

# Apparent Clearance of Sirolimus in Heart Transplant Recipients: Impact of Primary Diagnosis and Serum Lipids

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**Abstract:** The study was aimed to identify factors affecting sirolimus apparent clearance (CL/F) in *de novo* heart transplant recipients using a population pharmacokinetic approach. A total of 31 patients (7 female and 24 male) originally included in a formal clinical trial, contributed 524 sirolimus blood concentrations with the time after dose ranging from 11.08 to 31.83 hours. Sirolimus concentrations were measured using liquid chromatography-tandem mass spectrometry and data analysis was carried out using NONMEM (Globomax LLC, Hanover, MD) software. Factors screened included age, weight, gender, primary diagnosis, biochemical and hematological indices, cyclosporine dose, days post-transplant and potential interacting medications. The predictive performance of the final model was evaluated using a data-splitting method. Sirolimus apparent clearance (CL/F) was decreased by 20.8% for every 100 mg increase in cyclosporine daily dose and was 62.1% lower in patients with primary diagnosis of ischemic heart disease (IHD). Sirolimus apparent clearance was 37.8% lower when triglyceride was greater than 2 mmol/L. Based on the final model, the average values for sirolimus CL/F and apparent volume of distribution were 7.09 and 1350 L/h, respectively. Inter-subject variability in CL/F was 27.5% and residual random error was 24.1%. This study identified cyclosporine dose, hypertriglyceridemia and primary diagnosis of IHD as the most important factors affecting sirolimus CL/F. This information may assist in dose individualization of sirolimus in heart transplant recipients.

**Key Words:** apparent clearance, heart transplant, ischemic heart disease, NONMEM, sirolimus, triglyceride

(*Ther Drug Monit* 2006;28:818–826)

Sirolimus, is a mammalian Target-of-Rapamycin (mTOR) inhibitor that was initially obtained from the actinomycete strain *Streptomyces hygroscopicus*.<sup>1</sup> In September, 1999, sirolimus (Rapamune®; Wyeth-Ayerst Laboratories) was approved by the United States Food and Drug Administration (FDA) for the prophylaxis of organ rejection in kidney transplant recipients in combination with cyclosporine and

corticosteroids<sup>2</sup> and in 2003 for elimination of cyclosporine from a regimen of sirolimus, cyclosporine, and corticosteroids.<sup>3</sup> Sirolimus has a narrow therapeutic index<sup>2</sup> with side effects including dyslipidemia, leucopenia, thrombocytopenia, anemia and delayed wound healing.

Sirolimus is available in two oral forms: A solution and a solid tablet.<sup>3</sup> In healthy volunteers, the maximum blood concentration ( $C_{max}$ ) of sirolimus was attained within 1 h<sup>4</sup> with an oral bioavailability of approximately 15%.<sup>5</sup> In blood, sirolimus preferentially associates with erythrocytes and its blood-to-plasma ratio (B/P) is highly variable but dependent on drug concentration (mean  $\pm$  SD;  $36.5 \pm 17.9$  in stable kidney transplant recipients and  $79.9 \pm 45.8$  in healthy volunteers).<sup>6</sup> Sirolimus is mainly metabolized by cytochrome P450 3A4 enzymes located at the gut mucosa and liver<sup>7,8</sup> and biliary/fecal elimination is the major excretion pathway for this molecule.<sup>1</sup>

Pharmacokinetic properties of sirolimus including its clearance and elimination half-life, exhibit wide intra- and inter-subject variability. In 16 kidney transplant recipients under concomitant therapy with cyclosporine, sirolimus apparent clearance (CL/F) ranged from 42 to 339 mL/h/kg and elimination half-life from 39.3 to 86.5 h.<sup>9</sup> A long elimination half-life of approximately 62 h has also been reported in a group of 40 kidney transplant recipients who received sirolimus in addition to prednisone and cyclosporine for 14 days.<sup>10</sup> In the same patients, the average CL/F was  $208 \pm 95$  mL/h/kg indicating a 4.5-fold variability. Because of long elimination half-life, a relatively long time is required to achieve steady state, therefore it is recommended to use a loading dose of sirolimus to attain the steady state more rapidly.

Conventional pharmacokinetic studies have assessed the clearance of sirolimus in healthy volunteers or kidney transplant recipients, however factors affecting sirolimus clearance in heart transplant recipients has not been identified yet.<sup>1,9,10</sup> In addition, the single population pharmacokinetic study of sirolimus published to date, only includes conventional pharmacokinetic profiles obtained from 36 kidney transplant recipients but does not examine the influence of hematological or biochemical indices as covariates for sirolimus pharmacokinetic parameters.<sup>6</sup> The primary objective of this study was to develop a population pharmacokinetic model to estimate sirolimus CL/F and its intra- and inter-patient variability in heart transplant recipients. In addition, we have examined the influence of potential interacting medications and various demographic, hematologic and biochemic parameters on the sirolimus apparent clearance.

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## METHODS

### Patients and Data Collection

Data was collected for 31 *de novo* adult heart transplant recipients who were participating in a sirolimus heart transplant trial (0468-216-AU) at St. Vincent's Hospital, Sydney, Australia, one of the 5 centers involved in a multi-center phase III trial. The aim of the original clinical trial was to compare the efficacy of sirolimus in comparison with azathioprine, when co-administered with corticosteroids and cyclosporine, on acute rejection and transplant vasculopathy.<sup>11</sup> The duration of this trial was 24 months but beyond the 2-year endpoint, patients continued long-term treatment with sirolimus. Changes in immunosuppression (addition of mycophenolate mofetil or alterations in steroid dosage) were allowed beyond the end of trial although in general patients remained on the same treatment regimen. Permission for data transfer for the subgroup reported here was obtained from St. Vincent's Hospital Research-Ethics Committee and the Institutional Review Board at the University of Rhode Island, has approved the data analysis.

