

Supplementary Information

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Title: Development and Clinical Application of a Real-World Population Pharmacokinetic Model of Rivaroxaban in Asian Patients with Atrial Fibrillation

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Ultra-high-performance liquid chromatography with tandem mass spectrometry conditions for factor Xa inhibitors concentrations measurement.

Rivaroxaban plasma concentrations were measured using a UHPLC-MS/MS method adapted from our previously published work with modifications. Venous blood samples were collected in K₂EDTA tubes, centrifuged to obtain plasma, and stored at -80°C until analysis.

Sample Preparation

A 100- μL aliquot of plasma was deproteinized by adding 800 μL of 100% methanol containing 2 $\mu\text{g/mL}$ of [$^{13}\text{C}_6$]-rivaroxaban as the internal standard. The mixture was homogenized at 1000 rpm for 2 min (Geno/Grinder 2010), then centrifuged at 15,000 rcf for 5 min. Subsequently, 400 μL of the supernatant was transferred to a clean tube, dried using a centrifugal vaporizer (Thermo SpeedVac®), and stored at -20°C . Before analysis, samples were reconstituted with 200 μL of methanol and filtered through a 0.22- μm membrane filter.

Chromatographic and Mass Spectrometric Conditions

Analysis was performed using an Agilent 1290 UHPLC system coupled with an Agilent 6460 triple quadrupole mass spectrometer. Analyte separation was achieved on a Kinetex C18 column (2.1 $\times$ 50 mm, 2.6 μm) maintained at 55°C . The mobile phase consisted of 0.1% formic acid and 10 mM ammonium acetate in water (A) and in an isopropanol/acetonitrile mixture (9:1, v/v) (B), delivered at 0.35 mL/min. The mass spectrometer utilized a JetStream electrospray ionizer in multiple reaction monitoring (MRM) mode. The transitions monitored were 436.1 $\rightarrow$ 144.9, 436.1 $\rightarrow$ 72.9 for rivaroxaban, 442.1 $\rightarrow$ 144.9, 442.1 $\rightarrow$ 72.9 for [$^{13}\text{C}_6$]-rivaroxaban

Method Validation

The method was validated over a linear calibration range of 2–500 ng/mL. Intra- and inter-day precision (coefficient of variation) and accuracy (relative error) were all within the acceptable limit of 15% (20% at the lower limit of quantification). The robustness of the assay during the clinical study was monitored using pooled quality control samples, which yielded a coefficient of variation of $<5\%$.

Table S1. The classification of concurrent medications

Categorization	Medications
CYP3A4 or P-gp inhibitor	Amiodarone, dronedarone, verapamil, diltiazem, erythromycin, clarithromycin, fluconazole, ketoconazole, ritonavir
CYP3A4 or P-gp inducer	Carbamazepine, rifampicin, phenytoin, levetiracetam
CYP2J2 inhibitor	Danazol, flunarizine
BCRP inhibitor	Sulfasalazine, febuxostat, benzbromarone

Abbreviations: CYP, cytochrome P; BCRP, breast cancer resistance protein.

Table S2. The detailed formulation for lean body weight and lean body mass

Term	Formula	Equation
CrCL	C-G equation	Male: CrCL (mL/min) = $\frac{(140-\text{Age}) \times \text{BW}(\text{kg})}{72 \times \text{CRE}}$
		Female: CrCL (mL/min) = $\frac{(140-\text{Age}) \times \text{BW}(\text{kg})}{72 \times \text{CRE}} \times 0.85$
eGFR	MDRD-4 equation	Male: eGFR (mL/min/1.73m ²) = $175 \times \text{CRE}^{-1.154} \times \text{Age}^{-0.203}$
		Female: eGFR (mL/min/1.73m ²) = $175 \times \text{CRE}^{-1.154} \times \text{Age}^{-0.203} \times 0.742$
LBW	Janmahasatian formula	Male: LBW (kg) = $\frac{9270 \times \text{BW}(\text{kg})}{6680 + 216 \times \text{BMI}(\text{kg}/\text{m}^2)}$
		Female: LBW (kg) = $\frac{9270 \times \text{BW}(\text{kg})}{8280 + 244 \times \text{BMI}(\text{kg}/\text{m}^2)}$
LBM	James formula	Male: LBW (kg) = $1.1 \times \text{BW}(\text{kg}) - 128 \times \left[\frac{\text{BW}(\text{kg})}{\text{HT}(\text{cm})}\right]^2$
		female: LBW (kg) = $1.07 \times \text{BW}(\text{kg}) - 148 \times \left[\frac{\text{BW}(\text{kg})}{\text{HT}(\text{cm})}\right]^2$

Abbreviations: BW: bodyweight; BMI, body mass index; C-G equation, Cockcroft-Gault equation; CrCL, creatinine clearance; CRE, serum creatinine; eGFR, estimated glomerular filtration rate; HT, height; LBM, lean body mass; LBW, lean body weight; MDRD-4, four-variable Modification of Diet in Renal Disease.

