

Original Article

# A population pharmacokinetic study of tacrolimus in healthy Chinese volunteers and liver transplant patients

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**Aim:** To develop a population pharmacokinetic (PopPK) model of tacrolimus in healthy Chinese volunteers and liver transplant recipients for investigating the difference between the populations, and for potential individualized medication.

**Methods:** A set of 1100 sparse trough concentration data points from 112 orthotopic liver transplant recipients, as well as 851 dense data points from 40 healthy volunteers receiving a single dose of tacrolimus (2 mg, *po*) were collected. PopPK model of tacrolimus was constructed using the program NONMEM. Related covariates such as age, hepatic and renal functions that were potentially associated with tacrolimus disposition were evaluated. The final model was validated using bootstrapping and a visual predictive check.

**Results:** A two-compartment model of tacrolimus could best describe the data from the two populations. The final model including two covariates, population (liver transplant recipients or volunteers) and serum ALT (alanine aminotransferase) level, was verified and adequately described the pharmacokinetic characteristics of tacrolimus. The estimates of V<sub>2</sub>/F, Q/F and V<sub>3</sub>/F were 22.7 L, 76.3 L/h and 916 L, respectively. The estimated CL/F in the volunteers and liver transplant recipients was 32.8 and 18.4 L/h, respectively. Serum ALT level was inversely related to CL/F, whereas age did not influence CL/F. Thus, the elderly (≥65 years) and adult (<65 years) groups in the liver transplant recipients showed no significant difference in the clearance of tacrolimus.

**Conclusion:** Compared with using the sparse data only, the integrating modeling technique combining sparse data from the patients and dense data from the healthy volunteers improved the PopPK analysis of tacrolimus.

**Keywords:** liver transplantation; healthy volunteer; immunosuppressant; tacrolimus; population pharmacokinetics; serum alanine aminotransferase

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## Introduction

Liver transplant (LT)<sup>[1]</sup> technology was initiated in the 1950s and was primarily performed in the patients with end-stage liver disease caused by a variety of liver diseases, such as hepatocellular carcinoma (HCC), liver cirrhosis, chronic severe hepatitis, biliary atresia (which was common in children), and congenital metabolic diseases. HCC accounts for approximately 40% of the liver transplantation surgeries. The liver transplant is the only effective radical cure for all types of end-stage liver diseases and provides new technology, new ideas and new hope for patients. Meanwhile, immunosuppressants are the main preventive and treatment measures for organ

transplant rejections. The appropriate use of immunosuppressants is directly related to the survival of the liver transplant recipients.

After liver transplantation, a triple immunosuppressive regimen<sup>[2]</sup> centered on tacrolimus is usually adopted. Tacrolimus (Prograf<sup>®</sup>, FK506) is a potent macrocyclic lactone immune inhibitor<sup>[3,4]</sup> that was isolated from the soil by the Fujisawa Pharmaceutical Company in 1984. It is widely used to prevent immune rejection caused by the liver, pancreas, kidneys, heart, lungs and other solid organ transplantations and to treat some autoimmune diseases<sup>[2,5–10]</sup>. Tacrolimus has a clear First pass effect and incomplete oral absorption in the gastrointestinal tract, with an absolute oral bioavailability of 10%–17% in adult kidney transplant patients, 6%–22% in adult liver transplant patients and 5%–18% in healthy subjects<sup>[11,12]</sup>. Its binding rate with protein is approximately 99%, primarily binding with  $\alpha$ 1-acid glycoprotein and albumin, and it has a high affinity

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with red blood cells<sup>[13, 14]</sup>.

Tacrolimus is widely metabolized by a variety of mixed function oxidases<sup>[15, 16]</sup>, primarily the cytochrome P450 3A enzymes (CYP3A4 or CYP3A5) of the liver and intestine. It is biotransformed through demethylation and hydroxylation, with a primary metabolite of 13-demethyl tacrolimus. Tacrolimus is excreted at 30.7%–92.6% and 1.10%–2.30% from the bile and urine, respectively.

