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Population Pharmacokinetics of Cyclosporine in Clinical Renal Transplant Patients

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Abbreviations:

CsA, Cyclosporine; NONMEM, Nonlinear mixed-effect modeling;

POD, Post-operative days; TBIL, Total bilirubin level;

CBW, Current body weight; HCT, Hematocrit;

INHI, Concurrent metabolic inhibitors of cyclosporine;

CL/F, Oral clearance;

V/F, Apparent volume of distribution.

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Abstract

Population pharmacokinetics of cyclosporine (CsA) in the clinical renal transplant patients has been reported in the present study. A total of 2, 548 retrospective drug monitoring data points were collected from 120 renal transplant patients receiving CsA. Population modeling was performed using NONMEM (nonlinear mixed-effect modeling) program, using a one-compartment model with first-order absorption and elimination. The final regression model for CsA clearance (CL/F) with influence of six significant covariates, which is comprised of post-operative days (POD), total bilirubin level (TBIL, μM), current body weight (CBW，kg), age (Age, year), concurrent metabolic inhibitors of cyclosporine (INHI) and hematocrit (HCT, %), has been established and expressed as CL/F=28.5-1.24*POD-0.252*(TBIL-11)+0.188*(CBW-58) -0.191*(Age-42)-2.45*INHI-0.212*(HCT-28) (L/h). The values in parenthesis represent the median level for each of the corresponding covariates. The population estimates for CL/F (28.5 L/h), V/F (volume of distribution, 133 L) and inter-patient variability (CV% = 19.7%) for CL/F were achieved, respectively. The population model was further validated by internal and external approaches, and was demonstrated to be effective and stable. Moreover, simulation was conducted to facilitate the individualized treatment based on patient information and the final model.

Introduction

Cyclosporine (CsA) has been introduced into organ transplantation since the early 1980s and has shown to largely reduce the rate and severity of graft–versus-host disease, and to increase success in graft and survival of the patients (Hesselink et al., 2004). Today, CsA has become the backbone of immuno-suppression in clinical organ transplantation (Kyriakides and Miller, 2004). As a result, short-term and medium-term kidney allograft survivals have been greatly achieved. However, CsA application has exhibited high degree of inter-individual and intra-individual variability, either in pharmacokinetic and/or pharmacodynamic aspects. Furthermore, the therapeutic window (range of drug concentration for desired therapeutic effect) with acceptable tolerability is very limited (Abendroth, 2004; Armstrong and Oellerich, 2001). Levels below the window are associated with high risk of organ rejection, while levels above the window correlate with side effects, such as nephrotoxicity, infection, hepatotoxicity and tumor (Kasiske et al., 1988).

Many clinical pharmacokinetic studies of CsA have been conducted using ordinary pharmacokinetic methods, which were focused on individual parameter estimates, with multiple blood-sampling points (Trompeter et al., 2003; Banner et al., 2002). However, the pharmacokinetic properties of CsA changed greatly between patients and between investigations. It has been difficult to predict its disposition in a specific individual although pharmacokinetics and pharmacodynamics of CsA have been well reported in literature. In contrast to the traditional pharmacokinetic approach, population pharmacokinetics has great advantages in estimation of the population parameters and analysis on factors (i.e. influence of demographic parameters and physiological conditions on the pharmacokinetic parameters).

Population method is robust to predict drug's behavior based on specific individual information and, moreover, is ideal in analyzing the sparse data commonly obtained in clinic (Sheiner et al., 1977).

In the present study, medical history on 120 patients receiving renal transplant was retrospectively analyzed and population pharmacokinetics study of CsA in the patients was performed using NONMEM (nonlinear mixed-effect modeling). Consequently, the pharmacokinetic model was defined, using routing drug monitoring data, and could be employed to improve the clinical application of CsA.

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Methods

Patients and data collection. Plasma concentration data of CsA from 120 patients receiving renal transplantation in the past 4 years in Peking University First Hospital, Beijing, China, were collected. The patients were divided into two groups: 99 in index group for the construction of model and 21 in validation group for external validation. All the patients were treated with co-administration of CsA, mycophenolate mofetil (or azathioprine) and corticosteroid. CsA was administered orally in the soft capsule formulation twice daily. There were three brands of CsA: Neoral® (Novartis Pharmaceuticals Corporation, Switzerland), Tianke® (North China pharmaceutical Group Corporation, China) and Neocyspin® (Zhongmei Huadong Pharmaceutical Co., Ltd, China). Neoral® and Neocyspin® were composed of microemulsion formulation in soft capsule, while Tianke® was packed in a common soft capsule and turns into microemulsion in gastrointestinal tract spontaneously. Among the patients, 93 of the 120 patients in the study were administered with Neoral®, 25 with Tianke® and 7 with Neocyspin®, respectively. In addition, five patients were administered with the CsA products from two different manufacturers. In a routine protocol, methylprednisolone was first given for three days after operation, subsequently followed with prednisone on basis of once a day regimen. Methylprednisolone or dexamethasone was occasionally given for graft–versus-host disease as needed. The hepatic function and normal blood and biochemical parameters for the patients were examined and reported as normal (Table 1).

The influence of general covariates was analyzed as follows: age (Age), height, current body weight (CBW), body mass index (BMI), sex, post-operative day, hematocrit (HCT), concurrent metabolic inhibitors of CsA (INHI) and corticosteroid,

liver function tests [total bilirubin level (TBIL), alanine aminotransferase (ALT), alkaline phosphatase (ALP), (gamma)-glutamyl transferase (GGT)], different CsA dosage forms (DRUG). These covariates were selected according to the previous reports and clinical common sense.

Blood sampling and CsA analysis. Whole blood samples were collected immediately at predose (C_0), 2 hr (C_2) and every 2 or 3 days postdose according to clinical need until termination of the treatment or change to other immunosuppressants (usually tacrolimus). All samples were transferred into EDTA-vacutainer tubes, and CsA concentrations in whole blood were measured immediately utilizing fluorescence polarization immunoassay (FPIA) technology (TDx, Abbott Laboratories), which is capable of detecting CsA concentrations with 95% confidence for the samples containing ≥ 25.00 ng/mL and the values of CV were less than 4%.

Population model construction. Population pharmacokinetic analysis was performed using the NONMEM program (Version V Level 1.1). A one-compartment open model with first-order absorption and elimination was used to analyze CsA data. The model consisted of an absorption rate constant (Ka), clearance (CL/F), and apparent volume of distribution (V/F). Since almost all retrospective data were located at the two ends of absorption phase in the study, Ka, the constant of absorption was presumed to be $1.28h^{-1}$ based on literature values (Rui et al., 1995; Parke and Charles, 2000).

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The model was established using the forward inclusion – backward elimination method (Mandema et al., 1992). In the first step, the population pharmacokinetic analysis was conducted without any covariates in the basic model. Scatter plots of CL/F and V/F against each covariate helped to identify the trends and the regression pattern. In the second step, each candidate covariate was screened in turn by incorporating it into the basic model to develop the intermediate and full models and by observing decrease of the objective function value. The difference in objective function value (OFV) was kept as χ^2-distribution, and an OFV value greater than 3.84 associated with a *p*-value of 0.05 was used for statistical significance. There were also many indicators for the improvement of fit due to the addition of a parameter to the model: decrease in standard error of the parameter estimates, reduction in inter-patient and intra-patient variability, agreement between the observed and predicted concentrations, reduction in weighted residuals and uniformity of the scatter plot of weighted residuals versus predicted concentrations (Beal and Sheiner, 1989). The influences (Age, height, CBW, BMI, ALT, ALP, TBIL, GGT, and HCT) were included in the model as continuous covariates in a linear way. Discrete covariates, such as sex, INHI, DRUG and POD, were evaluated by stepwise inclusion of scaling factors: sex = 0 for males and sex = 1 for females; INHI = 1 for those coadministered with metabolic inhibitors of CsA (diltiazem or verapamil) and otherwise INHI = 0; DRUG = 0 for Neoral®, DRUG = 1 for Neocyspin® and DRUG = 2 for Tianke®; POD values are assigned in Table 2. In the final step, the influence of each covariate remaining in the full model was removed in turn, by fixing its value to zero. This process was repeated until the increase of objective function was less than the critical value of 10.83 ($p < 0.001$).