Table S3. Comparison of models using different modes of interindividual variability.

Model	OFV	PK parameter	IIV model	Omega	SE	RSE
1	5075.17	V	Additive	24.64	1.73	7.04
		CL	Additive	2.37	0.2	8.58
2	5015.54	V	Additive	18.39	1.41	7.65
		CL	Exponential	0.81	0.05	6.12
3	5016.4	V	Exponential	0.66	0.039	5.84
		CL	Additive	2.5	0.2	7.87
4	4968.1	V	Exponential	0.59	0.036	6.15
		CL	Exponential	0.71	0.052	7.22

Abbreviations: CL, clearance; IIV, interindividual variability; OFV, objective function value; Omega, standard deviation of random effects; PK, pharmacokinetics; RSE, relative standard error; SE, standard error; V, volume of distribution.

Table S4. Forward inclusion of covariates for the volume of distribution (V/F) model.

Forward inclusion			Backward elimination (Δ OFV in different models) ¹		
Covariate	Functional form	Δ OFV	I	II	III
Body surface area (BSA)	Proportional shift	-4.53			
	Exponential	-4.62			
	Power	-6.57			
Ideal bodyweight (IBW)	Proportional shift	-7.90			
	Exponential	-6.97			
	Power	-7.89			
Lean body mass (LBM)	Proportional shift	-5.83			
	Exponential	-7.09			
	Power	-7.94			
Lean bodyweight (LBW)	Proportional shift	-8.59			
	Exponential	-8.66	-2.43	+8.66	
	Power	-8.65			
Sex	Proportional shift	-7.89			
	Exponential	-10.12	-0.97		+10.01

A reduction in OFV greater than 3.84 was considered statistically significant, corresponding to a P-value < 0.05.

Abbreviations: OFV, objective function value.

¹ During backward elimination, the removal of either LBW or sex did not meet the significance threshold

(Model I). Due to potential collinearity between these variables, they were evaluated independently: Model II includes LBW only, and Model III includes sex only.

Table S5. Model selection process for clearance (CL/F).

Forward inclusion			Backward elimination
Covariate	Functional form	Δ OFV	Δ OFV
Age	Proportional shift	-6.35	
	Exponential	-8.58	-1.11
	Power	-7.98	
IBW	Proportional shift	-13.67	
	Exponential	-15.51	+0.17
	Power	-14.55	
LBM	Proportional shift	-6.41	
	Exponential	-7.15	
	Power	-7.48	
LBW	Proportional shift	-4.70	
	Exponential	-8.43	
	Power	-7.77	
CRE	Proportional shift	-7.20	
	Exponential	-8.48	
	Power	-9.92	
eGFR	Proportional shift	-26.60	
	Exponential	-27.50	
	Power	-27.60	
CrCL	Proportional shift	-29.68	
	Exponential	-29.49	
	Power	-30.28	+6.75
Sex	Proportional shift	-9.12	
	Exponential	-9.55	+1.30
CYP3A4/P-gp inhibitor	Proportional shift	-18.70	
	Exponential	-19.50	+16.48
BCRP inhibitor	Proportional shift	-5.28	
	Exponential	-5.62	+1.92

Abbreviations: BCRP, breast cancer resistance protein; CrCL, creatinine clearance; CRE, serum creatinine; CYP, cytochrome P; eGFR, estimated glomerular filtration rate; IBW, ideal bodyweight; LBM, lean body mass; LBW, lean bodyweight; OFV, objective function value; P-gp, P-glycoprotein.

Table S6. Association between various rivaroxaban exposure metrics and clinical outcomes.