The therapeutic window of tacrolimus is relatively narrow and has a significant individual variability<sup>[17, 18]</sup> in its pharmacokinetics and toxicology. Too high of a trough concentration can lead to side effects<sup>[19, 20]</sup>, and too low of a concentration may result in rejection<sup>[21]</sup>. Thus, it is particularly important to develop an individualized immunosuppressive therapy regimen through therapeutic drug monitoring. Although monitoring blood concentrations is an effective way to regulate the immunosuppressant dosages, clinical studies have found large differences in the desired dosages among different individuals to achieve and maintain the same immune inhibitor concentration<sup>[22]</sup>. Currently, immunosuppressive effects are empirically assessed by monitoring the whole blood trough concentration and the indexes of liver and kidney function. Thus, a certain amount of uncertainty exists in using immunosuppressant medications.

In recent years, compared with a large number of reports about the population pharmacokinetics (PopPK) of tacrolimus in other countries<sup>[23–27]</sup>, reports in China are still limited. The published research has focused on liver and kidney transplantation, and the majority of studies were performed in renal transplant patients, motivating us to perform this PopPK study in liver transplant patients. Moreover, PopPK studies of tacrolimus have usually been conducted in healthy subjects and patients of adult or children, but relevant studies of tacrolimus in the elderly have been rare. As a result, it is important to discover the disposition characteristics of tacrolimus in elderly liver transplant patients.

In this study, we retrospectively collected routinely monitored tacrolimus trough blood concentrations from 112 orthotopic liver transplant patients during their early postoperative days, as well as the rich concentration data from 40 healthy volunteers. These data were used to develop a PopPK model and explore the key covariates that affect the PK of tacrolimus. This study may potentially individualize therapy in Chinese liver transplant recipients, particularly for elderly patients. We also developed an integrative modeling strategy by combining the sparse data from patients and the rich data from healthy volunteers. We hypothesized that our method may facilitate a better understanding of the pharmacokinetic profiles of tacrolimus and other medicines for which only sparse data are available from patients.

## Materials and methods

One hundred and twelve liver transplant patients receiving allograft orthotopic liver transplantation for the first time were enrolled from March 2004 to August 2012 in the Organ Transplant Center of the General Hospital of the Armed Police

Forces. Forty healthy volunteers were from the PLA Second Artillery General Hospital. All subjects including liver transplant recipients and healthy volunteers provided informed consent, and all treatment was performed in accordance with the ethical standards of the Declaration of Helsinki and approved by the Hospital Ethics Committee.

The inclusion criteria for liver transplant patients were as follows: 1) no major existing primary diseases in preoperative days and a good recovery in postoperative period; 2) no serious complications during the surgery; 3) did not take any food or medicine, which may potentially affect the pharmacokinetics of tacrolimus; and 4) survived more than one year. The exclusion criteria for liver transplant patients included subjects with a rejection reaction, a primary graft failure or severe complications, such as an infection or even death after transplantation. All of the healthy volunteers in this study were male, and all of their examinations were normal.

The individual information for each subject was carefully recorded, including gender, age, body weight (BW), white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TBIL). If the subjects were healthy volunteers, the value of the SubPop was 0; otherwise, the value was 1.

## Drugs and software

Tacrolimus (Prograf<sup>®</sup>, FK506) capsules 0.5 mg or 1 mg (Astellas Pharma China, Inc), mycophenolate mofetil (MMF) dispersible tablets 0.25 g, (Hangzhou ZhongMeiHua Co, China), methylprednisolone injection 40 mg/bottle or 500 mg/bottle (Pfizer), and methylprednisolone tablet 4 mg (Tianjin TianYao Pharmaceuticals) were used. NONMEM Software: Version 7.2 (ICON, MD, USA), Perl-speaks-NONMEM (PsN, version 3.4.2) and R 2.15.3 (R-project.org) were used.

## Dosage regimen

All liver transplant recipients were given 500–1000 mg of a methylprednisolone injection during surgery. Patients initiated a triple immunosuppressive regimen of FK506, MMF and corticosteroids on the day following the surgery. The drugs were initially administered by nasal feeding and were gradually replaced by oral administration. The initial dose of tacrolimus was 0.05 mg·kg<sup>-1</sup>·d<sup>-1</sup> (twice daily). The dose was adjusted when necessary based on the results of therapeutic drug monitoring (TDM), biochemical parameters and physical conditions. The MMF dispersible tablets were administered as 750 mg bid after meals from the following day after surgery. A methylprednisolone injection was given simultaneously at a dose of 80 mg and was gradually decreased to 20 mg/d at a rate of 20 mg/d. Finally, oral methylprednisolone tablets were given at 16 mg/d to replace the injections and were gradually decreased to 4 mg/d at a rate of 4 mg/d. All healthy volunteers were given a single dose of tacrolimus (2 mg orally) in a randomized, two-formulation, double-cycle, cross-over design experiment.