The statistical model used to describe inter-individual variability in the pharmacokinetic parameter of CsA was expressed in Eqn. 1 (exponential):

$$Pij = P_{TV}j \cdot Exp(\eta ij) \qquad [1]$$

where Pij is the *j*-th basic pharmacokinetic parameter for the *i*-th individual, $P_{TV}j$ is the typical value of the *j*-th population parameter, and ηij is a random variable for *i*-th individual in *j*-th parameter distributed with a mean of 0, and variance of ωij.

A combined proportional and additive model was used to describe the residual variability (Eqn. 2):

$$C_{OBS} = C_{PRED} \cdot (1 + \varepsilon 1) + \varepsilon 2 \qquad [2]$$

where C_{OBS} and C_{PRED} are the observed and predicted blood CsA concentrations, respectively. ε1 and ε2 are randomly distributed terms in which each has zero mean and variances of σ1 and σ2, respectively.

Model validation. The contribution of individual on the modeling results and robustness of the final population model were assessed using internal and external validation strategies. Data-splitting method was employed as internal validation (Ishibashi et al., 2003). Patients in the index group were randomly divided into 10 subpopulations and each of them consisted of 90% of the patients in the original population. Each subset was analyzed by NONMEM with the final model to obtain the parameter estimates which were compared with those resulting from full data set. Next, the objective function value was calculated by applying each of the 10 subsets estimation into the full data set. These objective functions were compared with that from the full data set using the final model. Then Jackknife estimate was employed to

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calculate the 95% confidence interval (C.I.) for parameter estimates which were compared with those obtained by the NONMEM.

External method was also applied to validate the final model (Sheiner LB and Beal SL, 1981). Another 21 patients were included in the validation group (Table 1). The observed concentrations were compared with the corresponding predictions by NONMEM based both on the basic and final model. Predictive performance was assessed in terms of bias (mean predicted error, ME) and precision (mean squared prediction error, MSE and root mean squared prediction error, RMSE). Moreover, standardized prediction error (SPE) was also introduced in the model validation (Serrano BB et al., 1999). The indicators were defined as follows.

$$ME = \frac{1}{N}\sum_{i=1}^{N}(C_{PRED} - C_{OBS}) \qquad [3]$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(C_{PRED} - C_{OBS})^{2} \qquad [4]$$

$$RMSE = \frac{1}{N}\sum_{i=1}^{N}\sqrt{(C_{PRED} - C_{OBS})^{2}} \qquad [5]$$

$$SPE = \frac{C_{OBS} - C_{PRED}}{SD_{C_{PRED}}} \qquad [6]$$

where C_{OBS} and C_{PRED} are the observed and predicted blood CsA concentrations, respectively. $SD_{C_{pred}}$ is the standard deviation in the predicted values, while N is the total number of observations in the validation group.

Trial simulation. Simulations were carried out by NONMEM to facilitate the individualized treatment based on patient information and the final model. 175mg of CsA was orally administered twice a day. The value of all the key covariates was

assigned according to those in the index group. A standard patient with the average values for continuous key covariate (TBIL, CBW, Age and HCT) was simulated, and compared with other patients with different value of covariate (mean±2SD). The probability of the covariate value falling in the interval of mean±2SD is 95.5% and this range is wide enough to assess the influence of each covariate. The essential information for simulation is shown in Table 3. The simulation for each situation was conducted for 500 times.

Results

The plasma CsA concentration vs. time curves were well described using a one-compartment open model. The distribution of random residual errors was expressed using a combined model (additive and proportional) to best interpret intra-patient variability. The resulting population model with the covariates of CL/F (Age, CBW, POD, TBIL, HCT, and INHI) is shown in Eqn. 7,

$$\begin{aligned} CL/F = {} & 28.5 - 1.24 \cdot POD - 0.252 \cdot (TBIL - 11) + 0.188 \cdot (CBW - 58) \\ & - 0.191 \cdot (AGE - 42) - 2.45 \cdot INHI - 0.212 \cdot (HCT - 28) \end{aligned} \quad [7]$$

in which the influence scopes (TBIL, CBW, Age, HCT) were adjusted by their respective median values determined from the database (11 μmol/L for TBIL, 58 kg for CBW, 42 years for Age, and 28% for HCT, respectively). INHI was defined as 1 in the patients coadministered with metabolic inhibitors of CsA (diltiazem or verapamil), or as zero otherwise. POD value was estimated based on the post-operative day (Table 2). In addition, no statistical significance was observed in the presence of brand of CsA as a covariate on Ka. Inter-individual variability of V/F was considered as zero due to its insignificant values.

The population pharmacokinetic parameters with the model are listed in Table 4. The relative standard errors (% RSE) of estimation for the parameters were acceptable, with a range from 3.27% to 27.2%. The inter-individual variability for CL/F and Ka were 31.3% and 16.1%, respectively. Figure 1-A shows the relationship between the observed (OBS) and population model-predicted concentrations (PRED), and Figure 1-B shows the relationship between the observed (OBS) and individual model-predicted concentrations (IPRED). A good correlation in the plots was observed, suggesting that the resulting model fits the observed data well in the patients, although peak concentrations in several individuals were slightly

underestimated. The diagnostic plots of the model are also shown in Figure 2. The weighted residual values (WRES, -2.81, 4.72) for model-prediction concentration shown in the rectangular distribution were well acceptable. Moreover, the goodness of fit with the model had no significant difference among the patients.

The partial residual (η1) on CL/F with the key covariates was compared between the basic and resulting models (Figure 3). The trend in the parameter estimates from the basic model (left column) declined markedly in the model (right column). Height and BMI were observed to closely relate to η1, the inter-individual variability for CL/F. Close correlations were observed between Height and Age, between Height and CBW and between CBW and BMI (Table 5). Since Age and CBW were more closely related to a decrease in the values of the objective function (48.85, 55.23), Height and BMI were removed from the model.

A number of covariates are retained in the final model. As the covariates introduced into model, the variance of inter-individual variability (η1) for CL/F and objective function value (OFV) became smaller and smaller, and the magnitude of difference in η1 and OFV varied among the covariates. Table 6 shows the extent of each covariate explained η1 and OFV.

The population typical values of CL/F or V/F from the full data set and 10 individual subsets are shown in Figure 4. The result indicated that the CL/F_{TV} and V/F_{TV} estimates from the subsets were consistent with those resulted from the full data set (Mean ± S.E.). In addition, the objective function value with fixed parameter values estimated from the 10 subsets, ranged from 23126.3 to 23129.7, was not

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significantly different from that of the full data set (23126.0, P>0.05). The mean values and 95% C.I. for the two sets are indicated in Table 4 (the right half).

A great improvement in the predictive performance of the final model was achieved as compared to the basic one. Table 7 summarizes the precision errors from the basic and final model. The estimated average SPE (0.05) was very close to zero, and its standard deviation (0.97) was close to the expected value of 1.

The simulated steady state profiles for various situations are displayed in Figure 5. The range of concentrations varied when the covariate value changed.