### Drug Administration and Therapeutic Drug Monitoring

In 21 patients, oral sirolimus therapy was initiated with a loading dose of 15 mg, usually within 24 hours of transplant surgery, followed by a maintenance dose of 5 mg/day initially, with dose adjustment to maintain blood trough concentrations of 1 to 30 ng/mL. Halfway through the enrollment, because of concerns about the toxicity, the protocol was amended, for the last 10 patients, to a loading dose of 10 mg and maintenance dose of 3 mg/day to maintain sirolimus blood trough concentrations within a target range of 8 to 18 ng/mL.<sup>11</sup> Built into the study protocol, were guidelines for dose adjustment in the event of toxicities such as perturbation of hematological or lipoprotein parameters.<sup>11</sup> Sirolimus was administered 4 h after the morning dose of cyclosporine.

Sirolimus concentration, biochemical and hematological indices were monitored on every hospital visit. More than 6,000 dose and 614 sirolimus concentrations were extracted from the database. Concentration data were included in the population analysis only if corresponding dose and covariate data were available leaving 524 concentration points in total.

### Sirolimus Assay

As part of routine therapeutic monitoring, EDTA blood samples were collected and stored at  $-70^{\circ}$  to  $-80^{\circ}\text{C}$  in polypropylene tubes and was protected from light. The samples were then shipped to an external laboratory in Australia and analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) technique.<sup>12</sup> Accuracy and inter-day imprecision of the assay, for quality control samples, were 96.5 to 96.8% and 5.3 to 8.6%, respectively, over a working concentration range of 0.2 to 100 ng/mL.

### Immunosuppressants and Concomitant Maintenance Therapy

All patients received sirolimus in addition to cyclosporine (Neoral®) (Dose 50 to 725 mg/day) and oral prednisone (1 mg/kg/day, reducing to 0.1 mg/kg/day by month 6). By

protocol all patients received HMG-CoA reductase inhibitors (pravastatin 19, simvastatin 2, atorvastatin 10) sometimes during the study however 223 sirolimus dose and concentration data were obtained during the times that the patient was not on any statin therapy. Approximately 45% of the patients received diltiazem and 10% received azole antifungal agents for a short period of time during the study. Patients also took a wide range of other medications including anti-hypertensive (diltiazem, nifedipine, amlodipine, amiodarone), antibacterial (roxithromycin, erythromycin, clarithromycin, rifampin) and anti-fungal agents (fluconazole, itraconazole) according to the clinical need.

### Population Pharmacokinetic Modeling

Modeling was performed using NONMEM (version 5, level 1.1, GloboMax LLC, Hanover, MD) with double precision and first-order conditional estimation (FOCE) technique. PDx-POP (version 1.1, GloboMax) was coupled with NONMEM as an interface. A one-compartment pharmacokinetic model (NONMEM subroutine ADVAN2 and TRANS2) with first-order absorption and elimination was used to describe the concentration-time data. Because no data from the absorption phase was available, absorption rate constant ( $k_a$ ) was fixed and the effect of fixing this factor was evaluated using a stepwise increase in  $k_a$  value from 0.1 to 8.0 1/h. Preliminary analyses indicated that  $k_a$  fixed at 0.752 1/h yielded the minimum value of the NONMEM derived objective function and the smallest coefficient of variance for the estimates of apparent clearance (CL/F) and apparent volume distribution (V/F).

Estimation of CL/F of sirolimus using the population pharmacokinetic approach was conducted in three steps: 1) Base model development and exploratory data analysis; 2) covariate model development; followed by 3) selection and evaluation of the final full-population pharmacokinetic model. The study dataset was randomly split (in PDx-POP) using a 65:35 ratio into index (20 patients, 320 observations) and validation (11 patients, 204 observations) datasets used for model development and evaluation, respectively.

### Base Model Development and Exploratory Data Analysis

In the first step, the base model was developed without including patient covariates using the index dataset. The minimum objective function value (OFV) obtained in this step was used as a standard for assessing the impact of the inclusion and exclusion of different covariates in the subsequent models. Individual Bayesian estimates of apparent clearance ( $CL_j$ ) obtained using this model were further evaluated using graphical and statistical analyses to explore the correlation between sirolimus apparent clearance and various demographic parameters (patient age, weight, gender and ethnicity), clinical factors (concurrent medications and primary diagnosis leading to heart transplant) and biochemical (albumin, HDL-, LDL- and total cholesterol, triglycerides and total protein) and hematological (hematocrit and RBC) indices to identify possible covariates for subsequent analyses. Since most concentrations were obtained in the later phase of the dosing interval, the effect of covariates on V/F was not modeled.

Different models were evaluated to describe inter-patient variability in apparent clearance and residual error (proportional and additive error models). Based on the minimum objective function value and the distribution of residuals in the diagnostic plots of the base model, a proportional model for inter-patient variability (Equation 1) and a constant CV model for error distribution (Equation 2) were selected for these analyses. Such that;

$$CL_j = TVCL * \exp(\eta_{CL}) \quad (1)$$

$$C_{ij} = C_{ijPRED} * (1 + \varepsilon_{ij}) \quad (2)$$

where,  $CL_j$ , the estimate of apparent clearance of sirolimus in  $j$ th individual, TVCL, typical value of CL/F of sirolimus (also known as the population estimate),  $\eta_{CL}$ , difference between the true  $CL_j$  for the  $j$ th patient and the typical value for the population (TVCL) (also termed as the inter-subject variability).  $C_{ij}$ , the observed concentration,  $C_{ijPRED}$ , predicted concentration and  $\varepsilon_{ij}$ , the difference between  $C_{ij}$  and  $C_{ijPRED}$ . Both  $\eta_{CL}$  and  $\varepsilon_{ij}$  are randomly distributed with a mean zero and variance of  $\omega^2$  and  $\sigma^2$ , respectively.

