Acta Pharmacologica Sinica (2017) 38: 434–442
 1671-4083/17
www.nature.com/aps

Original Article

Development of a novel individualized warfarin dose algorithm based on a population pharmacokinetic model with improved prediction accuracy for Chinese patients after heart valve replacement

Yu-bin ZHU[1, #], Xian-hua HONG[1, 2, #], Meng WEI[3], Jing HU[1, 2], Xin CHEN[4], Shu-kui WANG[5], Jun-rong ZHU[1, *], Feng YU[2, *], Jian-guo SUN[6, *]

[1]Department of Pharmacy, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China; [2]School of Clinical Pharmacy, China Pharmaceutical University, Nanjing 210002, China; [3]Department of Pharmacy, Jinling Hospital Affiliated to Medical School of Nanjing University, Nanjing 210000, China; [4]Department of Cardiovascular Surgery, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China; [5]Department of Laboratory Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China; [6]Key Laboratory of Drug Metabolism & Pharmacokinetics, China Pharmaceutical University, Nanjing 210002, China

Abstract

The gene-guided dosing strategy of warfarin generally leads to over-dose in patients at doses lower than 2 mg/kg, and only 50% of individual variability in daily stable doses can be explained. In this study, we developed a novel population pharmacokinetic (PK) model based on a warfarin dose algorithm for Han Chinese patients with valve replacement for improving the dose prediction accuracy, especially in patients with low doses. The individual pharmacokinetic (PK) parameter - apparent clearance of *S*- and *R*-warfarin (CLs) was obtained after establishing and validating the population PK model from 296 recruited patients with valve replacement. Then, the individual estimation of CLs, VKORC1 genotypes, the steady-state international normalized ratio (INR) values and age were used to describe the maintenance doses by multiple linear regression for 144 steady-state patients. The newly established dosing algorithm was then validated in an independent group of 42 patients and was compared with other dosing algorithms for the accuracy and precision of prediction. The final regression model developed was as follows: Dose=-0.023×AGE+1.834×VKORC1+0.952×INR+2.156×CLs (the target INR value ranges from 1.8 to 2.5). The validation of the algorithm in another group of 42 patients showed that the individual variation rate (71.6%) was higher than in the gene-guided dosing models. The over-estimation rate in patients with low doses (<2 mg/kg) was lower than the other dosing methods. This novel dosing algorithm based on a population PK model improves the predictive performance of the maintenance dose of warfarin, especially for low dose (<2 mg/d) patients.

Keywords: Warfarin; population pharmacokinetic; dosing algorithm; Chinese patients; heart valve replacement

Acta Pharmacologica Sinica (2017) 38: 434–442; doi: 10.1038/aps.2016.163; published online 20 Feb 2017

Introduction

Warfarin is still regarded as the cornerstone of the anticoagulation regimen used for patients after valve replacement and with non-valvular atrial fibrillation. However, its optimal effectiveness and safety is limited by significant individual variability in dose response and the narrow therapeutic index, especially for patients who are warfarin sensitive (≤1.5 mg/d)[1]. Even with frequent INR monitoring, the percentage of patients within the therapeutic range of warfarin is generally <60% in usual care settings[2]. Therefore, an individual dosage adjustment of warfarin is necessary.

For many of the newly developed dose prediction models of warfarin, only approximately 40%–60% of individual variability of daily stable doses can be accounted for by basic demographic and genetic information[3–7]. Additionally, the models of Ohno, Wen and Huang *et al* tend to overestimate the low dose of warfarin (≤3 mg/d), and the overestimated rates [(estimate dose-actual dose)/actual dose×100%] are 57%, 48%, and

# These authors contributed equally to this work.
*To whom correspondence should be addressed.
E-mail junrong_zhu@aliyun.com (Jun-rong ZHU);
yufengcpu@163.com (Feng YU);
jgsun@cpu.edu.cn (Jian-guo SUN)
Received 2016-06-27 Accepted 2016-11-15

41%, respectively[4–6].

Recently, the study of population pharmacokinetic/pharmacodynamics (PPK/PPD) has offered a new opportunity for individualized treatment with warfarin. By applying a PPK/PPD model, the algorithm established by Sasaki *et al*[8] based on CYP2C9, VKORC1 and clinical factors is more effective than other pharmacogenomics (PG) algorithms in predicting the stable dose of warfarin in 49 Japanese patients, with a small prediction bias (mean prediction error, ME=0.01 mg) and a high degree of precision (root mean squared error, RMSE=0.44 mg), giving a strong correlation between actual and predicted maintenance doses (r^2=0.944). In Anna-Karin Hamberg's study, an adult PK/PD model for warfarin was adjusted for children using allometric scaling methods and increased the prediction proportion of children within ±20% of the actual dose to 70%[9].

In our earlier research, a warfarin dose algorithm based on pharmacogenetics and clinical factors has been obtained, which can explain 51.7% of the dose variability of warfarin and can predict doses ranging from 1.875 mg to 3.125 mg per day accurately[10]. However, Chinese patients are more sensitive to warfarin anticoagulation and are routinely administered low-intensity warfarin anticoagulation (target INR lower than 2 to 3). Therefore, in this study, we aimed to develop a population PK model-based dose algorithm to explain the dose variability of warfarin, especially for patients with low doses. Because S-warfarin is 3- to 5-fold more potent than *R*-warfarin[11], the plasma concentration of *S*- and *R*-warfarin in patients was studied separately to better illustrate the dose algorithm. Based on this model, the individual PK (apparent clearance of S-warfarin, CLs) parameters can be estimated. The maintenance dose of warfarin was described by multiple linear regression using the population PK parameters of S-warfarin, CLs, the values of INR and other clinical and genetic factors.