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Discussion

Aim of the study is to investigate factors that may influence the pharmacokinetics of CsA after the renal transplantation. Data from clinical drug monitoring in hospitalized renal transplantation patients are sparse and random. Population method is suitable to analyze this kind of data, to accommodate flexible treatment (such as dose fluctuations) and to estimate the factors that could change the pharmacokinetics as well as to compute the inter-patient and intra-patient variability.

Either proportional or additive fixed-effect model can be employed for the study. In the former model, variation of the pharmacokinetic parameter should increase as covariate value increases. In contrast, in the additive model, the scope of variation has no significant change with parameter value, and the upper boundary is in parallel with the lower boundary. For instance, scatter plots of CL/F versus CBW indicate that the relationship between CL/F and CBW was additive (Figure 6). The similar pattern for other covariates was characterized. In addition, four patterns of regression for the correlations were compared with different analyses in CL/F and CBW (linear, logarithm, power and exponent). It was noted that no significant differences among the analyses (Table 8) and no change in the objective function were observed. Hence, the covariate (COVR) was incorporated into the additive linear model as shown below:

$$P_{TV\,i} = P_{TV} + f_{COVR} \cdot (COVR_{MEDIAN} - COVR_i) \qquad [8]$$

where P_{TV}i and P_{TV} are *i*-th individual and population's typical value of PK parameter, $COVR_{MEDIAN}$ and COVRi are the population median and *i*-th individual's covariate value, and f_{COVR} is the scaling factor for influence of covariate, respectively.

It is not surprising that effect of body weight on CL/F of CsA was significant, which was consistent to the previous reports (Rui et al., 1995; Parke and Charles, 2000; Parke and Charles, 1998; Jacobson et al., 2003). A 0.118 L/h change on CL/F would occur on every kg variation on CBW, which was comparable to the value (0.101 - 0.183 L/h) previously reported (Jacobson et al., 2003). It is common that the patients can gain body weight by 10% after transplantation. The increase in body weight could be attributed to inappropriate food intake, decreased physical activity, as well as possibly high dose administration of steroid. Therefore, monitoring the body weight during the treatment is necessary.

It has been accepted that CsA is metabolized by cytochrome P450 3A4 (CYP3A4). Thus, pharmacokinetics of CsA can be altered by either induction or inhibition of the enzyme (Yates et al., 2003). Verapamil and diltiazem have been known to be potent competitive inhibitors of CYP3A4 in the metabolism of CsA (Jones et al., 1997; Pichard et al., 1990). As reported, CsA was usually co-administered with the inhibitors to improve therapeutic potency or to reduce cost of the treatment. The studies demonstrated that CL/F of CsA in the presence of verapamil or diltiazem decreased by 30 ~ 50% as compared to that in patients with CsA alone (Parke and Charles, 1998; Masri et al., 1994). CsA is known to be metabolized by CYP3A4 and, to a lesser extent, by CYP3A5. It is also a substrate for the cell efflux transporter, P-glycoprotein. Since CYP3A4, CYP3A5 and p-gp have been shown to exist in the liver as well as in the intestine, it seems possible that the metabolism of CsA would also occur at both organ sites. So, it is controversial that the difference of pharmacokinetics was mainly due to temporal changes in CL/F and/or F (bioavailability). Interestingly, Preuner et al reported that at low therapeutic

CsA concentrations diltiazem increased Cmax (maximum concentration) and AUC (areas under the curve) with minor change in Ctrough. However, at high CsA concentrations, an alternative metabolic pathway was detectable, not inhibited by diltiazem (Preuner et al., 1998). Other study also suggested that diltiazem enhances the absorption of CsA (Foradori et al., 1998). The phenomenon was similarly observed with verapamil (Tortorice et al., 1990).

It has been shown that CL/F for CsA can be affected by bilirubin level (TBIL). The clearance decreased as TBIL increases. Biliary route is believed to be the major elimination pathway of CsA in a manner proportional to bile production and liver function. Previous reports indicated that dysfunction in hepatic bile excretion with high plasma TBIL correlated closely with high blood concentrations of CsA (Sun et al., 2001). Besides, the present study showed that hematocrit (HCT) is a factor that changes CL/F, consistent with the previous report (Yee et al., 1988). This is due to probably the fact that about 50% of CsA bound to red blood cell and only free drug was clearable. The results including TBIL and HCT are in agreement with those from non-population analysis. Effect of HCT in hematopoietic stem cells transplant patients on CL/F was not evaluated due to infrequent HCT measure and the alteration for red blood cell transfusion (Jacobson et al., 2003). In contrast, routine and biochemical blood examination were performed in the present study and the frequent HCT measure at early post-transplant stage was high, which allowed measurement for all C0. Therefore, evaluation on relationship between CL/F and TBIL /HCT was feasible.

It was reported that POD, days post-operation, significantly altered CL/F for CsA (Rui et al., 1995; Jacobson et al., 2003). The value of CL/F declined after operation,

especially within the first three weeks. Our results from the proposed model agreed with these reports, in that CL/F for CsA decreased by 4.35% in week 1, 8.70% in week 2, and 13.05% in week 3 after renal transplant operation. Modeling a time-related change in CL/F greatly improved goodness of fit (Figure 7). However, attention should be paid that POD also had a great effect on oral absorption for CsA, since the altered gastrointestinal motility after operation was anticipated (Parke and Charles, 2000; Parke and Charles, 1998).

Large inter-individual variability for Ka (CV% = 179) was determined by NONMEM. This is probably due to insufficient retrospective concentration data that cannot provide adequate information for analysis on CsA absorption phase. However, since Ka values were collected from the previous study, differences of the characters between the populations from the previous study and the present study might exist. Since Neoral was used with most patients, the inter-individual variability for Ka caused by different product batches of CsA can be excluded.

The population model has been defined in the present study to contain several factors involved in CL/F for CsA. Therefore, the model validation is necessary. Our internal validation analysis confirmed that the model is robust and stable based on the coincidence between NONMEM estimate and Jackknife estimate (Table 4). No significant difference was observed between the subsets and the full data set. External validation is the most stringent test of a model. It is obvious that the performance (precision and accuracy) of the final model is better than that of the basic one in terms of ME, MSE, RMSE (Table 7). The 95% confidence interval of SPE includes zero, which indicates that the final model fits the observed concentrations well. Moreover,

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the SD of SPE (0.97) is close to 1, confirming that the contribution of predicted concentrations is in accordance with those of the observed values and the final model is valid.

Because only one covariate value was changed for each situation, the simulated concentrations did not vary greatly. However, the demographic background and dosing regimen in the real world is more complex and the concentrations will vary remarkably. Based on this study, special attention should be paid if a patient's key covariate value is abnormal. Simulation with the current final model will help to treat patient individually and ensure the concentrations varies within the therapeutic window.

The aim of this study is to demonstrate whether routine therapeutic drug monitoring data (C_0 and C_2) can be used to estimate the population parameters with NONMEM. It is popular to use C_0 and C_2 as markers in today's therapeutic CsA monitoring (Trompeter et al., 2003; Ray et al., 2003). The final model proposed in this study showed its competency in predicting CsA concentrations based on the patient information.

In conclusion, a population pharmacokinetic model for CsA in renal transplant patients receiving multiple oral doses has been successfully established. The model provides a useful tool that can be employed to estimate individual CL/F, V/F and Ka for the patients receiving CsA, and to adjust dosing regimens with covariate factors (POD, TBIL, CBW, Age, INHI, and HCT) that possibly interfere the population pharmacokinetic parameters.