### Development of a Covariate Model

In the second step, each of the covariates of interest was introduced individually into the basic model to assess the relative impact on estimates of apparent clearance. A decrease of OFV (from base model to covariate model) by 6.6 was considered to be statistically significant (at  $P < 0.01$  level) and this difference in OFV (between two models) was assumed to be asymptotically  $\chi^2$  distributed. In the next step statistically significant covariates were included into the base model in a forward selection manner to develop intermediate and full models. A total of 30 covariates were tested in the present study. The continuous covariates evaluated in this study were age, weight, post-transplant days, hematocrit, RBC, albumin, total protein, HDL-, LDL- and total cholesterol, triglyceride and cyclosporine dose. These were evaluated using four different approaches including:

$$\begin{aligned} TVCL &= \theta_{CL} + \theta_{cov} * \text{Covariate} \\ TVCL &= \theta_{CL} + \theta_{cov} * (\text{Covariate} - \text{Median}) \\ TVCL &= \theta_{CL} * (\text{Covariate})^{\theta_{cov}} \\ TVCL &= \theta_{CL} * \text{Covariate} \end{aligned}$$

where  $\theta_{CL}$ , the individual estimate of sirolimus CL/F,  $\theta_{cov}$ , the factor contributed by the covariate, TVCL, typical value of apparent clearance of sirolimus (also known as the population estimate). Categorical/discrete covariates examined included gender, ethnicity, concurrent medications (diltiazem, fluconazole, itraconazole, nifedipine, amlodipine, rifampin, roxithromycin, erythromycin, clarithromycin, amiodarone, prednisone, pravastatin, simvastatin and atorvastatin) and primary diagnosis leading to heart transplant (ischemic versus nonischemic heart disease). A number of continuous covariates including hematocrit, albumin, total protein, triglyceride, HDL-, LDL- and total cholesterol as well as post-transplant days were also analyzed after transformation into categorical variables. The cut-off range for data transformation was selected based on the usual clinical criteria defining normal versus abnormal levels. For example hypertriglyceridemia was defined as triglyceride level of  $\geq 2$  mmol/L.

### Selection of Final Full Model

In the final step covariates were eliminated from the full model developed in the previous step following the backward elimination technique and assessment of the significance ( $P < 0.01$ ) in the OFV. While selecting the final full model, the improvement in fit due to the addition of a parameter to the model was also assessed by the precision of the parameter estimate [standard error of the mean and 95% confidence interval (CI)] and by the reduction in inter-patient and residual variability. Scatter plots of weighted residual (WRES) versus sirolimus-predicted concentrations (PRED) were also drawn to check the goodness of fit in each model.

### Model Evaluation

The final population pharmacokinetic model which included influential covariates was evaluated using data from the validation dataset. Finally population and posterior Bayesian estimates of apparent clearance of sirolimus were estimated using the complete dataset (524 observations). Estimated population parameter values obtained using index, validation and whole dataset were compared. Predictive performance (based on bias and precision) of the final model using different datasets was also statistically compared to assess the robustness of the model.

### Statistical Analysis

All statistical tests were performed using SPSS® software version 13.0 (SPSS, Chicago, IL);  $P < 0.05$  considered significant. To establish normal distribution, Kolmogorov-Smirnov statistics were calculated for all parameters. Non-normally distributed data were reported as median and interquartile range. Independent samples  $t$ -test or Mann-Whitney U tests were used to compare the differences in parameters for normal and non-normally distributed data, respectively. The differences in proportions were calculated using Pearson  $\chi^2$  test.

## RESULTS

### Patient Demographic and Clinical Data

Demographic characteristic of the patient population is presented in Table 1. Seventeen patients (54%) required transplantation due to a primary diagnosis of ischemic heart disease (IHD). The demographic characteristics and biochemical indices of patients with a primary diagnosis of IHD have been compared to the other patients in Table 2. The IHD patients were somewhat older and had elevated levels of total and LDL-cholesterol and total triglyceride while the HDL-cholesterol concentration was significantly lower in this group (Table 2).

### Sirolimus Dose and Trough Blood Concentration

The majority of sirolimus concentrations were collected toward the end of the dosing interval with a median of 20 hours after dose administration and data were collected over an average of  $309 \pm 364$  post-transplant days (median: 169; range 4 to 1,845 days). Figure 1 depicts sirolimus concentrations and time after dose with the dotted lines representing

**TABLE 1.** Demographic and Biochemical Data of the Study Population (31 Heart Transplant Recipients)

Variables	Value	Range
Gender (male/female)	24/7	—
Age (yr)	49.0 ± 12.0	18.0–66.0
Weight (kg)	78.3 ± 12.7	51.0–102.5
Ethnicity (white/non-white)	29/2	—
Primary diagnosis (ischemic/non-ischemic heart disease)	17/14	—
Observations per patient	17.3 ± 8.9	2–30
Sirolimus dose (mg/day)	3.3 ± 1.9	0.5–5.0
Sirolimus dose weight adjusted (mg/kg/day)	0.49 ± 0.26	0.06–2.16
Sirolimus blood concentration (ng/mL)*	17.9 ± 9.8	0.2–74.4
Sampling time with respect to dosing time (hr)	20.1 ± 2.2	11.1–31.8
Cyclosporine dose (mg/day)	223.5 ± 127.5	50–725
Cyclosporine concentration† (ng/mL)	244 ± 106	39–500
Total protein (gm/L)	68.7 ± 7.0	43–86
Albumin (gm/L)	37.6 ± 4.7	19–48

Values are mean ± SD.

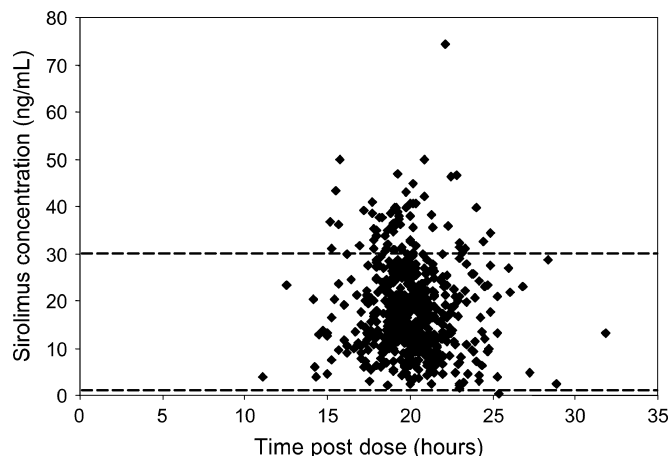
\*Sirolimus levels were measured using LC-MS/MS method.