Ischemic stroke, transient ischemic attack or systemic thromboembolism						
Covariates	Model 1 (Ct)		Model 2 (Cpk)		Model 3 (AUC)	
	aOR and 95% CI	P-value	aOR and 95% CI	P-value	aOR and 95% CI	P-value
Age (year)	0.97 (0.88, 1.07)	0.54	0.97 (0.88, 1.08)	0.58	0.97 (0.89, 1.07)	0.56
Male sex	2.16 (0.50, 9.36)	0.30	2.03 (0.45, 9.11)	0.36	2.02 (0.46, 8.86)	0.35
CrCL (mL/min)	1.00 (0.96, 1.04)	0.81	1.00 (0.96, 1.04)	0.91	1.00 (0.96, 1.04)	0.86
CHA2DS2-VASc score	1.45 (0.94, 2.28)	0.10	1.45 (0.92, 2.27)	0.11	1.45 (0.93, 2.27)	0.10
Exposure matric	0.99 (0.96, 1.02)	0.38	1.00 (0.99, 1.00)	0.50	1.00 (1.00, 1.00)	0.40
Major bleeding						
Covariates	Model 1 (Ct)		Model 2 (Cpk)		Model 3 (AUC)	
	aOR and 95% CI	P-value	aOR and 95% CI	P-value	aOR and 95% CI	P-value
Age (year)	1.06 (0.96, 1.18)	0.24	1.06 (0.96, 1.17)	0.26	1.06 (0.96, 1.17)	0.26
Male sex	1.31 (0.36, 4.75)	0.68	1.19 (0.32, 4.38)	0.79	1.24 (0.34, 4.52)	0.74
CrCL (mL/min)	0.97 (0.92, 1.02)	0.22	0.97 (0.92, 1.02)	0.19	0.96 (0.91, 1.02)	0.18
HAS-BLED score	0.73 (0.30, 1.73)	0.47	0.72 (0.31, 1.72)	0.47	0.73 (0.31, 1.73)	0.47
Exposure matric	1.00 (0.99, 1.02)	0.95	1.00 (0.99, 1.00)	0.52	1.00 (1.00, 1.00)	0.64

Abbreviations: aHR, adjusted hazard ratio; AUC, area under the concentration time curve; CI, confidence interval; Cpk, predicted peak concentration (at 3 hours); Ct, predicted trough concentration (at 24 hours).

Table S7. The incidence of clinical outcomes across different concentration groups.

Metrics	Event number	Follow-up (year)	Incidence ¹	P for log-rank test	HR and 95% CI	P-value ²
Trough concentration (Ct)						
Ischemic stroke, transient ischemic attack or systemic thromboembolism						
Lower (<12 ng/mL)	2	3.84 (2.89, 5.46)	1.78	0.66	1.69 (0.35, 8.16)	0.51
Within (12-137 ng/mL)	7	3.79 (2.37, 4.94)	1.01		Reference	N/A
Higher (>137 ng/mL)	0	3.33 (1.21, 3.93)	0		N/A	N/A
Major bleeding						
Lower (<12 ng/mL)	1	3.84 (2.89, 5.46)	0.89	0.64	0.77 (0.10, 6.19)	0.81
Within (12-137 ng/mL)	8	3.80 (2.21, 4.96)	1.15		Reference	N/A
Higher (>137 ng/mL)	1	3.33 (0.98, 3.93)	2.88		2.49 (0.31, 20.18)	0.39
Peak concentration (Cpk)						
Ischemic stroke, transient ischemic attack or systemic thromboembolism						
Lower (<178 ng/mL)	3	4.76 (2.02, 6.48)	1.13	0.68	0.88 (0.21, 3.71)	0.86
Within (178-343 ng/mL)	5	3.75 (2.02, 4.87)	1.33		Reference	N/A
Higher (>343 ng/mL)	1	3.68 (2.96, 4.22)	0.5		0.39 (0.05, 3.37)	0.39
Major bleeding						
Lower (<178 ng/mL)	2	4.76 (2.02, 6.48)	0.76	0.06	0.36 (0.07, 1.70)	0.20
Within (178-343 ng/mL)	8	3.75 (1.97, 4.89)	2.13		Reference	N/A
Higher (>343 ng/mL)	0	3.69 (2.96, 4.27)	0		N/A	N/A
Area under the concentration time curve (AUC)						
Ischemic stroke, transient ischemic attack or systemic thromboembolism						
Lower (<1860 ng*h/mL)	2	4.82 (2.77, 6.48)	0.92	0.49	0.69 (0.14, 3.36)	0.65