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Figure Legends

FIG. 1. Scatter plots of (A) population model-predicted concentrations (PRED, ng/mL) versus observed concentrations (OBS, ng/mL) and (B) individual model-predicted concentrations (IPRED, ng/mL) versus observed concentrations (OBS, ng/mL). The solid line is of a linear regression line and the broken line is of unity.

FIG. 2. Weighted residuals (WRES) versus population model-predicted concentrations (PRED, left) and the patient's identification number (ID, right). The solid line is a linear regression of the data.

FIG. 3. The relationship of the partial residual ($\eta 1$) for CL/F and the key covariates from the basic (left column) and the final model (right column).

FIG. 4. Population typical value of CL/F and V/F for full data set (●) and for 10 different subsets (○). The solid and broken lines are the parameter value and ± SE values from the full dataset, respectively.

FIG. 5. The simulated steady state profile for each situation. SIM Css is the simulated steady state concentration.

FIG. 6. Plot of CL/F versus CBW from the basic pharmacokinetic model. The solid line is of a linear regression line and the broken lines are the upper and lower boundary of the scope of CL/F.

FIG. 7. Weighted residuals (WRES) versus post-operative hours before (A) and after (B) POD were incorporated into the pharmacokinetic model of CL/F.

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TABLE 1 Patient demographic background

Characteristics	Number or mean (range)	
	Index group	Validation group
No. observations	2141	397
No. patients	99	21
Observations per patient	21.6 (6-61)	18.9 (8-35)
Age (year)	42.3 (16-66)	42.1 (24 - 66)
Height (cm)	165.1 (148-184)	166.3 (153 - 178)
CBW (kg)	57.9 (34-91)	66.3 (44 - 86)
BMI (kg/cm^2)	21.2 (14.5 - 33.3)	22.4 (16.3 - 33.5)
Sex (M/F)	46/53	9/12
Dose (mg/12hours)	25 - 275	50 - 260
Post-operative day	47 (9 - 202)	37 (12 - 95)
INHI (Y/N)[a]	1137/1004	175/222
TBIL (μM)	11.2 (1.1-34.1)	11.8 (4.4 – 39.6)
ALT (U/L)	30.1 (1-303)	31.5 (3 – 293)
ALP (U/L)	61.5 (2-389)	63.0 (2 - 380)
GGT (U/L)	37.0 (2-394)	37.1 (2 - 350)
HCT (%)	28.3 (10.9-49.3)	28.5 (17.3 – 42.0)
DRUG		
Neoral®	75	18
Tianke®	22	3
Neocyspin®	7	0

[a] account of concentrations concomitant with or without metabolic inhibitors of CsA.

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TABLE 2 Specification of POD

POD value	Post-operative Day
1	0-7
2	8-14
3	15-21
4	22-60
5	61-150
6	>150

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TABLE 3 Value for key covariates in simulation

ID	Post-operative day	TBIL (μM)	CBW (kg)	Age (years)	INHI	HCT (%)
121[a]	7	11.2	57.9	42.3	0	28.3
122	7	11.2	57.9	42.3	1	28.3
123	21	11.2	57.9	42.3	0	28.3
124	7	20.1	57.9	42.3	0	28.3
125	7	2.4	57.9	42.3	0	28.3
126	7	11.2	79.8	42.3	0	28.3
127	7	11.2	36.0	42.3	0	28.3
128	7	11.2	57.9	63.5	0	28.3
129	7	11.2	57.9	21.2	0	28.3
130	7	11.2	57.9	42.3	0	39.6
131	7	11.2	57.9	42.3	0	17.0

[a] Standard patient.

TABLE 4 Final population pharmacokinetic parameter estimates of CsA and the results of internal validation (Jackknife method)

	Estimates from final model (NONMEM estimate)			Results of internal validation (Jackknife estimate)		
Parameter	Estimate	%RSE[b]	95% C.I.[c]	Estimate	%RSE[b]	95% C.I.[c]
CL/F_{TV} (L/h)	28.5	3.27	26.7 -30.3	28.5	2.98	26.6-30.4
V/F_{TV} (L)	133	4.03	122 - 144	134	2.13	127-140
Ka_{TV}[a] (1/h)	1.28	-	-	-	-	-
f_{POD}	1.24	16.93	0.830 - 1.65	1.29	12.33	0.926-1.644
f_{TBIL}	0.252	17.06	0.168 - 0.336	0.306	11.44	0.226-0.386
f_{CBW}	0.188	18.62	0.119 - 0.257	0.217	18.88	0.124-0.309
f_{Age}	0.191	17.28	0.126 - 0.256	0.158	20.88	0.082-0.233
f_{INHI}	2.45	27.18	1.14 - 3.76	2.80	14.68	1.87-3.73
f_{HCT}	0.212	19.34	0.132 - 0.292	0.220	22.26	0.108-0.332
Inter-individual variability (% CV)						
CL/F	19.7			-		
Ka	179			-		
Residual error (CV% if proportional, S.D. if additive)						
σ1-Proportional	30.8			-		
σ2-Additive	42.4 (ng/mL)			-		

[a] Ka was fixed at 1.28 and the inter-individual variability of V/F was fixed at 0.

[b] %RSE is percent relative standard error (100% x SE/ Estimate) [c] 95% confidence interval

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TABLE 5 Linear correlation coefficient among Age, Height, CBW and BMI

	Age	Height	CBW
Height	-0.48		
CBW		0.62	
BMI			0.89

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TABLE 6 The influenced of each key covariate on the inter-individual variability for CL/F and objective function value (OFV)

Covariate	Decrease of ω1[a]	Decrease of OFV	*p*-value
POD	0.034	366.32	***[b]
TBIL	0.003	71.92	***
CBW	0.021	55.23	***
Age	0.016	48.85	***
INHI	0.001	50.21	***
HCT	0.015	45.84	***

[a] ω1 is the variance of inter-individual variability for CL/F. [b] <0.001

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TABLE 7 Prediction errors estimated in the validation group

	Basic model	Final model
ME (ng/mL)	-5.53	-2.26
(95%C.I.)[a]	(-21.8 – 10.8)	(-17.4 – 12.84)
MSE $(ng/mL)^2$	27359	23387
(95%C.I.)	(22408 – 32310)	(18820 – 27953)
RMSE (ng/mL)	115.1	101.1
(95%C.I.)	(103.5 – 126.9)	(89.7– 112.4)
SPE		
Value (95%C.I.)	-	0.05 (-0.04 - 0.14)
S.D.[b]	-	0.97

[a] 95% confidence interval [b] Standard deviation of SPE.

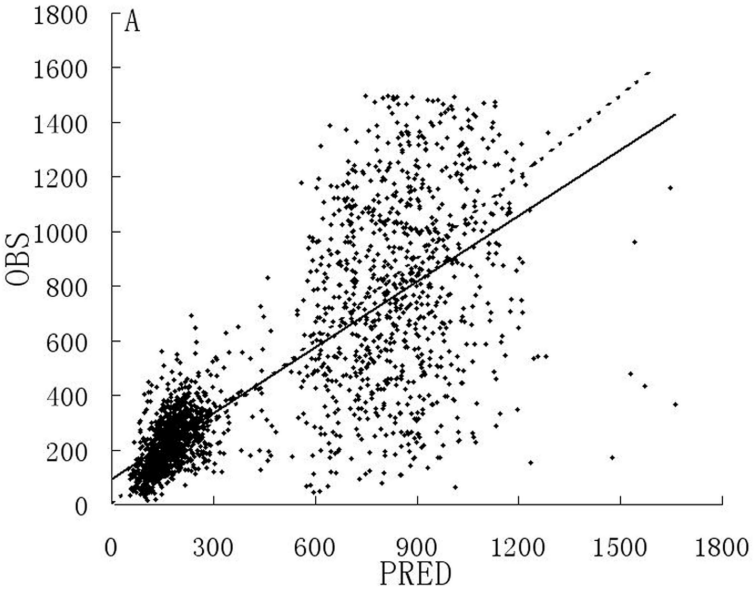
TABLE 8 Results from four regressions for CL/F and CBW

	Rsq[a]	Sigf[b]	b0	B1
Linear	0.075	0.000	16.09	0.12
Logarithm	0.084	0.000	-7.42	7.57
Power	0.086	0.000	5.95	0.33
Exponent	0.078	0.000	16.60	0.01

Independent: CBW; Dependent: CL/F

[a] Rsq–R square; [b] Sigf–significance.