†Clinically reported concentrations of cyclosporine measured using EMIT method.

target trough levels of 1 to 30 ng/mL. The average daily dose and blood concentration of sirolimus varied considerably over the post-transplant period with dose ranging from 0.5 to 5 mg/day and sirolimus concentration from 0.2 to 74.4 ng/mL (Table 1). A modest correlation was observed between

**TABLE 2.** Comparison of Demographic Characteristics, Biochemical Indices and Hematocrit Between Heart Transplant Recipients With Primary Diagnosis of Ischemic versus Non-ischemic Heart Disease

Variable	Primary Diagnosis		P Value
	Ischemic Heart Disease (n = 17)	Non-ischemic Heart Disease (n = 14)	
Gender (male/female)	14/3	10/4	0.38
Age (yr)	53.7 ± 6.6	40.9 ± 14.7	0.004
Weight (kg)	73 ± 11	72 ± 10	0.16
Sirolimus dose (mg/day)	3.16 ± 1.40	3.91 ± 2.02	<0.0001
Sirolimus dose weight adjusted (mg/kg/day)	0.44 ± 0.21	0.55 ± 0.29	<0.0001
Sirolimus concentration (ng/mL)	15.9 ± 8.3	20.3 ± 10.9	<0.0001
Cyclosporine dose (mg/day)	223 ± 129	229 ± 120	0.145
Cyclosporine concentration (ng/mL)	234 ± 112	255 ± 99	0.056
Hematocrit (%)	33 ± 4	33 ± 4	0.29
Total cholesterol concentration (mmol/L)	5.4 ± 1.4	4.9 ± 1.1	<0.001
LDL-cholesterol concentration (mmol/L)	2.9 ± 1.0	2.5 ± 1.0	<0.0001
HDL-cholesterol concentration (mmol/L)	1.3 ± 0.5	1.6 ± 0.5	<0.0001
Total triglyceride concentration (mmol/L)	2.6 ± 1.5	2.0 ± 0.9	<0.0001



**FIGURE 1.** Sirolimus blood concentration (524 observations) with respect to time after dose (dashed lines represent the target of 1 to 30 ng/mL).

sirolimus dose and trough blood concentration ( $r = 0.403$ ,  $P < 0.001$ ) indicating sirolimus dose can only predict 16% of the variability in sirolimus trough concentrations.

Interestingly sirolimus dose and blood concentration were also significantly lower in patients with IHD (Table 2). On closer analysis, it was found that serum triglyceride is negatively correlated with sirolimus dose ( $r = -0.126$ ,  $P < 0.001$ ) but not sirolimus concentrations ( $r = 0.025$ , NS) which may be a reflection of the clinical practice of lowering sirolimus dose when patients become dyslipidemic. Interestingly, neither cyclosporine dose nor concentrations were significantly different between the two groups (Table 2).

Furthermore the median (interquartile range) of sirolimus dose was 2.5 mg (2 to 5 mg) during diltiazem administration as compared with 4 mg (3 to 5 mg) in other periods ( $P < 0.00001$ ). Correspondingly, the concentration of sirolimus was 14.1 ng/mL (9.7 to 21.9 ng/mL) during diltiazem administration as compared to 18.9 ng/mL (12.6 to 26 ng/mL) at other times ( $P < 0.00001$ ).

### Base Pharmacokinetic Model and the Models Incorporating Patient Covariates

A one-compartment pharmacokinetic model with exponential distribution of inter-subject variability and additive residual error distribution was used to generate the predicted concentrations.

Table 3 represents the impact of addition of covariates to the base model. The addition of cyclosporine daily dose as a continuous covariate considerably reduced the OFV (Table 3). There was 20.8% decrease in sirolimus CL/F per 100 mg increase in cyclosporine daily dose. Among other potential covariates screened, only HDL-cholesterol and triglyceride were detected as significant covariates when included as discrete variables (Table 3). Patients with a high triglyceride level ( $\geq 2$  mmol/L) had a 37.8% lower apparent clearance when compared to patients with triglyceride level  $< 2$  mmol/L. In patients with higher HDL-cholesterol levels ( $\geq 2$  mmol/L), sirolimus CL/F was 36.4% lower. The inclusion of serum total