Materials and methods

Patient information and treatment

From December 2013 to August 2014, 338 Han Chinese patients who required long-term treatment with warfarin after valve replacement, with a target INR between 1.8 and 2.5, were recruited randomly from the Cardiovascular Clinic of the Nanjing First Hospital, Nanjing Medical University, China. For the 338 patients, 296 were used for establishing (cohort 1, *n*=242) and externally validating (cohort 2, *n*=54) the population PK model. The basic information of these 296 patients is summarized in Table 1. The patients were excluded if they had[10]: intracranial hemorrhage, hemorrhage of the digestive tract and urogenital system in the past 6 months, severe liver and kidney diseases, long-term use of non-steroidal anti-inflammatory drugs, antiplatelet and anticoagulant drugs except for warfarin, and thrombolytic drugs, pregnancy and malignant tumors, definite or suspected blood system diseases (except for mild, moderate anemia), platelet deficiency (platelet $<100\times10^9$/L), and known non-compliance with warfarin therapy or prescribed INR monitoring. The relevant information of these patients was available.

Two to three days after the operation, warfarin was administered at 8:00 PM every day. The dosing regimen was adjusted according to changes of the INR values. The follow-up visits of the patients lasted 6–12 months after discharge, and the steady state INR and maintenance doses were recorded.

The study was approved by the Ethics Committee of Nanjing Medical University. All of the volunteers signed a written informed consent form according to the principles of the Declaration of Helsinki.

The warfarin plasma concentration measurement and genotyping

The warfarin sampling time was 10 h after the previous administration, and the sampling interval varied from 24 h to 264 h. At least 1 to 3 samples were collected from each of the 296 patients from the total of 338 patients in this manner within 14 d after the heart valve replacement operation. The concentrations of *R*-warfarin and *S*-warfarin were then measured by high-performance liquid chromatography (HPLC). Separation was performed on the Chiralomix SA analytical column (4.6 mm×250 mm, 5 μm, Sepax Technologies, Inc, Delaware, USA) with the column temperature at 20 °C, and the mobile phase consisted of 20% ethanol:80% hexane with a flow rate of 1.0 mL/min. The detection wavelength was set at 308 nm. The detection range of the *R*- and *S*-warfarin concentration was 50–2000 ng/mL.

The sequencing of the fluorescence of the ligase detection reaction (LDR) that was applied for the genotyping of CYP2C9 and VKORC1-1639 of the 338 patients was performed on an ABI PRISM 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). All SNPs were assessed to ensure that they were in Hardy-Weinberg equilibrium. More details were provided in our previous study[10].

The Pharmacokinetic modeling

For 242 of the 296 patients, a total of 508 *R*-warfarin and 506 *S*-warfarin plasma concentrations were obtained and used for developing the population PK models. A one-compartment model with first-order absorption and first-order elimination was used for the PK modeling of *R*- and *S*-warfarin using NONMEN software (version 6.0)[12–17]. S-PLUS 6.1 (Insightful Corporation, Seattle, WA, USA) was used for model evaluation by the bootstrap method. The clearance (CL) and apparent distribution volume (V) were parameterized (in Equations 1–3)[11].

$$CL=\theta_{CL}\times\exp(\eta_{CL}) \quad (1)$$

$$V=\theta_{V}\times\exp(\eta_{V}) \quad (2)$$

$$Y=F\times\exp(EPS(1)) \quad (3)$$

where Y was the observed plasma concentration, θ_{CL} and θ_V were the population means for CL and V, respectively, F was the population-based predicted plasma concentration, η_{CL} and η_V were the inter-individual variability of CL and V, respectively, and EPS (1) was the residual error variability of Y.

The pharmacokinetic parameters CL and V and their inter-individual variation-η (η_{CL}, η_V) of the 242 patients were then calculated by the base PK models. The relationship between

Table 1. Baseline characteristics of the study population.

	Number or mean (±standard deviation) Cohort 1	Cohort 2	*P* value
Number of patients	242	54	
Female, *n* (%)	140 (57.9)	20 (37.0)	0.014*
Age (year)	55.06±11.73	56.09±13.40	0.607
Weight (kg)	62.93±11.22	66.81±9.00	0.026*
BSA (m^2)[a]	1.65±0.18	1.72±0.14	0.006*
BMI (kg/m^2)	23.63±3.23	23.86±2.89	0.657
Number of the replaced heart valve	1.23±0.42	1.30±0.46	0.364
Smoking or drinking history, *n* (%)	52 (21.5)	23 (42.6)	0.006*
Previous thromboembolism, *n* (%)	16 (6.6)	5 (9.3)	0.543
Hypertension, *n* (%)	74 (30.6)	18 (33.3)	0.727
Diabetes mellitus, *n* (%)	12 (5.0)	2 (3.7)	1.000
Atrial fibrillation, *n* (%)	96 (39.7)	18 (33.3)	0.5
Atria sinistrum thrombus, *n* (%)	22 (9.1)	2 (3.7)	0.349
CYP2C9 genotype, *n* (%)	–	–	0.779
*1/*1	218 (90.1)	50 (92.6)	
*1/*3	24 (9.90)	4 (7.40)	
VKORC1-1639 genotype, *n* (%)	–	–	
AA	210 (86.8)	43 (79.6)	0.209
AG	32 (13.2)	11 (20.4)	
WBC ($10^9/L$)	10.69±3.59	11.26±3.65	–
HB (g/L)	102.74±16.97	107.30±14.58	–
PLT ($10^9/L$)	166.63±80.00	192.81±92.83	–
TP (g/L)	57.61±9.18	59.49±9.37	–
Albumen (g/L)	33.86±9.87	34.32±4.81	–
Number of concomitant medicines that can strengthen the effect of warfarin[b]	1.61±1.06	2.73±1.16	–
Number of concomitant medicines that can weaken the effect of warfarin[c]	1.95±0.70	2.21±0.72	–

BSA, body surface area; BWI, body mass index; WBC, white blood cell; HB, hemoglobin; PLT, platelet; TP, total protein.

