table 1).

The correlation between single MPA concentration values at time points later than 2 h and full MPA AUC_{0-12h} values are summarized in Table 1. The best value for r^2 (0.686) for a model containing only a single concentration was obtained for MPA concentrations at 8 h (Table 1). Equations for estimation of MPA AUC values and details of the limited sampling strategies evaluated in this study are summarized in Table 1. Linear regression analysis plots of the estimated AUC vs the corresponding measured full MPA AUC_{0-12h} values for models 1, 7, and 10 are displayed in Fig. 2. The bias of LSS-derived estimates was analyzed by calculating the mean prediction error for the estimates i.e., the mean for the residuals [difference between ln(estimated AUC) and ln(measured AUC)]. The distribution for coefficients and a statistical summary for the distribution of the residuals for models 1, 7, and 10 are summarized in Table 2. Prediction errors for the abbreviated AUC profiles are summarized in Table 3. The median and mean ± SD for the prediction error for model 10 were 3.0% and 6.1% ± 19%, respectively. For

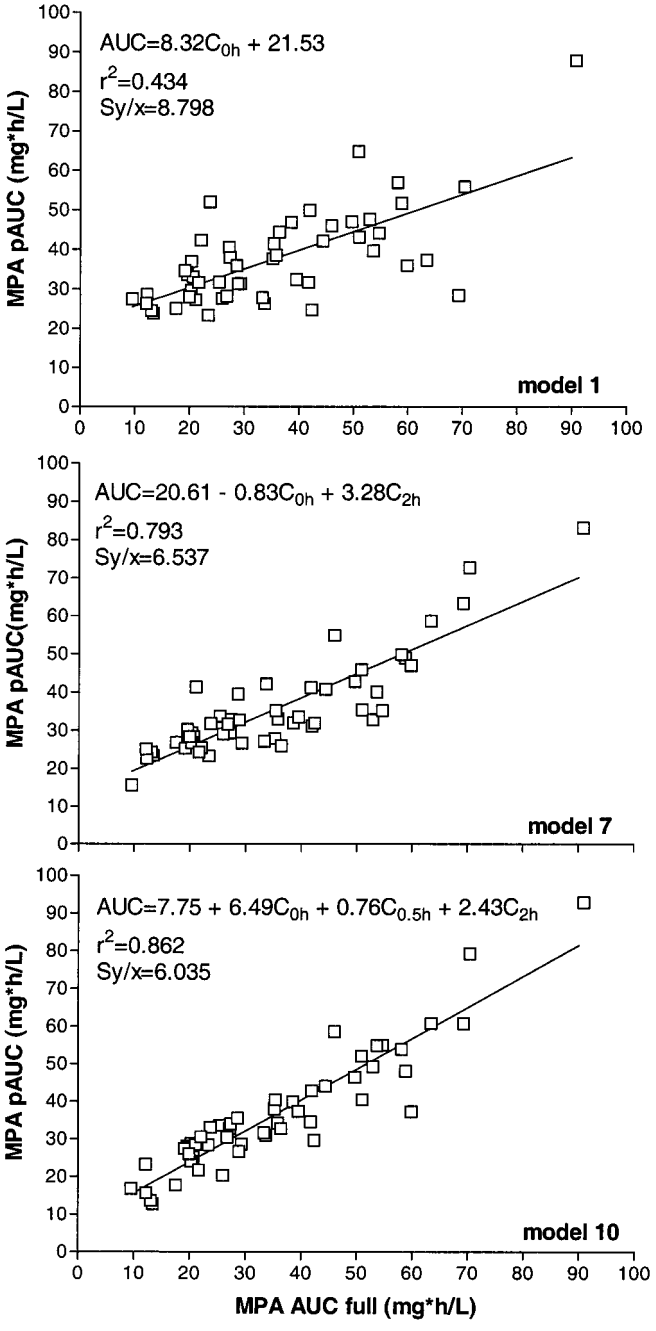

Fig. 2. Linear regression plots of MPA AUC values predicted using regression models 1 (*top*; single sample is predose MPA concentration), 7 (*middle*; two samples: predose MPA concentration and 2-h MPA concentration), and 10 (*bottom*; three samples: predose MPA concentration and 0.5- and 2-h MPA concentrations) vs the corresponding 50 MPA AUC values calculated from the full sets of 12 timed samples by the linear trapezoidal rule.

this model, in 41 of 50 (82%) of the profiles, the estimation of the values was within ± 15% of the value using all 12 samples over 12 h. For the other models, the estimate was within ± 15% of the actual value in only ≤62% of the 50 profiles.