**TABLE 3.** Results of Univariate Analyses (n = 31)

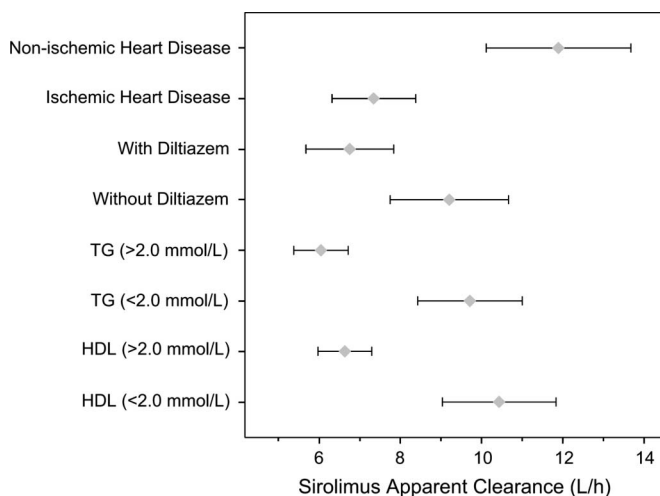
Covariate Category	Covariate in the Model	Covariate Scaling	$\Delta$ OFV	P Value
Demographic	Age	—	0	NS
	Weight	—	-1	NS
	Gender	—	2	NS
Hematological and biochemical	Hematocrit*†	Hematocrit <35% = 0; others = 1	-1	NS
	RBC count	—	0	NS
	Albumin*†	Albumin <35 g/L = 0; others = 1	0	NS
	Protein	—	0	NS
	Total cholesterol*†	Total cholesterol $\geq$ 5.5 mmol/L = 0; otherwise = 1	-6	NS
	HDL†	HDL $\geq$ 2.0 mmol/L = 0; otherwise = 1	-50	<0.01
	LDL†	LDL $\geq$ 4.7 mmol/L = 0; otherwise = 1	0	NS
Concurrent medication	Triglyceride (TG)†	TG $\geq$ 2 mmol/L = 0; otherwise = 1	-45	<0.01
	Cyclosporine dose	Dose in mg	-70	<0.01
	Diltiazem (n = 16)	Diltiazem co-administered = 0; others = 1	-23	<0.01
	Statins‡	Statin co-administered = 0; others = 1	0	NS
Others	Primary diagnosis	Ischemic heart disease = 1; non-ischemic heart disease = 0	-49	<0.01
	Days post-transplant	Days post-transplant >60 days = 0; otherwise = 1	0	NS

\*95% confidence interval includes null value, therefore covariate was excluded from further analysis.

†Covariate was also investigated as a continuous variable but did not change OFV significantly.

‡All patients (n = 31) received a statin (pravastatin, simvastatin or atorvastatin) sometimes during the study however 223 sirolimus dose and concentration data were obtained during the times that the patient was not on any statin therapy.

cholesterol concentration with a cut-off value of 5.5 mmol/L provided a marginally significant decrease in OFV (Table 3) but the 95% CI of the parameter estimate included the null value and hence was excluded from further analysis. Albumin, hematocrit and LDL-cholesterol levels were not detected as significant covariates when included either as continuous or discrete variables. Figure 2 shows the effect of HDL cholesterol, triglycerides, diltiazem and primary diagnosis of ischemic heart disease on apparent clearance of sirolimus using univariate models.



**FIGURE 2.** Effect of HDL cholesterol, triglycerides, concurrent medications (diltiazem) and primary diagnosis (ischemic heart disease versus non ischemic heart disease) on apparent clearance of sirolimus. Mean value and 95% confidence interval (error bars) are shown. Values of apparent clearance were obtained using univariate model.

The *CYP3A4* enzyme inhibitors, diltiazem, fluconazole and itraconazole were influential covariates in the single covariate model analyses. However, with fluconazole the 95% CI of the parameter estimates included the null value, hence it was excluded from the analysis. Other concurrent medications (mentioned in the Methods section) which were investigated for their possible influence on sirolimus CL/F were not detected as significant covariates because of an inadequate number of drug exposure. In the exploratory analyses the impact of post-transplant day as a significant covariate was evaluated as either continuous or discrete covariates (various scaling including  $\leq$ 15 days versus >15 days post-transplant,  $\leq$ 30 days versus >30 days post-transplant,  $\leq$ 60 days versus >60 days post-transplant were tested) but this variable was not detected as significant covariate for sirolimus CL/F (Table 3).

The primary diagnosis of IHD was detected as a significant covariate for sirolimus apparent clearance. Patients having transplant due to nonischemic heart disease had a 62.1% higher CL/F as compared to patients with IHD however other demographic parameters (including patient's age, weight and gender) were not detected as significant covariates.

Covariates included in the forward step of the model building included cyclosporine daily dose, co-administration of diltiazem or itraconazole, triglycerides level and primary diagnosis of IHD. Whilst HDL-cholesterol and co-administration of diltiazem or itraconazole were detected as significant covariates for sirolimus CL/F earlier, inclusion of these covariates into the combined model did not change OFV significantly.

In the final backward elimination step of the evaluation of covariates, significant increase in OFV was observed when cyclosporine daily dose, triglyceride concentration or primary diagnosis of IHD was eliminated from the model. Therefore, only three covariates were included in the final population pharmacokinetic model (Table 4).

**TABLE 4.** Population Parameter Estimates of the Final Pharmacokinetic Model (n = 31)

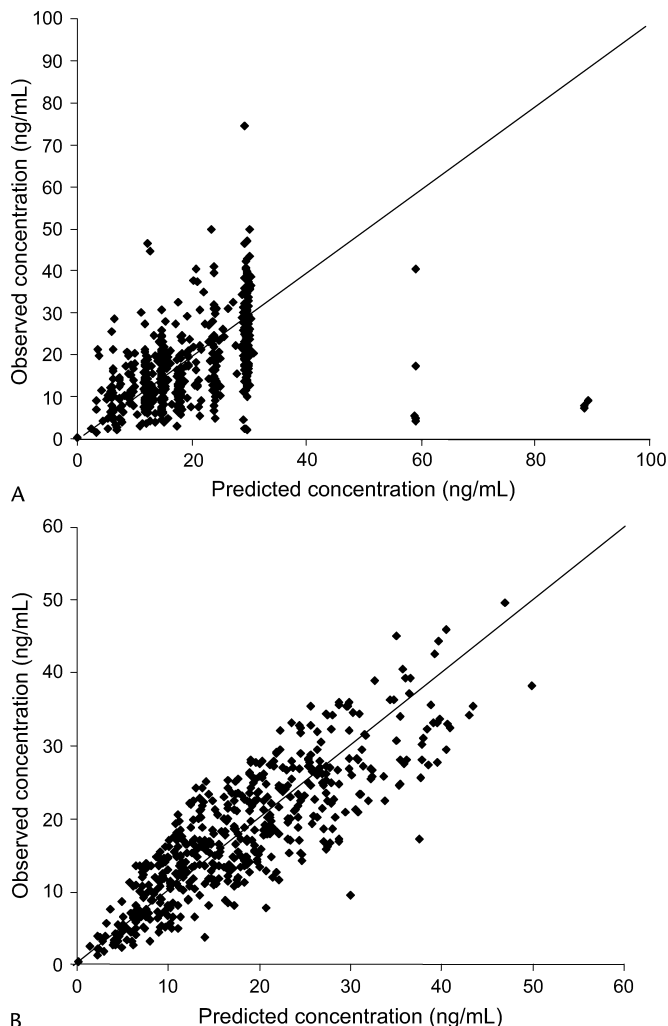
Parameters	Parameter Value	95% Confidence Interval	Precision of Estimate (% CV)
Apparent clearance, CL/F ( $\theta_{CL}$ ) (L/h)	7.09	6.75–7.41	13.0
Apparent volume of distribution, V/F ( $\theta_V$ ) (L)	1350.0	1180.8–1519.2	35.6
Absorption rate constant ( $\theta_{KA}$ ) (1/h)	0.752 (fixed)	—	—
Factor for cyclosporine dose ( $\theta_{CYSD}$ ) (1/mg)	0.0147	0.0138–0.0156	17.9
Factor for triglycerides ( $\theta_{TG}$ )	1.37	1.20–1.54	35.6
Factor for IDH ( $\theta_{IHD}$ )	2.20	1.87–2.52	42.3
Inter-subject variability in CL/F (%)		27.5	
Residual random error (%)		24.1	