### Blood sample collecting and analysis

A peripheral venous blood sample (1–2 mL) was collected from the liver transplant recipients before taking tacrolimus on the morning of their third day after surgery. The samples were measured using a monoclonal antibody-based microparticle enzyme immunoassay (MEIA). The calibration curve was linear over a range of 1.5–30 µg/L, and the accuracy and precision met the requirements. For healthy volunteers, whole blood samples were intensively drawn at 0 h prior to drug administration and 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24, 48, and 72 h after drug administration. The concentrations of tacrolimus in these samples were determined using HPLC-MS. The assay was assessed to be linear over the range of 0.1–25 µg/L, and the accuracy and precision met the requirements<sup>[28]</sup>.

### Population pharmacokinetic model development

First-order conditional estimation with interaction (FOCEI) estimation<sup>[29–31]</sup> of the NONMEM (ADVAN4 module) was used to develop the model. Both a one- and two-compartment model with first-order absorption were used to describe our data. The random effects of the population pharmacokinetic study were composed of inter-individual variability and residual random effects. Random effects between individuals are expressed exponentially or additively, whereas residual random effects refer to the difference between the observed values and the predicted ones caused by unknown factors and are expressed additively, proportionally, or by a combination of the two. The volume of distribution in the peripheral compartment ( $V_3/F$ ) was fixed as the estimate from the healthy Chinese volunteer group.

Covariates were investigated based on diagnostic plots and the value of the objective function (OFV). When introducing a covariate into the model, if the change in the OFV ( $\Delta$ OFV) was greater than 3.84, the factor was indicated to significantly improve the model ( $P < 0.05$ ) and thus be included. All of the covariates were introduced individually to examine their effects on the model. At the end, all of the factors that significantly impacted the model were retained, and the full model was established.

The effect of the covariates in the full model was further evaluated using more stringent criteria ( $\Delta$ OFV > 7.88,  $P < 0.005$ ). The covariates were excluded from the full regression model one by one to confirm their significance in the model. An increase of more than 7.88 in OFV indicated that the factor had a significant effect on the model and should thus be retained in the model; otherwise, the factor should be excluded from the model. The final model was eventually developed after excluding the unnecessary covariates.

In this study, the continuous covariates were included by using Eqs 1 or 2:

$$P_i = \theta_{TV} \times [1 + \theta_{cov} \times (\text{Covariate}_i - \text{Covariate}_{\text{median}})] \times e^{\eta_i} \quad (1)$$

$$P_i = \theta \times \left[ \text{EXP} \left( \frac{\text{Covariate}_i}{\text{Covariate}_{\text{median}}} \times \theta_{cov} \right) \right] \times e^{\eta_i} \quad (2)$$

where  $i$ ,  $P_i$ ,  $\theta_{TV}$  [or  $\theta^* \text{EXP}(\theta_{cov})$ ] and  $\theta_{cov}$  were the individual's ID, individual parameter, typical value of the correspond-

ing parameter and the correlation coefficient for the relevant covariate, respectively.

For the categorical covariates, such as gender and SubPop, they were evaluated using Eq 3:

$$P_i = (\theta + \theta_{cov} \times \text{Covariate}_i) \times e^{\eta_i} \quad (3)$$

where  $\theta$  was the estimate of the parameter for the reference group.  $\theta_{cov}$  describes the effect contributed by the other group.

Eventually, the robustness, reliability and prediction performance of the final model were evaluated by bootstrapping and a visual predictive check (VPC). Through the bootstrap analysis, resampling was performed 1000 times. The median and 95% confidence interval of the parameters were obtained and compared with the estimates of the final model from the NONMEM. For the VPC, the concentration *vs* the time curve of tacrolimus was simulated 1000 times using the final model. Consequently, the median and 90th percentile interval of the simulation were graphically compared with the observed values to evaluate the model's predictive performance.

### Results

A total of 1100 routinely monitored tacrolimus whole blood trough concentration data were retrospectively collected from 112 liver transplant patients, in which 36 patients were older than 65 years. A total of 851 tacrolimus whole blood concentration data were collected from 40 healthy Chinese male volunteers in a bioequivalent study. The demographic and biological characteristics of the two populations in this study are summarized in Table 1.