[a] BSA (m^2)=0.0061×Height (cm)+0.0128×Weight (kg)−0.1529;

[b] Concomitant medicines that can strengthen the effect of warfarin include: β-receptor blockers, Amiodarone, Omeprazole, Cefazolin, Statins;

[c] Concomitant medicines that can weaken the effect of warfarin include: Vitamin K, Spironolactone, Budesonide, Digoxin, Methylprednisolone, Cortisone and Prednisone;

*: The difference was significant; –: The two-sample *t* test was not performed as the final result may be greatly influenced, due to its variation with the time and the different recorded days of therapy of each patient.

η (η_{CL}, η_V) and the categorical covariates (gender, smoking, drinking history, number of the replaced valves, hypertension, diabetes, atrial fibrillation, thrombus in the atrium sinistrum, deep vein and pulmonary embolism history, concomitant medications, and the CYP2C9 and VKORC1-1639 genotypes) and continuous covariates (age, height, body surface area, body mass index, white blood cell, hemoglobin, platelet, total protein, and albumin) were analyzed by ANOVA and Spearman analysis, respectively. Each candidate covariate was screened in turn by adding it into the base model. The potential covariates were included nonlinearly into the corresponding PK parameter models in forward inclusion and only those that made the objective function value (OFV) decrease more than 3.84 (P<0.05, df=1) were retained. Then, the retained covariates were removed from the models in backward elimination and only those that made the OFV increase more than 6.63 (P<0.01, df=1) were finally retained.

Model validation

The population averages of the pharmacokinetic parameters CL and V (θ_{CL} and θ_V) were fixed by the FIXED statements in NONMEN control documents after establishing the final PK models for the 242 patients. Then, the 85 *R*-warfarin and 82 *S*-warfarin plasma concentrations obtained from the remaining 54 patients (cohort 2) were iterated by NONMEN and the predictive plasma concentrations of warfarin were obtained. The prediction result of the 54 patients (cohort 2) was then compared with that of the 242 patients (cohort 1) to evaluate the accuracy and precision of external validation of the final PopPK models, by comparing WR (population weight residual), AWR (population absolute weight residual), IWR (individual weight residual) and IAWR (individual absolute weight residual) and the scatter-plots of DV-PRED (observed concentration *vs* population predicted concentration) and DV-IPRED (observed concentration *vs* individual predicted

concentration) between cohort 1 and cohort 2. The calculation formulas were listed in Equations 4–7.

$$WR_{ij}=(CP_{ij}-PRED_{ij})/PRED_{ij} \quad (4)$$

$$AWR_{ij}=|CP_{ij}-PRED_{ij}|/PRED_{ij} \quad (5)$$

$$IWR_{ij}=(CP_{ij}-IPRED_{ij})/IPRED_{ij} \quad (6)$$

$$IAWR_{ij}=|CP_{ij}-IPRED_{ij}|/IPRED_{ij} \quad (7)$$

where CP_{ij} was the jth observed concentration of the ith patient, $PRED_{ij}$ was the jth population prediction concentration of the ith patient, and $IPRED_{ij}$ was the jth individual prediction concentration of the ith patient calculated by the POSTHOC method.

Establishment of the maintenance dosing algorithm

After 6–12 months of follow-up visits for the 296 patients, a total of 186 patients reached a steady-state of anticoagulation with available steady state INR values and maintenance doses. Among the 186 patients, 144 were randomly selected as "cohort 3" for developing the dose equation, and the remaining 42 patients constituted "cohort 4" for validation of the dose equation. The individual PK parameter for the 144 steady-state patients (apparent clearance of S-warfarin, CLs) could be estimated by Bayesian forecasting based on the population PK model developed in the 242 patients[18]. Then, a multiple linear regression analysis was performed to evaluate the association between the individual PK parameter-CLs, the observed individual INR value, age and the actual maintenance dose with SPSS19.0 software.

Validation of the dosing algorithm and comparison with other dosing algorithms

The newly established dosing algorithm was then validated in another independent group of 42 patients, and the correlation analysis was performed between the predicted and the actual dose. Then, the mean prediction error (ME) and root mean squared error (RSME) of these 42 patients obtained by the new dosing algorithms were used to evaluate and compare the accuracy and precision with other dosing algorithms. A lower ME represents a smaller bias between the predicted and the actual dose. A lower RMSE represents a higher degree of prediction precision. The formulas of ME and RMSE were listed as follows:

$$ME=1/N\sum_{j=1}^{N}(V_{pred,j}-V_{obs,j})$$

$$RMSE=\sqrt{1/N\sum_{j=1}^{N}(V_{pred,j}-V_{obs,j})^2}$$

where N was the number of subjects, and $V_{pred,j}$ and $V_{obs,j}$ were the jth predicted and observed value, respectively, such as maintenance dose, INR, Cps, *etc*.

Results

Patient information

In total, 338 patients were recruited in this study, of which 296 patients were used for the PopPK modeling and algorithm establishment, and the remaining 42 patients were used for external validation. The basic information of the 296 patients was presented in Table 1, of which 242 patients were randomly selected as "cohort 1" for developing the PopPK model, and 54 of them constituted "cohort 2" for external validation of the PopPK model. The Chi-square test or the two-sample *t*-test was performed to compare the differences of the basic information for the 2 cohorts.

The population pharmacokinetic modeling

The plasma concentration of *R*- and *S*-warfarin could both be successfully fitted using the one-compartment model (ADVAN2 TRANS2). The significant covariates by correlation analysis were introduced into the models in certain forms. Their reservation in the models was based on the change of OFV and the inter- and intra-individual variation of the PK parameters. The final PK models with 242 patients were obtained by the forward stepwise inclusion and backward stepwise elimination methods. The final PK models and the population PK parameters of the final PK models are shown in Table 2 and Table 3.