Model parameters:  $TVCL = \theta_{CL} - \theta_{CYSD} * CYSD - \theta_{TG} * (1 - TG) + \theta_{IHD} * (1 - IHD)$ ;  $CL = TVCL * EXP(ETA(1))$ ;  $TVV = \theta_V$ ,  $V = TVV$ ,  $KA = \theta_{KA}$ ,  $S2 = V$ .  
 TVCL, typical (population) estimate of apparent clearance; TVV, typical (population) estimate of apparent volume of distribution; CYSD, cyclosporine dose (mg).  
 TG, 0, if triglyceride is  $\geq 2$  mmol/L, otherwise 1, IHD = 1, if primary diagnosis is ischemic heart disease, otherwise 0.

**Model Evaluation**

There were no statistically significant difference in demographic characteristics (age:  $51.0 \pm 10.4$  yr versus  $48.0 \pm 13.2$  yr, weight:  $77.0 \pm 13.4$  kg versus  $76.0 \pm 9.6$  kg, gender: 80.0% female versus 81.8% male) of the patients in the index and validation datasets. Quantitative evaluation (mean prediction error, ME; median prediction error, MdnE; root mean square error, RMSE; and mean absolute error, MAE) of the model performance using the validation dataset showed the model to be precise and free from bias (95% CI included zero). Estimates of ME and MdnE (measures of bias) using sirolimus trough blood concentration predictions from the final model and data from the validation dataset were small and the 95% CI included zero (ME:  $-0.39$  to  $0.50$  ng/mL; MdnE:  $-0.11$  to  $0.78$  ng/mL). The measures of precision (RMSE and MAE) associated with the validation group were small (RMSE: 5.2 and MAE: 4.1 ng/mL). The distribution of observed and model predicted sirolimus concentrations is depicted for base model in Figure 3A and for the final model in Figure 3B. Predicted trough blood concentrations of sirolimus based on the final model shows a uniform distribution of points along the line of identity.

Finally, using the developed model population estimates of the parameters, inter-patient and residual variability were also estimated for the complete dataset (Table 4). The estimated population parameters values calculated using the whole, index and validation datasets were comparable (Table 5). This shows that the data splitting did not affect the population parameter estimation in this analysis. Based on the residual variability data, the model will have a prediction error of 4.3 ng/mL and a sirolimus trough blood concentration of 17.9 ng/mL. Using the complete dataset, the mean posterior Bayesian estimates of sirolimus CL/F was 7.09 L/h (95% CI 6.75 to 7.41 L/h) and V/F was 1,350 L (95% CI, 1,180 to 1,592 L).



**FIGURE 3.** Model-predicted values calculated from (A) Base model and (B) Final model versus observed blood concentration of sirolimus (ng/mL) in 31 heart transplant recipients (solid line represents line of identity).

Considering that the maintenance dose of a drug can be estimated using the following Equation 3:

$$Dose = (C_{pss})(CL/F)(\tau) \tag{3}$$

where Dose is the dose of drug required to achieve a blood concentration ( $C_{pss}$ ), CL/F is the value of apparent clearance and  $\tau$  is the dosing interval; information on the extent of change in the sirolimus CL/F will reduce the guesswork in sirolimus dose adjustment to achieve a desired therapeutic level. The final model for CL/F is shown in Equation 4:

$$TVCL = 7.09 - 0.01472 * CYSD - 1.37 * (1 - TG) + 2.20 * (1 - IHD) \tag{4}$$

where TVCL, typical (population) estimate of apparent clearance, CYSD = cyclosporine dose (mg/day), TG = 0, if triglyceride is  $\geq 2$  mmol/L, otherwise 1, IHD = 1, if the

**TABLE 5.** Population Parameter Estimates of the Pharmacokinetic Model Using Index, Validation and Whole Datasets (Data in Parenthesis are 95% Confidence Interval of the Mean)

Parameters	Unit	Index Dataset (n = 20)	Validation Dataset (n = 11)	Whole Dataset (n = 31)
No of observations	—	320	204	524
Apparent clearance, CL/F ( $\theta_{CL}$ )	L/h	6.81 (4.89–8.73)	7.49 (6.82–8.16)	7.09 (6.75–7.41)
Apparent volume of distribution, V/F ( $\theta_V$ )	L	1580.0 (916.0–2240.0)	1430.0 (991.0–1870.0)	1350.0 (1180.8–1519.2)
Absorption rate constant ( $\theta_{KA}$ )	1/h	0.752 (fixed)	0.752 (fixed)	0.752 (fixed)
Factor for cyclosporine dose ( $\theta_{CYSD}$ )	1/mg	0.0156 (0.0145–0.0168)	0.0117 (0.0103–0.0131)	0.0147 (0.0138–0.0156)
Factor for triglycerides ( $\theta_{TG}$ )	—	2.2 (2.3–3.2)	3.8 (2.7–4.9)	1.4 (1.2–1.5)
Factor for primary diagnosis ( $\theta_{IHD}$ )	—	2.65 (0.93–4.37)	2.39 (1.43–3.35)	2.20 (1.87–2.52)
Inter-subject variability in CL/F	%	25.6	31.3	27.5
Residual random error	%	21.3	25.3	24.1