FIG. 1.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1500
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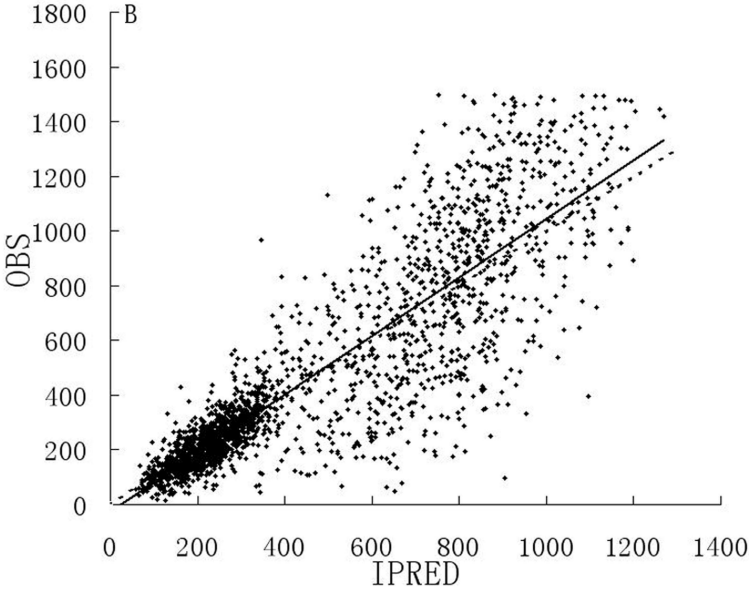
PRED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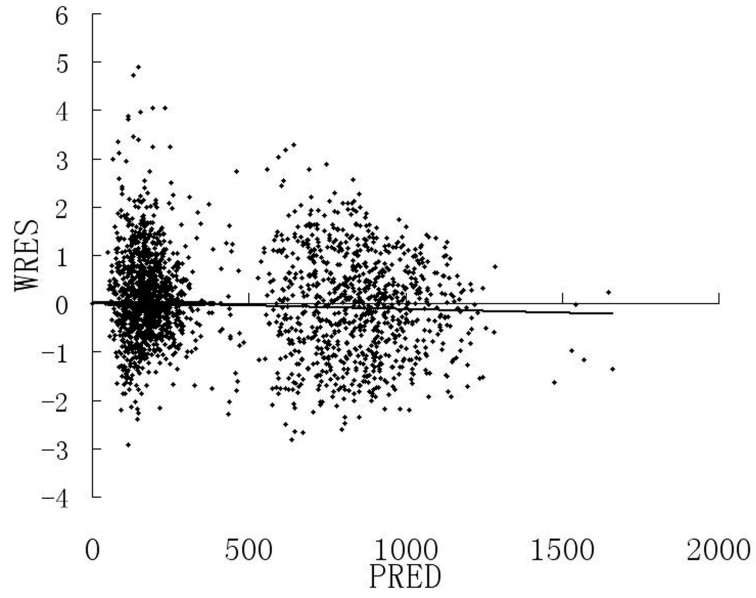
1200
1400
IPRED

FIG.2.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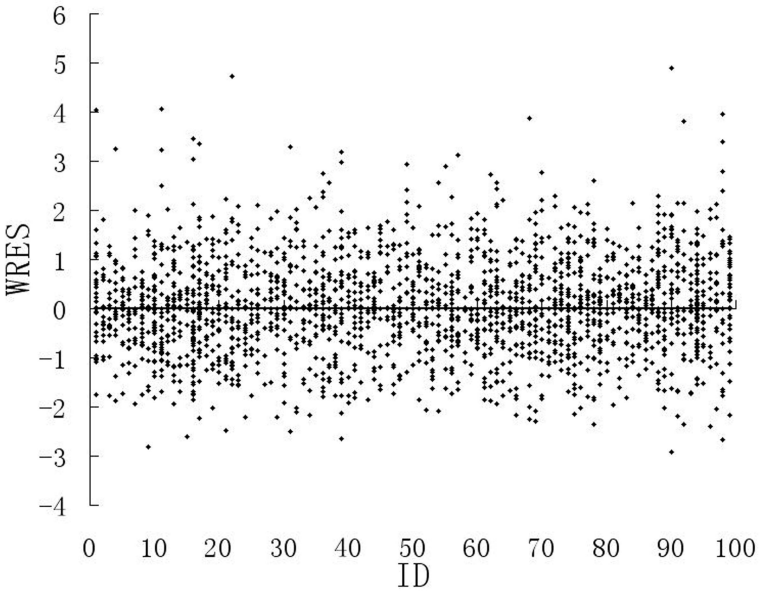
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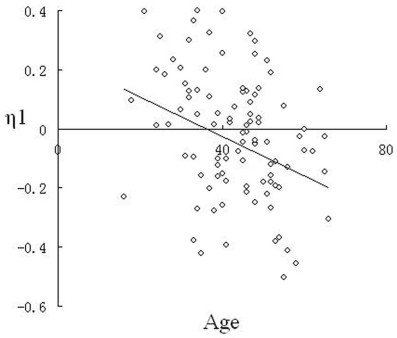