The goodness of fit diagnostic plots for the final population

Table 2. The final PK models of *S*- and *R*-warfarin.

Model	Model parameter	Formula	
		R-warfarin	S-warfarin
The fixed effect model	TVCL	θ_{CL}	$\theta_{CL}\times(1-CYP2C9\times\theta_{CYP2C9})$
	TVV	$\theta_V\times(1+GEND\times\theta_{GEND})$	$\theta_V\times(1+(BSA-1.66)\times\theta_{BSA})$
The random effect model		$Ln(P_j^0)=ln(P_j)+\eta^{Pj}$	
		$Ln(C_{ij}^0)=ln(C_{ij})+\varepsilon_{ij}$	

TVCL was the population value of clearance; TVV was the population value of distribution volume; GEND stands for gender, when the object is male, GEND is 0, when the object is female, GEND is 1; BSA stand for the body surface area; CYP2C9: when it is the genotype of CYP2C9*1/*1, then CYP2C9 is 0, when it is the genotype of CYP2C9*1/*3, then CYP2C9 is 1; θ_{CL} and θ_V were the population averages for CL and V, respectively; θ_{CYP2C9} stands for the effect of CYP2C9*1/*3 (% reduction) on the apparent clearance of *S*-warfarin; θ_{BSA} stands for the effect of body surface area (% change/m^2) on the apparent clearance of *S*-warfarin; θ_{GEND} stands for the effect of the gender of female on the (% change) on the apparent distribution volume of *R*-warfarin. P_j^0 and P_j was the jth predicted and actual PK parameter respectively, η^{Pj} was the inter-individual variation of P_j. C_{ij}^0 and C_{ij} was the jth predicted and actual plasma concentration of the ith patient respectively, ε_{ij} was the intra-individual variation of C_{ij}.

Table 3. The population PK parameters of the final warfarin PK models.

	R-warfarin				*S*-warfarin			
	Parameter	Value	95% Confidence interval	CV	Parameter	Value	95% Confidence interval	CV
OFV	–	2330.017	–	–	–	2065.694	–	–
CL (L/h)	θ_{CL}	0.258	(0.229, 0.287)	5.54%	θ_{CL}	0.586	(0.536, 0.636)	4.32%
	–	–	–	–	θ	0.322	(0.137, 0.507)	28.7%
V (L)	θ_V	26.2	(22.0, 30.4)	7.94%	θ_V	22.9	(20.0, 25.8)	6.33%
	θ	-0.319	(-0.454, -0.184)	21.1%	θ	1.41	(0.932, 1.89)	17.0%
Inter-individual variation	ω_{CL}	0.221	(0.128, 0.314)	21.0%	ω_{CL}	0.118	(0.0654, 0.1706)	22.3%
	ω_V	0.154	(0.0874, 0.221)	21.6%	ω_V	0.0771	(0, 0.155)	50.7%
Intra-individual variation	σ	0.0184	(0.0127, 0.0241)	15.4%	σ	0.0316	(0.0237, 0.0395)	12.6%

OFV stands for the object function value; CL is the apparent clearance; *V* is the apparent distribution volume; θ_{CL} and θ_v were the population averages for CL and V, respectively; θ_{CYP2C9} stands for the effect of CYP2C9*1/*3 (% reduction) on the apparent clearance of *S*-warfarin; θ_{BSA} stands for the effect of body surface area (% change/m^2) on the apparent clearance of *S*-warfarin; θ_{GEND} stands for the effect of the gender of female on the (% change) on the apparent distribution volume of *R*-warfarin. ω_{CL}, ω_v stand for the variance of the inter-individual variation of CL and V, respectively; σ stands for the variance of the intra-individual variation of plasma concentrations. CV: Coefficient of variation, CV=SD/Mean*100%.

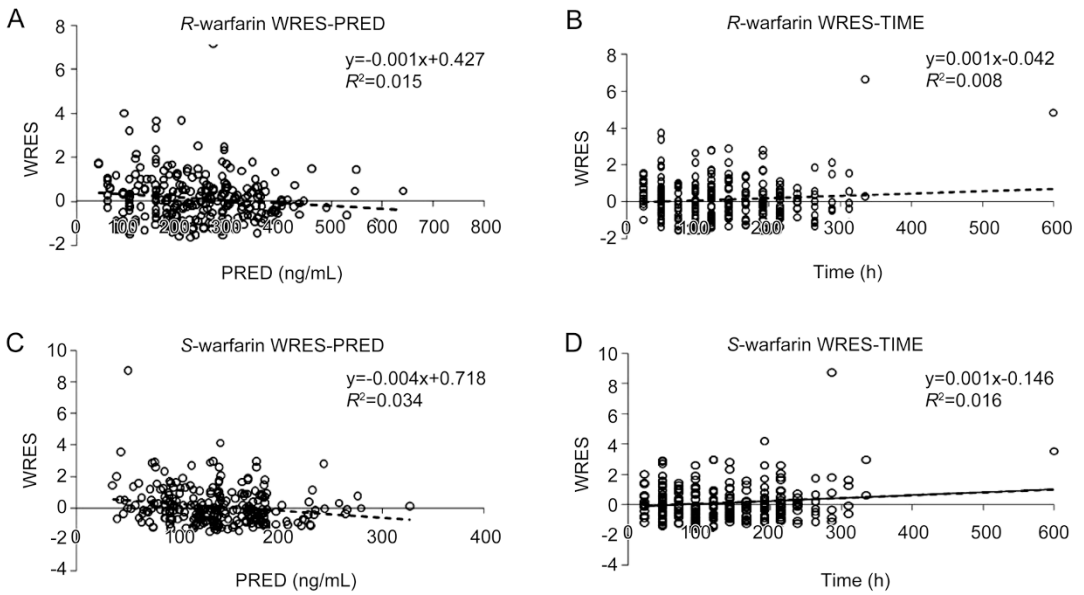

Figure 1. Weighted residuals - Population predicted plasma concentration and time (WRES-PRED and WRES-TIME). (A, B) for *R*-warfarin and (C, D) for *S*-warfarin.