Model parameters:  $TVCL = \theta_{CL} - \theta_{CYSD} * CYSD - \theta_{TG} * (1 - TG) + \theta_{IHD} * (1 - IHD)$ ;  $CL = TVCL * EXP(ETA(1))$ ;  $TVV = \theta_V$ ,  $V = TVV$ ,  $KA = \theta_{KA}$ ,  $S2 = V$ .

TVCL, typical (population) estimate of apparent clearance; TVV, typical (population) estimate of apparent volume of distribution; CYSD, cyclosporine dose (mg); TG = 0, if triglyceride is  $\geq 2$  mmol/L, otherwise 1, IHD = 1, if primary diagnosis is ischemic heart disease, otherwise 0.

primary diagnosis leading to heart transplant is ischemic heart disease (IHD), otherwise 0.

For example, using Equation 4, the apparent clearance of sirolimus can be estimated at approximately 2.78 L/h for a hypothetical patients on 200 mg/day cyclosporine dose, primary diagnosis of IHD and hypertriglyceridemia, whereas, 4.15 L/h for the same patient with normal triglyceride value. Sirolimus CL/F can be estimated to be 6.35 L/h for the similar patient but without IHD as primary diagnosis of heart failure. These values can be then used in Equation 3 to estimate the sirolimus dose required to achieve a desired concentration of sirolimus.

## DISCUSSION

We report the results of the first population pharmacokinetic analysis of sirolimus that included serum biochemistry and primary diagnosis as covariates for sirolimus clearance in any type of organ transplantation. Indeed, to date, no other publications, using either conventional or population pharmacokinetic methods, had described sirolimus pharmacokinetics in heart transplant recipients. We have found that daily dose of cyclosporine, concentration of serum triglyceride and ischemic heart disease as primary diagnosis of heart failure leading to transplantation were the major factors influencing sirolimus dose-concentration relationship.

This analysis found a reasonable estimate for the sirolimus CL/F and V/F values. The average CL/F was calculated to be 7.1 L/h which is close to those estimated by others.<sup>6,9,10</sup> In a conventional pharmacokinetic study of 16 kidney transplant recipients, the average oral clearance of sirolimus was estimated at 106.9 mL/h/kg which translate to an apparent clearance of 7.5 L/h in a 70 kg individual.<sup>9</sup> Moreover, in a population pharmacokinetic analysis in 36 kidney transplant recipients, sirolimus apparent clearance was calculated to be 8.9 L/h.<sup>6</sup> Furthermore, despite the fact that this study was neither aimed nor powered to calculate the V/F for sirolimus, the value of 1,350 L calculated is comparable to a volume of distribution value of  $12 \pm 5$  L/kg ( $840 \pm 350$  L for a 70 kg individual) estimated by Zimmerman et al.<sup>10</sup> In addition only a modest correlation was

observed between sirolimus dose and trough blood concentration which is comparable to a correlation coefficient of 0.59 observed by Kahan et al.<sup>13</sup>

The patients included in this study were participating in a randomized multi-center clinical trial. The trial was aimed to assess the effects of sirolimus, in comparison with azathioprine, on the occurrence of acute allograft rejection and coronary artery diseases.<sup>11</sup> This trial showed the incidence of biopsy-proven acute rejection was significantly lower in sirolimus-treated patients. In addition the results of intracoronary ultrasound revealed that sirolimus patients exhibited less extensive transplant coronary disease with significant preservation of coronary artery lumen.<sup>11</sup> The positive point about this analysis is that we have made use of some sirolimus dose and trough concentration data collected meticulously in the context of a formal clinical trial and have attempted to extract clinically relevant information from it. This study was not designed as a population pharmacokinetic investigation, nevertheless has yielded reasonable estimates for CL/F and V/F values. In future clinical trials, sparse concentration-time sampling can be built into the study protocol so further information about the absorption phase of sirolimus can be obtained.

We have estimated a decrease of 20.8% in the sirolimus CL/F for every 100 mg increase in the cyclosporine daily dose. The effect of cyclosporine dose on the CL/F of sirolimus has not been reported before however significant drug interaction between cyclosporine and sirolimus is a well known phenomenon.<sup>1,3,14,15</sup> In a study in rats, it was observed that cyclosporine increases the concentration of orally but not intravenously administered sirolimus suggesting this interaction occurs in gastrointestinal tract rather than in the liver.<sup>3</sup> In healthy volunteers, sirolimus bioavailability was increased by 230% when sirolimus administered orally at the exact same time with cyclosporine.<sup>14</sup> Even when it was administered 4 hours after cyclosporine, the AUC for sirolimus was 80% higher than without cyclosporine administration.<sup>14</sup> Based on this, it was recommended that sirolimus and cyclosporine doses to be taken 4 hours apart which was the case in the patients included in our study. With the cyclosporine dose ranging from 50 to 725 mg/day, it can be anticipated that a large degree of variability in sirolimus trough concentrations

can be attributed to the variation in cyclosporine dose. This observation has potential importance for sirolimus dose adjustment in cyclosporine dose minimization or elimination protocols. Based on our findings, it can be recommended that to maintain a desirable trough concentration, sirolimus dose must be increased by approximately 20% for every 100 mg decrease in the cyclosporine daily dose.