Discussion

There is a significant relationship between the dose-interval MPA AUC and risk for acute rejection based on retrospective investigations of MPA concentration vs biopsy-confirmed rejection rates in renal and heart transplant patients and on prospective investigations of MPA concentrations vs biopsy-confirmed rejection rates in renal transplant patients *(8–12)*. Subsequent studies have confirmed the increased risk for acute rejection associated with decreased values for MPA AUC and, in addition, have reported an increased risk for hematologic side effects associated with increasing MPA AUC values *(13–16)*. There is an emerging consensus that individualizing MMF dosage to achieve a target MPA AUC within the range 30–60 mg · h/L will provide a lower risk for acute rejection and hematologic side effects *(13–16, 25)*. Because there is a >10-fold range in the MPA AUC values achieved using fixed daily doses of MMF *(12–14, 17)*, a therapeutic drug monitoring approach would be needed to keep all patients in the 30–60 mg · h/L range, supporting the importance of MPA therapeutic drug monitoring as a standard of practice.

Measurement of MPA AUC_{0-12h} using a full set of samples (e.g., 8–14 timed samples) is very demanding of skilled personnel time and laboratory resources and requires considerable quantities of the patient's blood and at least 12 h of time in a medical center. In our experience, the three samples in the 2-h postdose time period defined by this new sampling scheme provide a testing strategy that our clinical colleagues find is a practical approach, whereas sampling schemes that include a greater number of samples or a larger time interval are unacceptable (T. Pawinski, unpublished observation).

A conclusion drawn by other investigators about abbreviated sampling schemes is that inclusion of a 6-h timed sample is critical to obtaining an abbreviated sampling model with the best predictive performance. For example, coefficients of determination (r^2) of 0.87, 0.74, and 0.76 were obtained for models with sampling times of 1, 2, and 6 h; 0, 0.5, and 2 h; and 0, 1.5, and 6 h, respectively, in an investigation involving 61 patients *(19)*. Bland–Altman analysis of these data showed that the mean error for the model with the best r^2 value was ± 9.5 mg · h/L *(19)*. It is unclear whether this conclusion would have been reached by use of a cross-validation approach, which is the recommended standard of practice for the evaluation of a CsA LSS *(26)*. In another investigation, the r^2 value was 0.84 for the best model tested, a 4-sample model with sample times of 0, 1, 3, and 6 h, but was only 0.63 for a model that used five samples obtained within 2 h of the MMF dose (0, 0.25, 0.75, 1.25, and 2 h) *(21)*. The predictive performance analyses reported for these two models were 95% CIs of −26.3% to 32.5% and −45.3% to 52.7%, respectively *(21)*. The improved performance achieved by the addition of the 6-h timed sample in these two studies was attributed to the fact that in both studies the patients did not fast overnight *(27)*. Meal consump-

Table 3. Prediction errors for the abbreviated MPA AUC profiles.