PK models of *R*- and *S*-warfarin are shown in Figure 1 and 2. The scatter-plots of RES-PRED (Residuals – Population predicted plasma concentration), WRES-PRED (Weighted Residuals-Population predicted plasma concentration), WRES-TIME (Weighted Residuals–Time) show that the residual points are uniformly distributed around the line Y=0 and that the fit is good. The range of residuals of the final population PK model was smaller than that of the basic model. The plot of DV-PRED showed that the prediction error of the final population PK model was much smaller than the basic model, meaning that the introduction of the covariates improved the final model. The plot of DV-IPRED showed that the individual prediction points are closely distributed around the diagonal line, with good linearity (the correlation coefficient R^2 values were 0.971 and 0.899, respectively, for *R*- and *S*-warfarin).

Validation of the final population pharmacokinetic model

The population and the individual values of *R*- and *S*-warfarin plasma concentrations were estimated in cohort 1 (242 patients) and cohort 2 (54 patients), respectively. The scatter-plots are shown in Figure 3.

The result showed that the correlation between DV (dependent variable, the observed concentrations in this study) and IPRED (the individual predictive value) by Bayesian forecast-

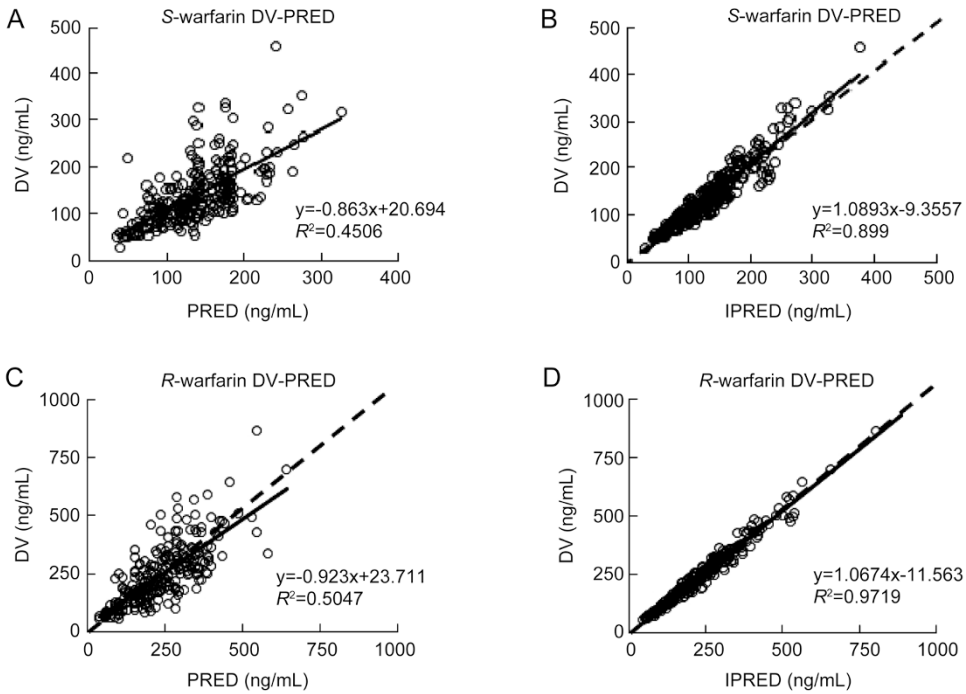

Figure 2. Observed plasma concentration *vs* Population predicted plasma concentration or individual predicted plasma concentration (DV-PRED or DV-IPRED). DV stands for the observed plasma concentration of warfarin. The dotted line stand for line y=x. PRED stands for the population predicted plasma concentration of warfarin based on the PK models. IPRED stands for the individual predicted plasma concentration of warfarin based on the PK models. (A, B) for *S*-warfarin and (C, D) for *R*-warfarin.