Identification of primary diagnosis of ischemic heart disease and hypertriglyceridemia as the two important factors contributing to sirolimus CL/F are the most important findings of this study and has not been reported before. In fact, patients requiring heart transplantation due to a primary diagnosis of non-IHD had CL/F values that was 62.1% higher than patients with IHD and patients with hypertriglyceridemia (triglyceride  $\geq 2$  mmol/L) had a 37.8% lower CL/F when compared to patients with  $< 2$  mmol/L triglycerides levels.

Apparent clearance is a parameter that relates to both absorption and metabolism therefore alteration in either of the two phases can explain the change in this parameter. Sirolimus oral bioavailability is 35% higher when it is taken with a high-fat meal<sup>16</sup> indicating a bile flow-dependent absorption pattern and a high affinity of this molecule for fatty complexes. Hypertriglyceridemic patients may habitually consume higher fat meals which may then result in an increased absorption of sirolimus. In addition sirolimus itself can increase total cholesterol and triglyceride levels<sup>13</sup> and patients with a history of ischemic heart disease are more prone to hypercholesterolemia.<sup>17</sup>

In addition, it can be anticipated that similar to other immunosuppressive agents,<sup>18</sup> sirolimus may also be a drug with low intrinsic clearance therefore its hepatic clearance will be dependent upon the extent of drug binding to blood cells and plasma proteins. Our observation on lower sirolimus clearance in patients with IHD and hypertriglyceridemia can be explained by speculating that patients with dyslipidemia may have reduced hepatic clearance of sirolimus because of lower unbound fraction that is available for metabolism and elimination. Unfortunately reliable estimation of sirolimus plasma protein binding or unbound concentration has not been possible to date because in plasma, sirolimus undergoes ester hydrolysis and converts to a ring opened form, seco-sirolimus<sup>19</sup> therefore generating erroneous results. The only study to date has used tritium-labeled sirolimus to investigate plasma protein binding of sirolimus.<sup>20</sup> It was found that 60% of drug in plasma is associated with lipoprotein deficient fraction while remaining 40% was bound to HDL ( $19.5 \pm 3.9\%$ ), LDL ( $20.5 \pm 5.9\%$ ) and VLDL ( $1.2 \pm 0.5\%$ ). The proportion of LDL-bound sirolimus increased in parallel with the total concentration of the drug whereas the concentration of drug in lipoprotein-deficient plasma decreased<sup>20</sup> as the total drug concentration increased showing that sirolimus has higher affinity to bind to lipoproteins than albumin or other plasma proteins. However, this study did not account for the conversion of sirolimus to seco-sirolimus; therefore its results must be interpreted with caution.<sup>20</sup>

Sirolimus is metabolized by cytochrome P450 3A4 isoenzymes and is a substrate for p-glycoproteins.<sup>1</sup> The clinicians participating in the trial were cautioned about the potential interaction between inhibitors (erythromycin, azole antifungals) and inducers (nifedipine, rifampin) of this system

hence this database only included a small number of patients on these agents. Also, by trial protocol, all patients preemptively received lipid lowering agents which precluded the likelihood of finding the effects of lipid lowering agents on sirolimus apparent clearance. The only interacting drug taken by a relatively large number of patients was the calcium channel blocker diltiazem which showed to significantly reduce sirolimus CL/F in the univariate analysis. In a drug interaction study between sirolimus and diltiazem in healthy volunteers, it was shown that the apparent oral clearance and volume of distribution of sirolimus decreased 38% and 45%, respectively.<sup>21</sup>

In this study, we did not find patient's age, weight, gender or ethnic origin to be significant covariates for sirolimus apparent clearance. During phase 2 and 3 clinical trials of sirolimus, it was observed that healthy African-American subjects had 34% higher sirolimus apparent clearance than Caucasian subjects.<sup>22</sup> Also, it was shown that healthy female patients had 20% higher clearance as well as pediatric patients who had 90% higher clearance than healthy adult individuals.<sup>22</sup> The failure to detect the effect of demographic characteristics on the apparent clearance of sirolimus most probably is a result of the small number of demographically homogenous patients included in this study.

The analysis was performed using NONMEM software which is the gold standard in population pharmacokinetic analysis. A one-compartmental model with first-order absorption and elimination was used to describe sirolimus concentration-time data. Preliminary analysis (data not presented) showed that the concentration time data is best fitted by a one-compartment model. This could possibly be due to the fact that available concentration data were obtained during the elimination phase of sirolimus, therefore, there was not sufficient information to describe the absorption and distribution phases using multi-compartment model. The manuscript by Ferron et al<sup>6</sup> uses a two compartment model however, the concentration-time data in this study included seven concentrations collected during a 12-hour dosing interval therefore enabling them to model both distribution and elimination phases of sirolimus.

The FOCE method was used in order to allow residual error to be evaluated based on conditional parameter estimates rather than typical values.<sup>23,24</sup> We have recently reported a population pharmacokinetic analysis of tacrolimus in liver transplant recipients which essentially used the same modeling approach.<sup>25</sup> Furthermore, this approach has been reported to give better estimates when only trough concentrations are available for model development.<sup>26</sup>

## CONCLUSION

In conclusion, a population pharmacokinetic model for estimation of oral clearance of sirolimus in heart transplant recipients was developed. Cyclosporine dose, hypertriglyceridemia and primary diagnosis of ischemic heart disease were identified as important covariates for sirolimus apparent clearance. This information can be utilized for sirolimus dose adjustment to achieve optimal trough concentrations. In the light of this study, further conventional pharmacokinetic studies are recommended to fully characterize pharmacokinetic

parameters of sirolimus in heart transplant patients with a primary diagnosis of IHD and in hypertriglyceridemic patients.

### ACKNOWLEDGMENTS

We are grateful to Dr Rajagopalan (Bayers Pharmaceuticals, CT) for his input into the population PK analysis and Dr Joseph Scarola (Wyeth Pharmaceuticals, PA) for his invaluable help in obtaining data from the Clinical Trial Database. The authors have no conflict of interest to report and did not receive any funding from Wyeth Pharmaceuticals for this study.

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