Model	Sampling times, h	n	Prediction error, %			Compared with full AUC values,[a] n		
			Mean ± SD	Median	95% CI	>15%	±15%	<−15%
1	0	50	24.1 ± 50.1	8.7	−58.9 to 189.2	18	21	11
2	0.5	50	20.2 ± 56.4	5.8	−67.4 to 132.6	20	18	12
3	1	50	24.3 ± 55.1	6.7	−61.6 to 128.4	17	17	16
4	2	50	14.0 ± 47.2	5.1	−47.3 to 115.9	17	18	15
5	0, 0.5	50	10.0 ± 37.7	6.2	−58.2 to 123.7	12	24	14
6	0, 1	50	10.3 ± 39.2	0.6	−59.7 to 122.6	12	23	15
7	0, 2	50	11.9 ± 50.6	3.4	−52.8 to 190.0	10	28	12
8	0, 0.5, 1	50	9.8 ± 30.7	6.8	−59.1 to 62.5	10	30	10
9	0, 1, 2	50	11.0 ± 29.8	11.2	−48.4 to 67.8	16	26	8
10	0, 0.5, 2	50	6.1 ± 19.0	3.0	−33.1 to 32.0	5	41	4
11	3	50	26.8 ± 67.4	9.5	−80.4 to 173.7	23	16	11
12	4	50	25.1 ± 59.8	8.9	−75.4 to 145.6	10	18	22
13	6	50	13.3 ± 34.5	7.6	−62.3 to 134.1	15	25	10
14	8	50	11.5 ± 32.1	7.1	−50.4 to 109.2	11	26	13
15	9	50	14.6 ± 32.8	6.9	−72.1 to 135.3	14	24	12
16	10	50	10.8 ± 30.4	5.4	−55.2 to 121.4	14	23	13
17	11	50	12.2 ± 28.8	7.4	−60.4 to 118.6	11	27	12
18	12	50	14.2 ± 35.4	7.2	−80.1 to 159.4	15	23	12
19	0, 3	50	10.3 ± 31.7	4.8	−48.9 to 103.4	13	26	11
20	0, 4	50	10.8 ± 28.1	4.7	−41.5 to 76.9	12	29	9
21	0, 6	50	11.4 ± 29.8	5.3	−38.6 to 54.2	11	31	8
22	0, 8	50	11.9 ± 30.6	6.6	−53.1 to 90.4	13	27	10
23	0, 9	50	10.4 ± 30.8	5.2	−60.4 to 123.7	11	25	14
24	0, 10	50	12.1 ± 31.7	6.3	−60.3 to 101.3	11	27	12
25	0, 11	50	10.7 ± 30.6	5.4	−42.2 to 73.1	10	30	10
26	0, 12	50	10.1 ± 39.4	7.1	−68.7 to 111.8	10	26	14

[a] Number of LSS-estimated MPA AUC values that were within 15% (±15%), more than 15% higher (>15%), or more than 15% lower (<−15%) than the values obtained for the 50 full 12-point MPA AUC_{0-12h} values.

tion causes an increase in t_{max} and a decrease in c_{max}, but no significant change in value for the 12-h MPA AUC *(28)*. According to the authors' suggestion, the predictive performance of 2-h limited sampling schemes is diminished by not including maximum concentrations for at least some of the profiles *(27)*. Inclusion of the 6-h sample would eliminate most, if not all such cases and thereby improve the accuracy of the prediction model. In practice, we favor adopting a rule of not using the abbreviated profile to estimate MPA AUC if the predose concentration is unusually high, indicating noncompliance with the procedure (dosing inadvertently started before obtaining the predose sample or lack of overnight fasting). In our investigation, food intake of each study participant's choosing was permitted 1 h after the oral MMF dose, following an overnight fast. This produced an average t_{max} of 2.1 ± 2.7 h (median, 1 h; range, 0.5–11 h) that is greater than that cited by Willis et al. (1.71 ± 1.22 h) *(21)*, making a delay in t_{max} less likely to be the overriding factor for establishing an accurate abbreviated sampling model.

We believe that the statistical method used to establish the model deserves serious consideration for its importance in deriving a robust limited sampling estimation model. A commonly used approach for establishing estimation models is to perform a multiple stepwise linear regression on the total set of full AUCs *(19)*. When we used that approach, we obtained a r^2 value of 0.74 and a prediction error of 7.6% ± 26.7%, (median, 6.5%; 95% CI, −51.9% to 67.5%), and the model estimated MPA AUC to within 15% of the full value in 56% of the profiles. Our estimation model using the repeated cross-validation approach was significantly better, with a r^2 value of 0.862, prediction error of 6.1% ± 19%, (median, 3.0%; 95% CI, −33.1% to 32%), and estimation of MPA AUC to within 15% of the value (when all 12 samples are used to calculate MPA AUC) in 82% of the profiles. To test for the effect of adding a 6-h sample to our 3-sample model, we used the repeated cross-validation approach to derive the model for this case. Indeed, some improvement was achieved by adding the 6-h sample: the r^2 was 0.891, the prediction error was 3.5% ± 19.2% (median, 2.9%; 95% CI, −42.6% to 59.2%), and the estimated MPA AUC values were within 15% of the full MPA AUC result in 86% of the profiles. Thus a small improvement in the predictive performance was achieved, although the degree of improvement over the three samples in a 2-h model is small and would not justify adding a fourth sample and a total