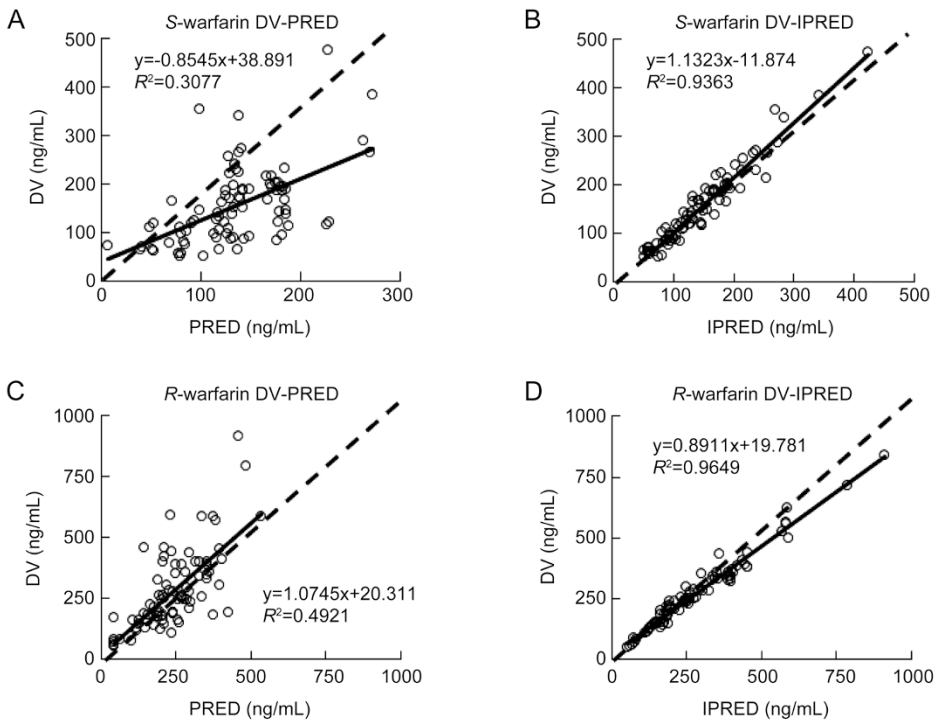

Figure 3. The comparison of the DV-PRED and the DV-IPRED scatter-plot of cohort 1 and cohort 2. (A and C) Scatter plot of observed *vs* population predicted plasma concentration of *S*- and *R*-warfarin in 54 patients from the validation group (cohort 2). (B and D) Scatter plot of observed *vs* individual predicted plasma concentration of *S*- and *R*-warfarin in 54 patients from the validation group (cohort 2).

ing was stronger than that of PRED (the population predictive value). The points in the DV-IPRED scatter-plots were distributed uniformly around the diagonal line, with a similar slope and intercept of the regression line, indicating a good prediction effect of the final PK models for the *R*- and *S*-warfarin concentration of the patients in cohort 1 and cohort 2. Meanwhile, the WR, IWR, AWR, and IAWR of each observed concentration and their median and average values in cohort 1 were calculated, which were compared with those in cohort 2, to evaluate the prediction accuracy of the external validation (Table 4).

Table 4. The comparison of prediction accuracy in cohort 1 and cohort 2.

	Cobort 1		Cohort 2		*P* value
Error	Mean	Median	Mean	Median	
WR	0.04	-0.01	0.05	-0.16	0.914
AWR	0.30	0.23	0.47	0.25	0.352
IWR	-0.01	-0.01	-0.04	-0.05	0.172
IAWR	0.10	0.07	0.13	0.08	0.078

The result showed that there was no significant difference of IWR and IAWR between the two cohorts, and their mean and median values were similar, indicating the similar prediction accuracy between the two cohorts.

Building the maintenance dosing algorithm

During the 6–12 month follow-up visits for the 296 discharged patients, the INR values of 186 patients (shown in Table 1) reached steady state (cohort 3 for developing the dose equation, n=144; cohort 4 for external validation, n=42. The Chi-square test or the two-sample t-test was performed to compare the differences of the basic information for the 2 cohorts). The individual prediction of CLs (the clearance of *S*-warfarin) and CLr (the clearance of *R*-warfarin) for the 144 patients in cohort 3 could be obtained by Bayesian forecasting.

Although the plasma concentrations of *R*-warfarin were almost as much as 2-fold of *S*-warfarin, the CLr did not have a correlation relationship with the steady-state dose; therefore, CLr was not included in the final dose algorithm. With respect to *S*-warfarin, in this study, unlike the research of Hamberg[12] and Lane[13], gender, age and weight were not found to have an influence on the clearance of *S*-warfarin and were not included in the parameter estimates of CLs. As a result, they may not be entered into the final dose prediction formula. However, for the other dose equations[4–8], gender, age and weight were important factors that affected the dosage of warfarin, so these three factors should be included in the dose prediction formula. In the end, CLs, VKORC1 genotypes, the steady-state INR values, gender, age and weight were all used for the multivariate regression. The final multiple regression model, selected by means of the stepwise backward elimination procedure, is summarized in Table 5. The equation was obtained as: Maintenance Dose=-0.023×AGE+1.834×VKORC1 +0.952×INR+2.156×CLs. The target INR value ranges from 1.8 to 2.5, showing a moderately strong correlation between the predicted and actual maintenance dose (R^2=0.665, P<0.05).

Table 5. The multiple linear regression model of warfarin maintenance dose using pharmacokinetic (PK) parameters with the full dataset of the 144 steady-state patients.

Variable	Unstandardized coefficients		Standardized coefficients	*t*	*P* value
	B	SE	Beta		
Constant	0.550	0.739		0.745	0.459
AGE	-0.023	0.006	-0.284	-3.392	<0
VK	1.834	0	0	8.933	<0
INR	0.952	0	0.268	3.761	<0
CL	2.156	0	0	4.636	<0

CLs: Apparent clearance of S-warfarin; INR: International normalized ratio of prothrombin time; SE: Standard error; CI: Confident interval.

Validation of the dosing model and comparison with other dosing algorithms

The result of external validation of the algorithm in the independent group of 42 patients is shown in Figure 4, with a high correlation coefficient (R^2=0.716) between the predicted and the actual maintenance dose. The result also revealed that the equation tended to overestimate for doses less than 2 mg and underestimate for doses more than 4 mg. The comparison of the predictive performance of the current and other dosing methods for the 42 patients is shown in Table 6. The result showed that the ME and RSME of our prediction algorithm were smaller than almost all of the other 4 models. Although our equation tended to overestimate for doses lower than 2 mg/d (Figure 4), the results of subgroup analysis of the 18 patients (<2 mg/d) in cohort 4 showed that the prediction accuracy and precision of our equation were still better than the other 5 dose formulas (Table 7).