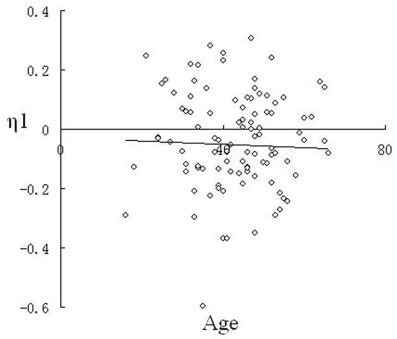

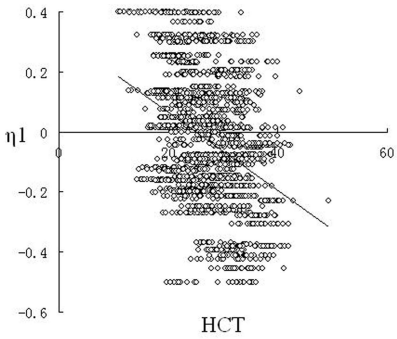

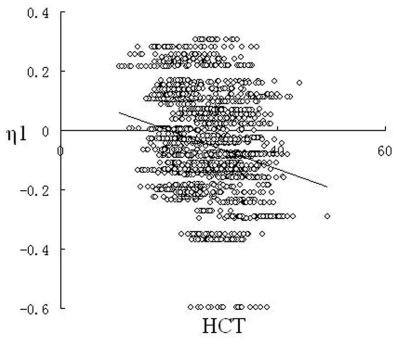

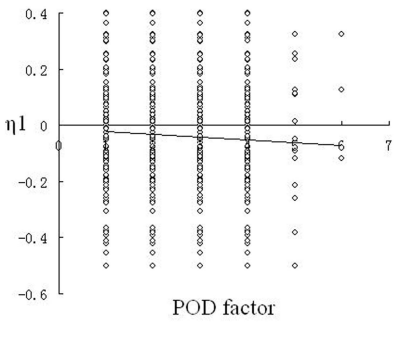

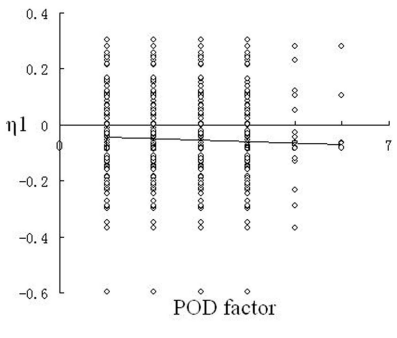

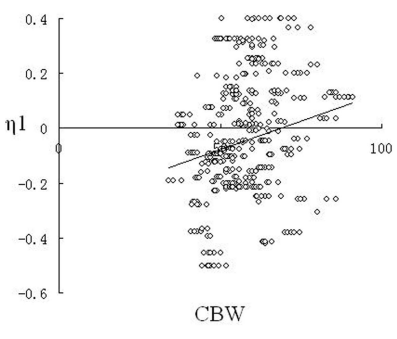

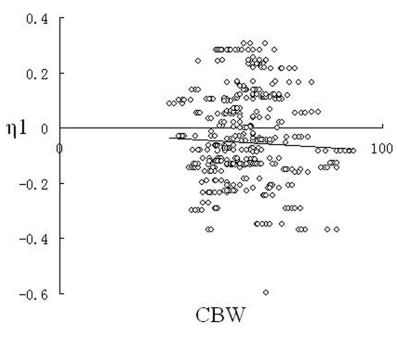

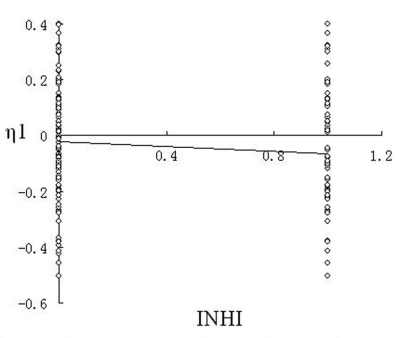

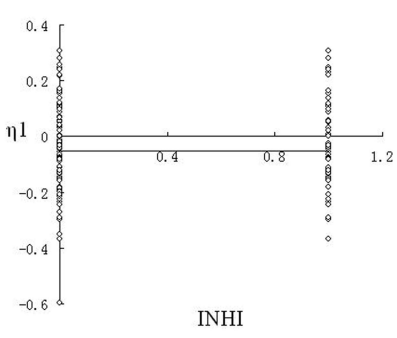

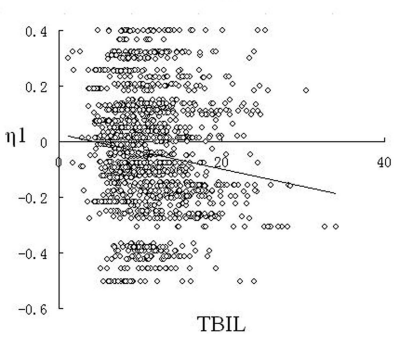

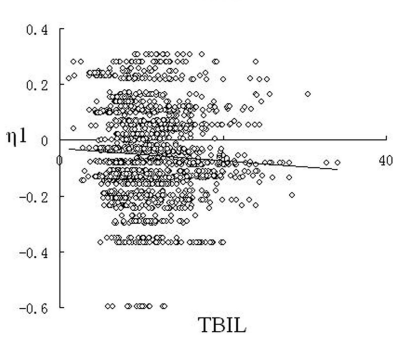

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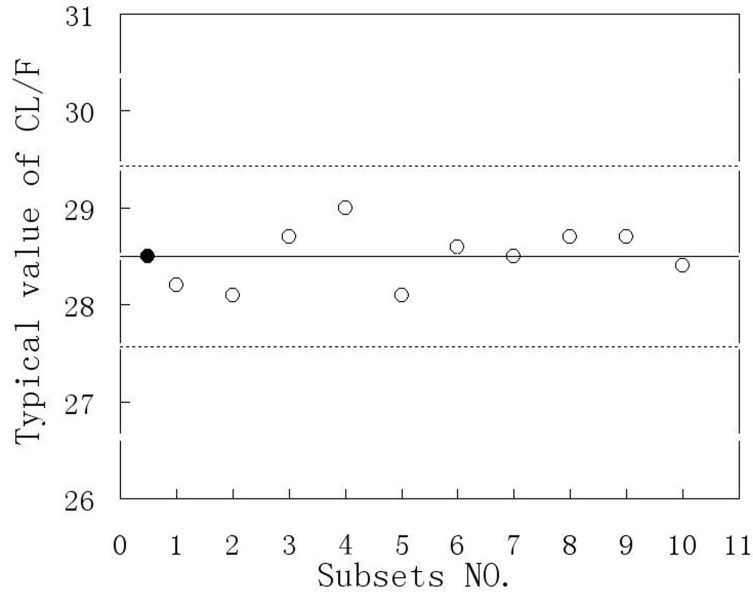
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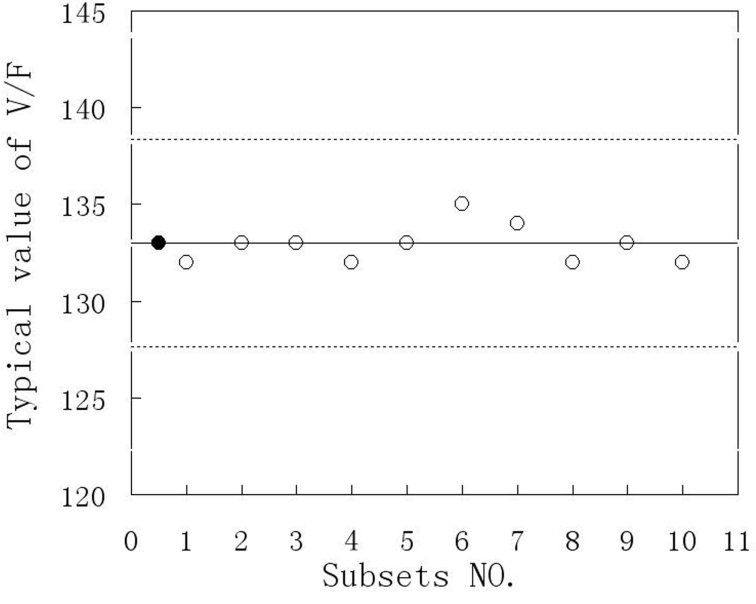