time of 6 h to the procedure. In addition, the exercise presented here applied a much more stringent challenge in applying regression results to data points not included in the regression, repeated 50 times using random division of the data sets to reduce the impact of sampling variation on the assessment. Fitting regressions to the entire data set causes issues involving model selection and bias *(26)*.

Another recommended abbreviated sampling strategy includes samples collected at 0 and 75 min and 4 h *(13)*. To test for the possibility that a 3-sample model based on these time points would provide an even better estimation of the MPA AUC based on all 12 timed samples, we evaluated an additional set of 3 timed MPA concentrations: 0, 1, and 4 h. The 1-h sample was chosen because the timed samples in our investigation did not include 75 min and the former was the closest in time to the latter. The 3-time point model produced by the repeated cross-validation approach is: MPA AUC=5.03 + $3.36c_{0h}$ + $1.61c_{1h}$ + $5.44c_{4h}$. The r^2 value for the regression analysis of MPA AUC estimated by this 3-sample model vs the 50 full MPA AUCs is 0.748 and the prediction error is as follows: mean ± SE, 7.3% ± 28.7%; median, 3.5%; 95% CI, −35.1% to 76.9%. In this case, 50% of the estimated MPA AUC values were within 15% of the full MPA AUC values. Thus the use of this abbreviated sample model did not improve on the predictive performance of the 0, 30 min, and 2 h model. Other investigators *(13)* have reported that an abbreviated sampling model that includes 4 h, such as 0, 75 min, and 4 h, provides reliable prediction of the dose interval MPA AUC (r^2 = 0.76 for first month posttransplantation; r^2 = 0.83 thereafter) for renal transplant patients whose concomitant immunosuppression was afforded by CsA. We do not know the reason for the differences in the results of the two studies. Among the possibilities are the fact that a different concomitant immunosuppressant was used in these two studies (CsA vs tacrolimus), different techniques were used for developing the estimation model, and differences in the timing of the middle sample used for the 4-h model (75 min vs 60 min). Further studies will be required to establish which one or more of these variables contribute(s) to the observed differences and whether the nature of concomitant immunosuppression affects the values of the model equation coefficients. In addition, when contemplating what estimation model to use for patients who are recipients of an organ transplant other than a kidney, further testing and validation are recommended before using the 0, 30 min, 2 h model developed here, or any other algorithm for estimation of the MPA AUC.

David and Johnston *(26)*, in a critical discussion of LSS for estimation of the dose-interval AUC for CsA, emphasized the importance of cross-validation in the evaluation of a LSS. Validation requires dividing a data set into a training set, used to derive model parameter estimates, and a testing set, used to evaluate the predictive abilities of the model arising from the associated training set. In the present study, we used repeated cross-validation, similar to the bootstrap procedure, by randomly assigning data sets to either the training set or the evaluation set, as if a group of independent investigators had each randomly chosen their own training and testing sets and then pooled their results. This produced a distribution of prediction residuals that will be less sensitive to the choice of observations allocated to the training and evaluation sets because each observation will be in either set many times. This procedure enables a more meaningful comparison of the different potential models (i.e., the differing number of sample time points and different time points), based on a criterion of shortest range or tightest clustering (smallest SD) of the prediction residuals. The conclusions regarding the use of three sampling times within the first 2 h after a dose of MMF described here are similar to those found from a set of MPA AUC data for a cohort of renal transplant patients who were receiving MMF and concomitant CsA immunosuppression (M. Hale, unpublished observation). In the latter case, although the equation coefficients derived were different, the development of a reliable model based on three samples obtained within the first 2 h after a dose of MMF was accomplished.

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