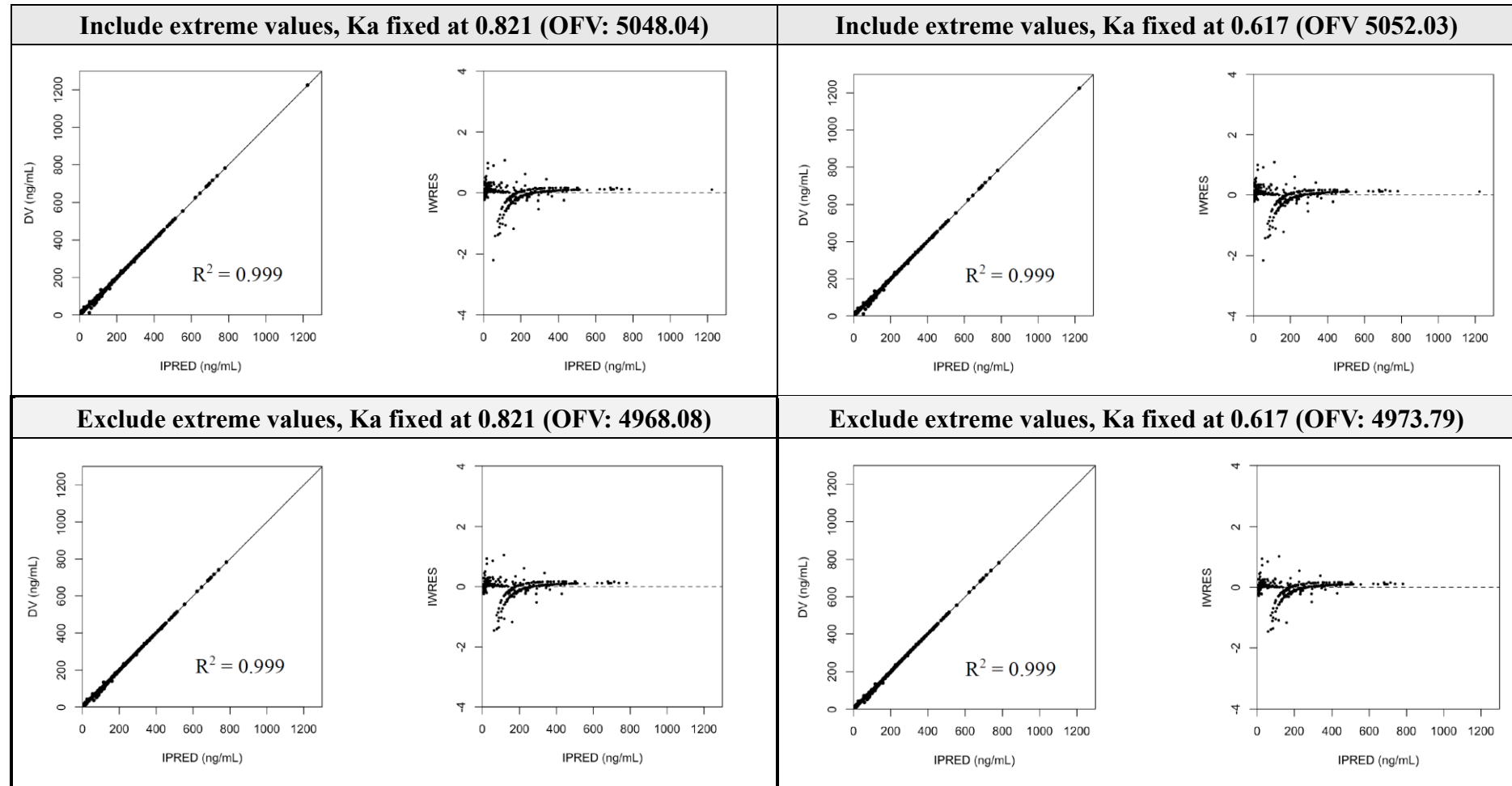
Within (1860-5434 ng*h/mL)	7	3.76 (2.19, 4.85)	1.34		Reference	N/A
Higher (>5434 ng*h/mL)	0	3.55 (2.15, 4.05)	0		N/A	N/A
Major bleeding						
Lower (<178 ng/mL)	3	4.82 (2.77, 6.48)	1.39	0.13	1.23 (0.30, 4.99)	0.77
Within (178-343 ng/mL)	6	3.77 (2.06, 4.87)	1.15		Reference	N/A
Higher (>343 ng/mL)	1	3.55 (2.15, 4.05)	0.98		0.96 (0.10, 7.15)	0.89

¹Incidence rates are presented per 100 person-years.