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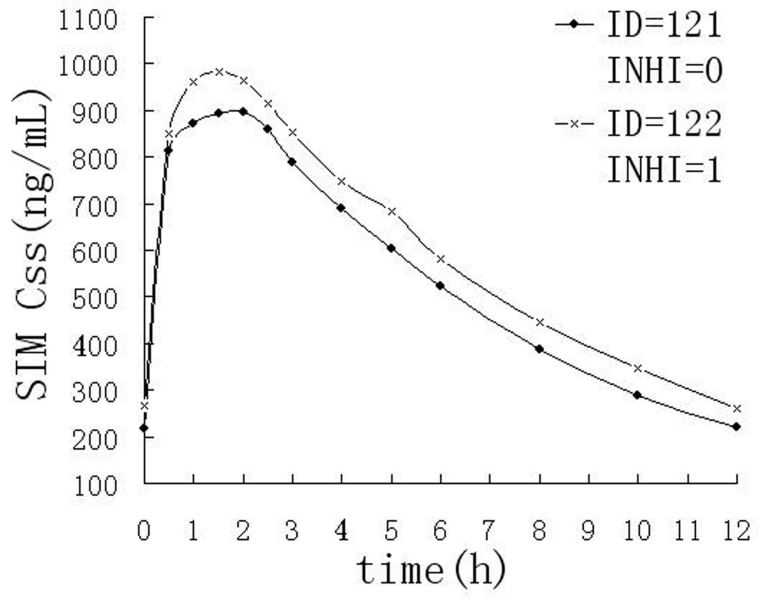
FIG.5.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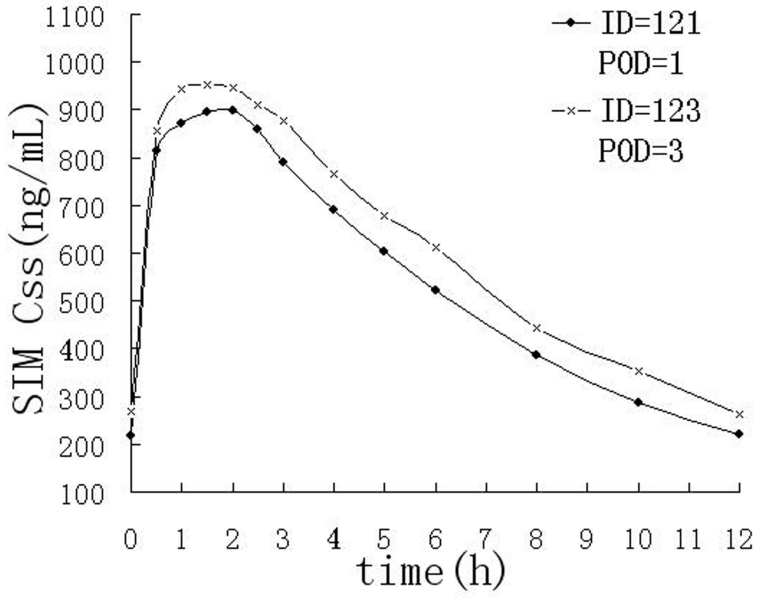
time(h)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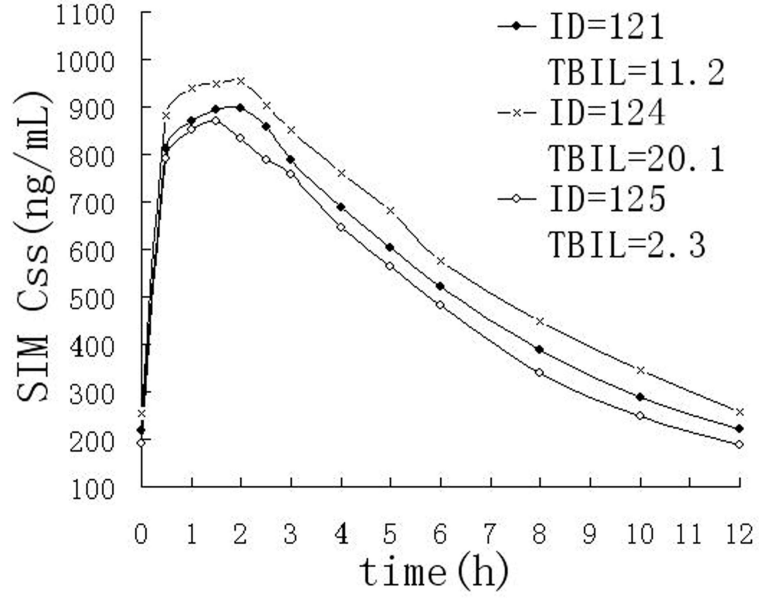
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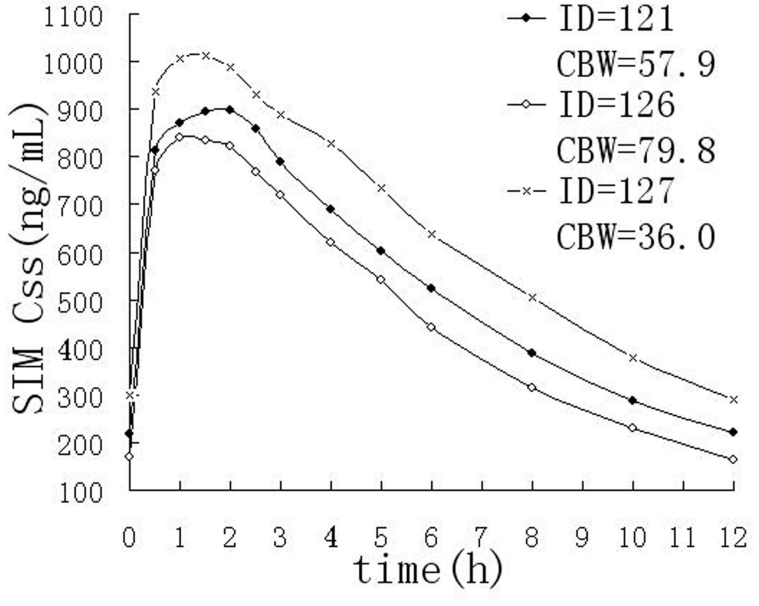
time(h)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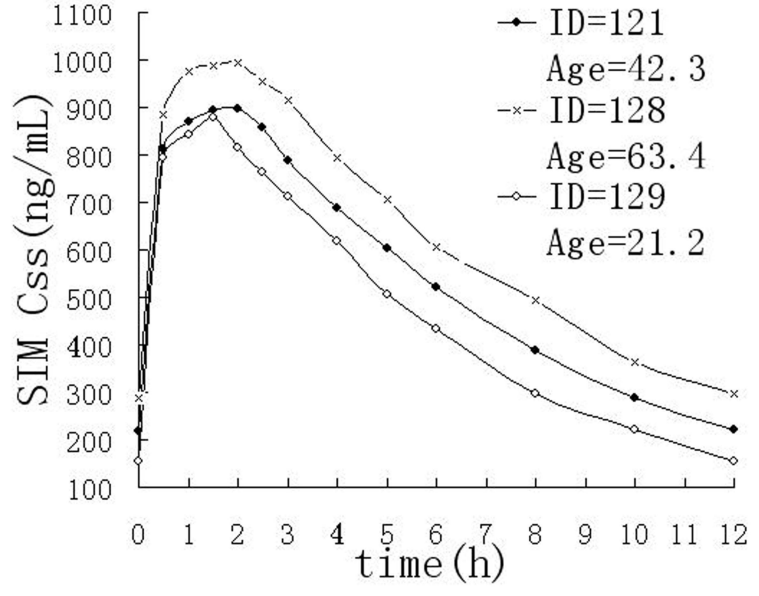
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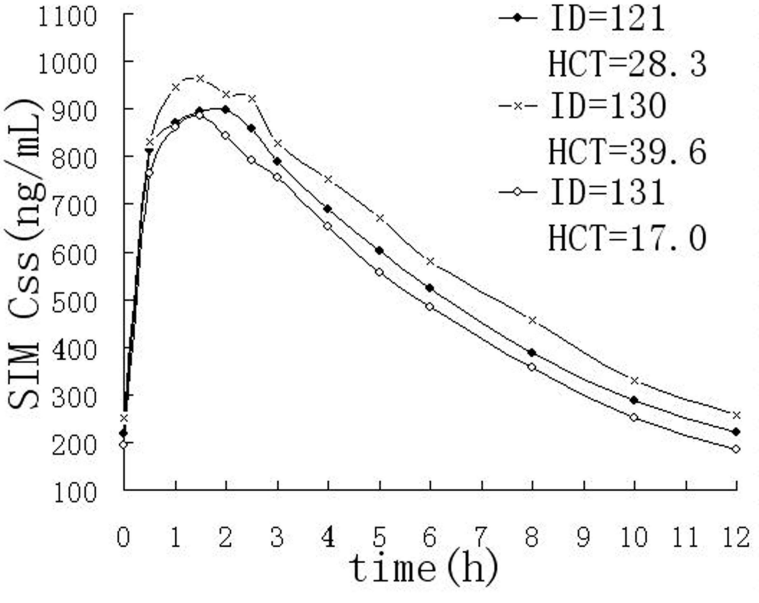
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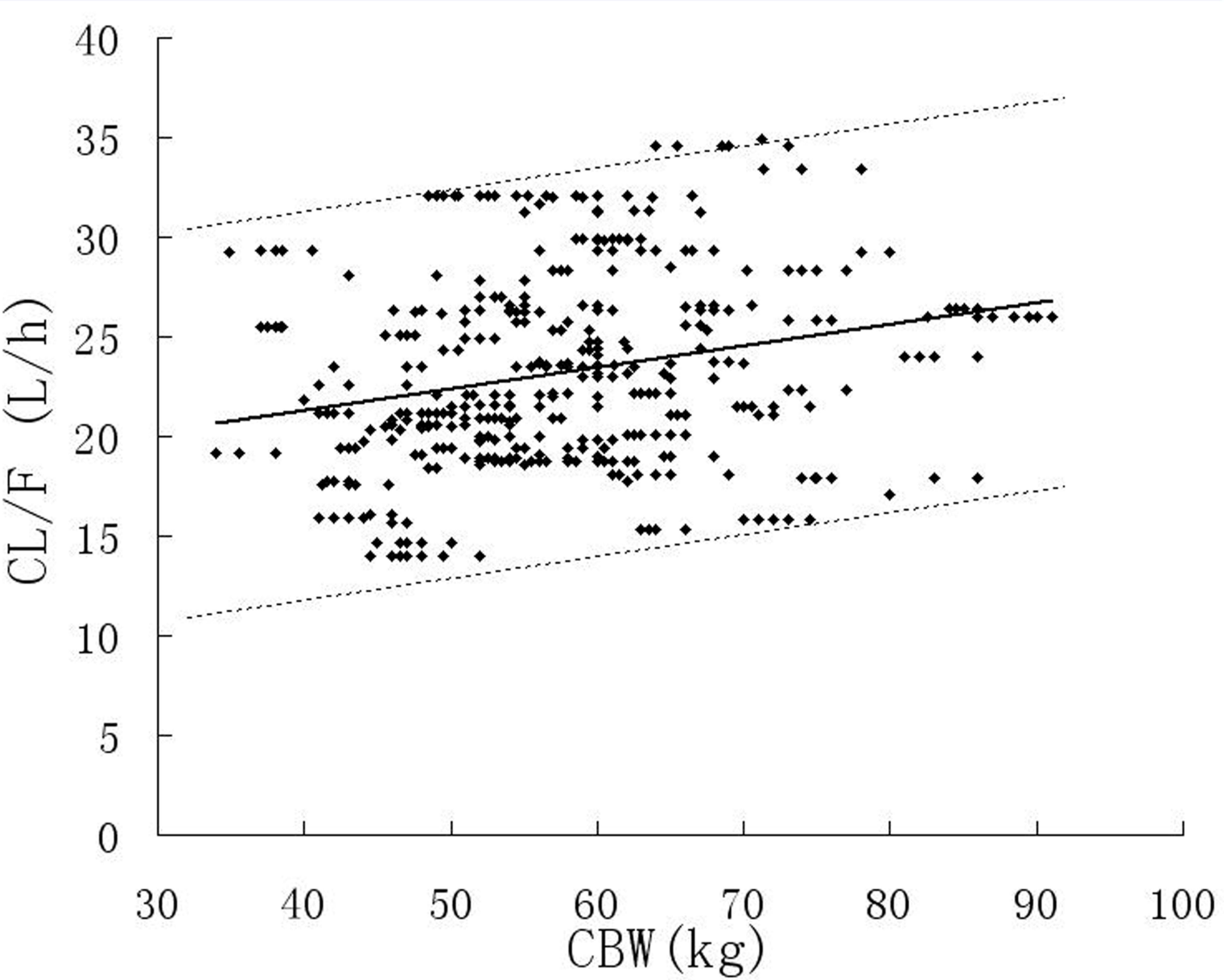
time(h)