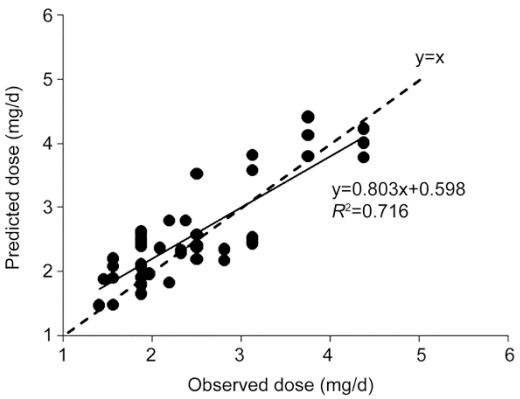

Figure 4. Scatterplot of the observed dose versus the predicted dose of the 42 patients for external validation.

Table 6. The comparison of the predictive performance of the current and the other dosing methods for the 42 patients of another independent group.

	ME	RMSE
Current method	-0.20	0.24
Takahashi *et al*[17]	1.64	1.75
Miao *et al*[7]	-0.28	0.55
Ohno *et al*[4]	0.84	0.98
Huang *et al*[6]	0.48	0.69

ME: Mean prediction error; RMSE: Root mean squared error.

Table 7. The comparison of the predictive performance of the current and the other dosing methods for the 18 low dose (<2 mg/d) patients from cohort 4.

	ME	RMSE
Current method	-0.03	0.31
Takahashi *et al*[17]	1.74	1.87
Miao *et al*[7]	0.03	0.32
Ohno *et al*[4]	1.16	1.23
Huang *et al*[6]	0.74	0.86

ME: Mean prediction error; RMSE: Root mean squared error; Overestimate rate=(predictive dose–actual dose)/actual dose×100%.

Discussion

Although the therapeutic index of warfarin is narrow, the research of Sjögren[19] in 77 423 patients recently showed that as long as the proportion of time in the therapeutic range was high, warfarin will continue to be a valid treatment option in the era of newer oral anticoagulants. In this study, we established a new dosing prediction model, a population PK-PD model, by using the individual PK parameter (CLs) forecasted by the Bayesian method, the INR value of the steady state, and the age of the patients. The new dosing model gave higher individual variation rates (71.6%) than the general gene-guided dosing models (50%)[7, 20].

The basic *R*- and *S*-warfarin PK model could be fitted better by a one-compartment model compared with the two-compartment model, with a decrease in the relative standard error (SE%) of clearance from 29.6% to 4.1%, which was similar to the study of Hamberg[12] and Lane[13]. This was the first time that the population PK models of *R*- and *S*-warfarin for the Han Chinese patients were developed. During the PK modeling of *R*-warfarin, no influence factor was found for CLr, and gender was identified as influencing the distribution volume, with the distribution volume of women 31.9% lower than men. However, in building the maintenance dosing algorithm, CLr and volume did not have a correlation relationship with the steady-state dose. As *S*-warfarin's PK parameter is better correlated with the steady-state dose compared to *R*-warfarin, we did not value the model predictive performance of the total warfarin.

The prediction accuracy of the plasma concentration of *S*-warfarin was improved by the Bayesian forecasting, with the correlation coefficient between the predicted and the actual value improving from 0.4506 to 0.8990 prior and post Bayesian inference. The mean value of individual CLs with Bayesian inference was 0.586 L/h, which was comparable with the previously reported clearance of *S*-warfarin (0.61 L/h) in a Chinese population[21]. Taking these results into consideration, the individual population PK parameters by Bayesian forecasting were acceptable for the maintenance dose prediction modeling of warfarin in Chinese patients.

Recent studies[22] have found that the algorithms from Caucasian and racially mixed populations tended to perform better in the higher dose group (≥4.5 mg/d), and algorithms from Asian populations performed better in the intermediate dose group (1.5–4.5 mg/d). None of the algorithms performed well in the lower dose group (≤1.5 mg/d). In our research, the prediction precision of our model for the low-dose (<2 mg/d) patients was improved, with the value of ME and RMSE lower than the other 4 gene-guided algorithms[4, 6, 7, 17] in the validation group. In this research, the prediction accuracy and precision (predicted dose-actual dose)/actual dose×100%) for the 18 patients with low dose (<2 mg/d) in cohort 4 (n=42) was better than the other 4 models (ME: -0.03 mg *vs* 1.74 mg, 0.03 mg, 1.16 mg, 0.74 mg; RMSE: 0.31 mg *vs* 1.87 mg, 0.32 mg, 1.23 mg, 0.86 mg). By comparing the difference of the values of CLs, INR and age of the 4 models (the Chi-square test or the two-sample nonparametric Mann Whitney U test were performed to compare the differences of the basic information for these 2 groups), there was a significant difference in age (56.11±9.98 years *vs* 48.71±11.54 years, P=0.044) and VKORC1-1173 (P=0.005) between the two groups. This result indicated that age and VKORC1-1173 genotypes may be important potential factors that improve the prediction rate for the patients with low dose (<2 mg/d). However, CYP2C9, the primary enzyme of warfarin metabolism, which is a commonly accepted factor in most reported models about warfarin dose prediction, was included in the equation $CLs=\theta_{CL}\times(1-CYP2C9\times\theta_{CYP2C9}$; for CYP2C9: when it is the genotype of CYP2C9*1/*1, then CYP2C9 is 0; when it is the genotype of CYP2C9*1/*3, then CYP2C9 is 1).

The result of the multiple linear regression modeling showed that the correlation coefficient between the prediction dose and actual dose was 0.6658, indicating that there are still "unknown factors" determining individual maintenance doses. In addition, the measurements of the drug concentration and INR levels might require extra costs and a delay until the therapeutic dose can be estimated, but it would be worth it for the low dose Chinese patients with a high risk of bleeding.

In conclusion, we established a new algorithm based on the PK/PD model, with a high accuracy of warfarin dose prediction for Han Chinese patients, especially for low dose (<2 mg/d) patients.