A two-compartment model with first-order absorption described our data better. The inter-individual variability was evaluated exponentially, and the residual error was estimated proportionally. The parameter estimates obtained using NONMEM are shown in Table 2.

The regression equations of the population pharmacokinetic base model of tacrolimus follow below.

$$CL/F = \theta 1 \times e^{\eta 1} \quad (4)$$

$$V_2/F = \theta 2 \times e^{\eta 2} \quad (5)$$

$$Q/F = \theta 3 \times e^{\eta 3} \quad (6)$$

$$V_3/F = \theta 4 \times e^{\eta 4} \quad (7)$$

$$K\alpha = \theta 5 \times e^{\eta 5} \quad (8)$$

$$ALAG1 = \theta 6 \quad (9)$$

All of the covariates listed in Table 1 were investigated in this study. The results of stepwise forward addition and backward elimination are shown in Table 3. The SubPop and ALT were found to significantly improve the fitting of our model and were included. The effect of other covariates was also evaluated on the base model, including age, gender, BW, HGB, HCT, and TBIL on the parameter of apparent clearance (CL/F), but no significant improvement was observed. The estimates of the final model are listed in Table 2, and the basic goodness-of-fit plots are shown in Figure 1. The typical values of the volume of distribution in the central compartment ( $V_2/F$ ), the inter-compartmental clearance (Q/F), the volume of distribution in peripheral compartment ( $V_3/F$ ), the constant

**Table 1.** The demographic and laboratory index of the liver transplant patients and healthy Chinese volunteers. Mean±SD (range).

Characteristics	Liver transplant patient group	Healthy Chinese volunteer group
<b>Demographic</b>		
Male	86	40
Female	26	-
Age, year	58.4±11.6 (28-78)	28.7±3.47 (24-37)
BW, kg	69.0±11.8 (44-97)	62.5±6.46 (52-76)
<b>Laboratory</b>		
POD, day	19.38±17.75 (2-137)	-
WBC, 10 <sup>9</sup> /L	7.72±4.19 (1.06-28.93)	6.24±1.19 (3.54-11.19)
HGB, g/L	97.5±16.0 (53-159)	150.3±9.45 (126-170)
HCT, %	29.1±4.77 (15.4-46.4)	42.3±2.58 (34.9-48.6)
PLT, 10 <sup>9</sup> /L	120.9±85.3 (6-713)	215.3±45.4 (92-311)
ALT, IU/L	146.4±290.0 (5-6300)	33.0±20.9 (8.5-125.3)
AST, IU/L	70.9±232.6 (7-6580)	31.5±11.8 (12.7-91.7)
TBIL, μmol/L	50.6±53.2 (5.5-503.1)	12.5±7.09 (4.88-58.05)
N <sub>0</sub> of blood samples	1100	851

BW, body weight; POD, post-operation day; WBC, white blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin.

**Table 2.** Population pharmacokinetic parameter estimates and bootstrap results.

Parameters	Base model		Final model		Bootstrap	
	Estimate	RSE (%)	Estimate	RSE (%)	Median	95% CI
OFV	-755	-	-839	-	-859	-1111- -605
CL/F (L/h)	20.5	5.00	32.8	9.30	32.8	27.1-39.5
V <sub>2</sub> /F (L)	21.8	18.1	22.7	17.4	23.2	15.1-31.2
Q/F (L/h)	81.8	9.50	76.3	8.50	76.6	64.4-90.4
V <sub>3</sub> /F (L)	916*	-	916 *	-	916*	-
K <sub>a</sub> (h <sup>-1</sup> )	0.404	6.50	0.419	5.40	0.420	0.375-0.464
ALAG1 (h)	0.405	4.40	0.404	4.40	0.403	0.366-0.440
CL_SubPop	-	-	0.562	9.60	0.563	0.466-0.694
CL_ALT	-	-	-0.0237	27.6	-0.0241	-0.0405- -0.0137
<b>Inter-individual variability (% coefficient of variation)</b>						
CL-IIV (%)	53.8	8.00	46.6	7.60	46.1	39.8-53.8
V <sub>2</sub> -IIV (%)	57.5	19.0	57.3	18.9	55.4	30.2-76.3
Q-IIV (%)	50.3	13.4	46.0	12.5	45.1	33.1-56.3
V <sub>3</sub> -IIV (%)	100	8.80	93.5	9.00	93.2	78.1-111
K <sub>a</sub> -IIV (%)	0*	-	0*	-	0*	-
<b>Residual error</b>						
Proportional (%)	40.4	3.60	39.8	3.60	39.7	36.8-42.5