FIG.6.



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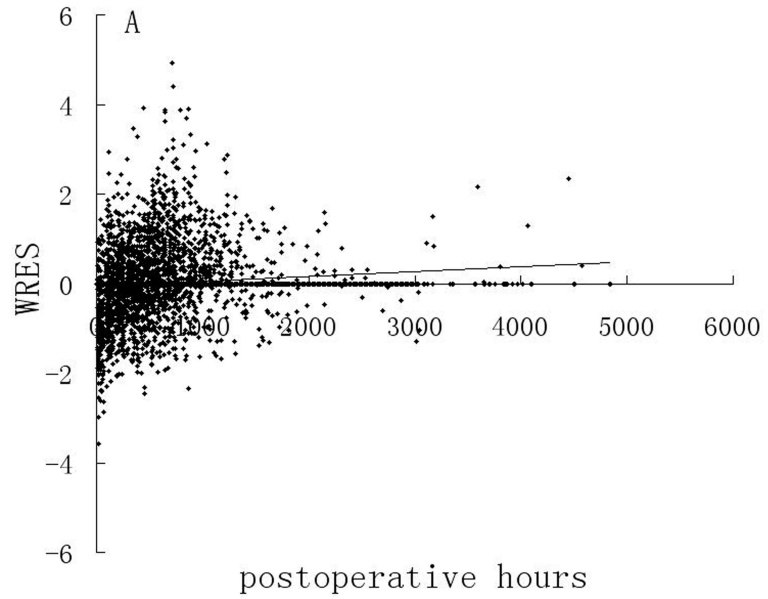
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1000
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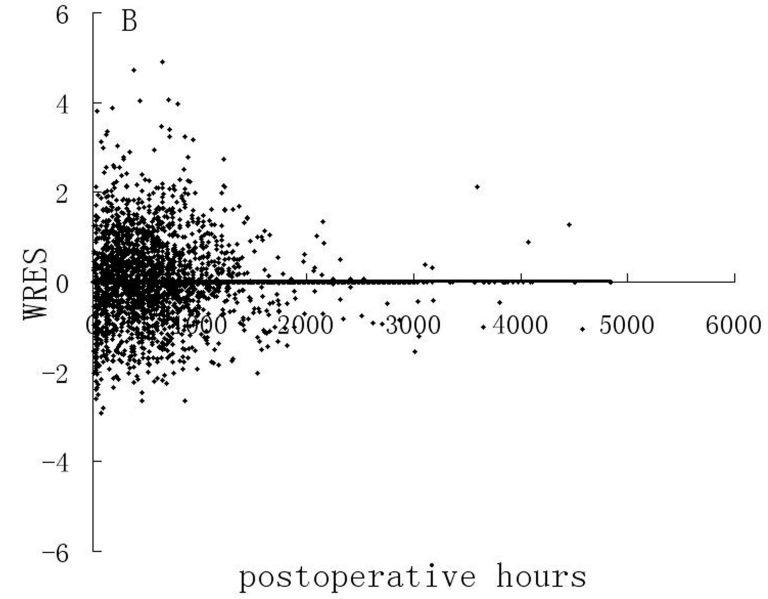
4000
5000
6000
postoperative hours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6000
postoperative hours