Acknowledgements

We thank all of the clinicians, nurses and patients who participated in this study. This study was supported by the Department of Cardiovascular, Nanjing First Hospital, Nanjing Medical University. In addition, the authors wish to thank Prof Li-yan MIAO from the First Hospital Affiliated to Soochow University for her assistance with data support. The research was supported by the Science and Technology Program of Nanjing, China (No 2014sc517048).

Author contribution

Jun-rong ZHU and Feng YU designed the research; Yu-bin ZHU and Xian-hua HONG performed the experiments and wrote the article; Jian-guo SUN, Xian-hua HONG, and Jing HU analyzed the data; and other colleagues helped to collect the sample and clinical data.

References

1 Yuan HY, Chen JJ, Lee MT, Wung JC, Chen YF, Charng MJ, *et al*. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. Hum Mol Genet 2005; 14: 1745–51.

2 Shu WY, Li JL, Wang XD, Huang M. Pharmacogenomics and personalized medicine: a review focused on their application in the Chinese population. Acta Pharmacol Sin 2015; 36: 535–43.

3 Singh O, Sandanaraj E, Subramanian K, Lee LH, Chowbay B. Influence of CYP4F2 rs2108622 (V433M) on warfarin dose requirement in Asian patients. Drug Metab Pharmacokinet 2011; 26: 130–6.

4 Ohno M, Yamamoto A, Ono A, Miura G, Funamoto M, Takemoto Y, *et al*. Influence of clinical and genetic factors on warfarin dose requirements among Japanese patients. Eur J Clin Pharmacol 2009; 65: 1097–103.

5 Wen MS, Lee M, Chen JJ, Chuang HP, Lu LS, Chen CH, *et al*. Prospective study of warfarin in dose requirements based on CYP2C9 and VKORC1 genotypes. Clin Pharmacol Ther 2008; 84: 83–9.

6 Huang SW, Chen HS, Wang XQ, Huang L, Xu DL, Hu XJ, *et al*. Validation of VKORC1 and CYP2C9 genotypes on interindividual warfarin maintenance dose: a prospective study in Chinese patients. Pharmacogenet Genomics 2009; 19: 226–34.

7 Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: proposal for a new dosing regimen in Chinese patients. Eur J Pharmacol 2007; 63: 1135–41.

8 Sasaki T, Tabuchi H, Higuchi S, Ieiri I. Warfarin-dosing algorithm based on a population pharmacokinetic/pharmacodynamic model combined with forecasting. Pharmacogenomics 2009; 10: 1257–66.

9 Hamberg AK, Friberg LE, Hanséus K, Ekman-Joelsson BM, Sunnegårdh J, Jonzon A, *et al*. Warfarin dose prediction in children using pharmacometric bridging-comparison with published pharmacogenetic dosing algorithms. Eur J Clin Pharmacol 2013; 12: 1275–83.

10 Wei M, Ye F, Xie D, Zhu Y, Zhu J, Tao Y, *et al*. A new algorithm to predict warfarin dose from polymorphisms of CYP4F2, CYP2C9 and VKORC1 and clinical variables: derivation in Han Chinese patients with non valvular atrial fibrillation. Thromb Haemost 2012; 107: 1083–91.

11 Hirsh J, Fuster V, Ansell J, Halperin JL. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. J Am Coll Cardiol 2003; 41: 1633–52.

12 Hamberg AK, Wadelius M, Lindh JD, Dahl ML, Padrini R, Deloukas P, *et al*. A pharmacometric model describing the relationship between warfarin dose and INR response with respect to variations in CYP2C9, VKORC1, and age. Clin Pharmacol Ther 2010; 87: 727–34.

13 Lane S, Al-Zubiedi S, Hatch E, Matthews I, Jorgensen AL, Deloukas P, *et al*. The population pharmacokinetics of *R*- and *S*-warfarin: effect of genetic and clinical factors. Br J Clin Pharmacol 2012; 73: 66–76.

14 Hamberg AK, Dahl ML, Barban M, Scordo MG, Wadelius M, Pengo V, *et al*. A PK-PD model for predicting the impact of age, CYP2C9 and VKORC1 genotype on individualization of warfarin therapy. Clin Pharmacol Ther 2007; 81: 529–38.

15 Yuen E, Gueorguieva I, Wise S, Soon D, Aarons L. Ethnic differences in the population pharmacokinetics and pharmacodynamics of warfarin. J Pharmacokinet Pharmacodyn 2010; 37: 3–24.

16 Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP, *et al*. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 2005; 106: 2329–33.

17 Takahashi H, Wilkinson GR, Nutescu EA, Morita T, Ritchie MD, Scordo MG, *et al*. Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. Pharmacogenet Genomics 2006; 16: 101–10.

18 Peter S Craig, Michael Goldstein, Jonathan C Rougier, Allan H Seheult. Bayesian forecasting for complex systems using computer simulators. J Am Stat Assoc 2001; 96: 717–29.

19 Sjögren V, Grzymala-Lubanski B, Renlund H, Friberg L, Lip GY, Svensson PJ, *et al*. Safety and efficacy of well managed warfarin. A report from the Swedish quality register Auricula. Thromb Haemost 2015; 113: 1370–7.

20 Sangviroon A, Panomvana D, Tassaneeyakul W, Namchaisiri J. Pharmacokinetic and pharmacodynamic variation associated with VKORC1 and CYP2C9 polymorphisms in Thaipatients taking warfarin. Drug Metab Pharmacokinet 2010; 25: 531–8.

21 Cai W, Jing H, Chen B, Shen Y, Lin S. Pharmacokinetics of warfarin enantiomers in patients with cardiac valve replacement. Chin Pharm J 1997; 32: 152–4.

22 Liu Y, Yang J, Xu Q, Xu B, Gao L, Zhang Y, *et al*. Comparative performance of warfarin pharmacogenetic algorithms in Chinese patients. Thromb Res 2012; 130: 435–40.