\*: FIX.

of absorption ( $K_a$ ) and the time lag (ALAG1) derived from the final model were 22.7 L, 76.3 L/h, 916 L, 0.419 h<sup>-1</sup> and 0.404 h, respectively. Unsurprisingly, the estimate of CL/F was different between the groups, with values of 32.8 L/h (healthy volunteers) and 18.4 L/h (patients). ALT was inversely related to CL/F in the patient group. In Figure 1, the plots of observa-

tions *vs* population predictions and observations *vs* individual predictions show a symmetric distribution around the identity line. The conditional weighted residuals (CWRES) *vs* the predictions plot suggested an appropriate error model. The CWRES distributed evenly around the zero line.

The regression equations of the final model were obtained

**Table 3.** Forward inclusion and backward elimination process.

Model No	Model description	OFV	ΔOFV	Significant
1	Base model	-755.12		
Forward Inclusion				
2	Add ALT on CL in model 1	-804.62	-49.50	YES
2	Add HCT on CL in model 1	-755.58	-0.46	NO
2	Add HGB on CL in model 1	-758.07	-2.95	NO
2	Add GEND on CL in model 1	-755.89	-0.77	NO
3	Add BW on CL in model 2	-808.10	-3.48	NO
3	Add SubPop on CL in model 2	-839.47	-34.85	YES
4	Add TBIL on CL in model 3	-842.73	-3.26	NO
4	Add AGE on CL in model 3	-839.56	-0.09	NO
Backward elimination				
5	Remove ALT on CL from model 3	-798.77	40.70	YES
6	Remove SubPop on CL from model 3	-804.62	34.85	YES

$$V_2/F=\theta_2\times e^{\eta_2} \quad (11)$$

$$Q/F=\theta_3\times e^{\eta_3} \quad (12)$$

$$V_3/F=\theta_4\times e^{\eta_4} \quad (13)$$

$$K_a=\theta_5\times e^{\eta_5} \quad (14)$$

$$ALAG1=\theta_6 \quad (15)$$

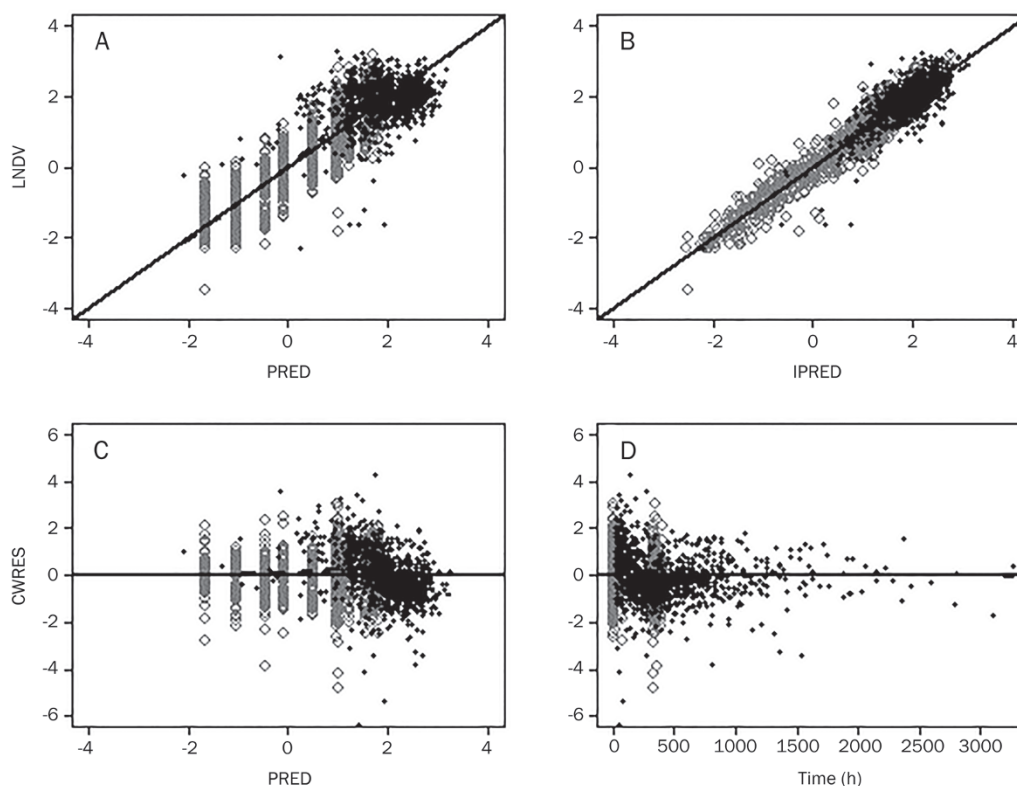
The robustness of the final model was validated using a bootstrap method. The percentage of successful minimizations and covariance was 81.9%. The median and 95% CI of the parameters from the bootstrap analysis were in good agreement with the estimates from the final model obtained using NONMEM (Table 2). The graphical results of VPC are presented in Figure 2, which shows the predictive property of the model was good with most of the observation points falling in the 90% prediction interval. Because there were limited observations after approximately 1500 h, the VPC plot of the liver transplant patients was cut off at 1500 h.

### Discussion

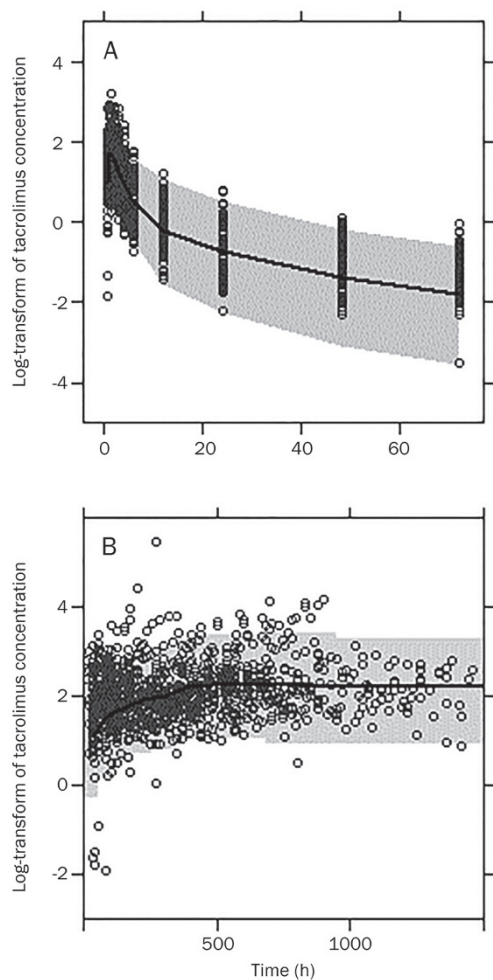
China has one of the highest incidences of end-stage liver disease across countries, and new cases of hepatic cancers account for approximately 55% of all cases worldwide. Many people died of various types of liver disease such as liver cirrhosis and hepatic cancer. Male accounted for 76.79%, and

after backward elimination as follows:

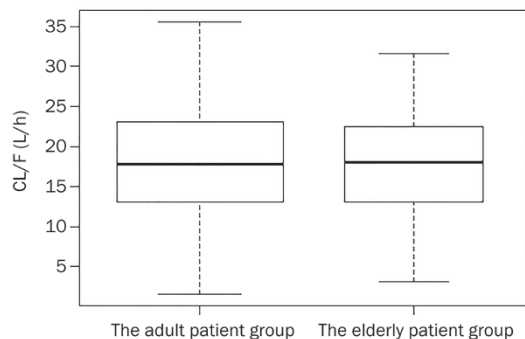
$$CL/F=\theta_1\times\theta_7^{\text{SubPop}}\times\left[\text{Exp}\left(\frac{ALT}{40}\times\theta_8\right)\right]^{\text{SubPop}}\times e^{\eta_i} \quad (10)$$



**Figure 1.** Basic goodness-of-fit plots of the final model. The grey circles represent observations from healthy volunteers and the black dots represent observations from liver transplant subjects. (A) The plot of observations (LNDV, natural log of data) vs population predictions. (B) The plot of observations (LNDV) vs individual predictions. (C) The plot of conditional weighted residuals (CWRES) vs predictions. (D) The plots of conditional weighted residuals (CWRES) vs time. The solid lines in A and B are identity lines. The solid lines in C and D are zero lines. The dashed lines represent regression lines by using locally-weighted polynomial regression. DV, PRED, and IPRED values had been log-transformed.



**Figure 2.** Visual predictive check (VPC) for the final model. Circles are the observations. The solid line represents the medians of the simulation values, the shaded area represents the 90% predictive interval. (A) The VPC plot for the healthy Chinese volunteers; (B) The VPC plot for the liver transplant patients (time <1500 h).



**Figure 3.** The comparison between the adult (<65 yr) and elderly ( $\geq 65$  yr) patient groups in apparent clearance (CL/F).

female accounted for 23.21% of the 112 cases of orthotopic liver transplant patients in this study. Primary hepatic can-

cer, liver cirrhosis and chronic severe hepatitis, hepatic cancer recurrence after resection or interventional treatment, and alcoholic cirrhosis in this study accounted for 50.9%, 30.6%, 6.48%, 4.63%, and 2.70% of the diseases in this study, respectively, whereas the other liver diseases accounted for less than 5%. Primary hepatic cancer accounted for half of these cases, and liver transplantation is the only effective method to treat hepatic cancer and other end-stage liver diseases. Thus, early diagnosis and early treatment for hepatic cancer are particularly important.

Tacrolimus is metabolized in the liver and primarily excreted by bile. Renal tacrolimus clearance only accounted for 1.1%–2.3%. Therefore, the status of the patient's liver and biliary tract plays a very important role on its pharmacokinetics. Of the seven biochemical factors in the patient group (Table 1), only WBC and PLT remained in the normal range within the early postoperative period, whereas HGB and HCT were lower and the rest of the values were higher than normal. In previous studies, covariates including HCT, ALT (or AST) were added to the final model<sup>[32–34]</sup>. In this study, ALT was added to the model and showed a significant influence on the elimination of tacrolimus for the liver transplant patient group. No influence of ALT on CL/F was included in the final model for the healthy volunteer group because no evident relationship between them was observed based on graphic evaluation. HCT was also evaluated in the model, but it was not significantly influential. Moreover, POD was excluded from our model because of its high correlation with the hepatic biochemical parameters (ALT in our model).

Most of the previously reported population pharmacokinetic models of tacrolimus for the transplant patients were one-compartment models<sup>[26, 35–37]</sup>. Limited by the sparse data from this population, the reliability, prediction performance and robustness in these models could hardly be expected to be high. Tacrolimus is a fat-soluble drug and can easily distribute to the peripheral compartment. Therefore, a two-compartment model (as demonstrated in this study) was more reasonable to describe tacrolimus pharmacokinetic behavior. The volume of distribution in the peripheral compartment for tacrolimus between the healthy volunteers and the liver transplant patients can be reasonably assumed to be similar based on previous publications<sup>[38–40]</sup>. A two-compartment model of tacrolimus was developed by combining the dense data from the healthy volunteers and the sparse data from the liver transplant patients, which allowed us to capture more information on pharmacokinetic behaviors, especially in the patients.

Nearly one-third of the subjects from this liver transplant population were over 65 years old. As a covariate, age showed a non-significant effect on CL/F in the liver transplant patient population. CL/F from the elderly ( $\geq 65$  years) and adult patient (<65 years) groups in the final model are compared in Figure 3. No significant difference exists between the elderly and adult groups for tacrolimus pharmacokinetics among liver transplant patients. Compared with other studies, the model developed may be more representative, relatively stable and suitable for a wider population range of liver transplant

patients.

In conclusion, we developed a PopPK model of tacrolimus, in which we retrospectively collected trough concentrations from 112 orthotopic liver transplantation patients and rich concentration data from 40 healthy volunteers. The pharmacokinetics of tacrolimus between the healthy subjects and the liver transplant patients were revealed to be different in CL/F, whereas the pharmacokinetics between the elderly and adult patient groups was shown to be similar within the patient population. More importantly, compared with using the sparse data only, the integrating modeling technique combining sparse data from the patients and rich data from the healthy volunteers improved the PopPK analysis of tacrolimus. This method could be potentially expanded to other similar medications that could only be sparsely monitored in the clinic.

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### Author contribution

Wei LU, Yan-xia LU, and Qing-hong SU designed the research and wrote the paper; Qing-hong SU, Ke-hua WU, and Yu-peng REN analyzed data and plotted figures; Liang LI and Tian-yan ZHOU provided technical assistance.

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