²P-values are derived from the Cox-proportional hazard model.

Abbreviations: CI, confidence interval; HR, hazard ratio.

Table S8. The Goodness-of-fit plot and objective function values across different inclusions groups and different absorption rate constant groups.



Abbreviations: DV, direct observation value; IPRED, individual predicted concentration; IWRES, individual weighted residuals; Ka, absorption rate constant; OFV, objective function values; RSE, relative standard error; RV, residual variability

Table S9. Forward inclusion of covariates for the volume of distribution (V/F) model.

Covariate	Functional form	Pearson correlation coefficient	P value
AST	Proportional shift	0.086	0.396
	Exponential	0.085	0.403
	Power	0.100	0.324
ALT	Proportional shift	0.052	0.513
	Exponential	0.082	0.301
	Power	0.098	0.215
T-bil	Proportional shift	-0.206	0.208
	Exponential	-0.144	0.383
	Power	-0.248	0.129
BUN	Proportional shift	-0.211	0.008
	Exponential	-0.234	0.003
	Power	-0.263	<0.001
Alb	Proportional shift	-0.423	0.080
	Exponential	-0.233	0.352
	Power	-0.274	0.272
HCT	Proportional shift	0.107	0.136
	Exponential	0.088	0.220
	Power	0.080	0.266

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; HCT, hematocrit; Alb, serum albumin; T-bil, total bilirubin.

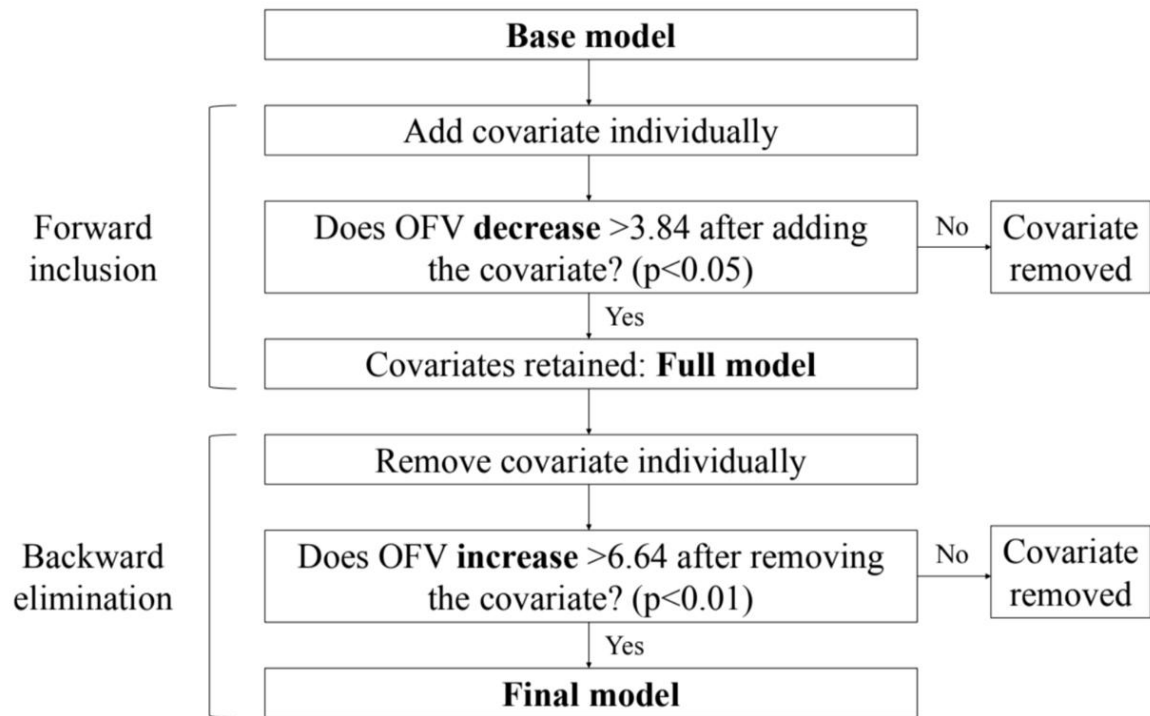


Figure S1. The process of population pharmacokinetic model development.

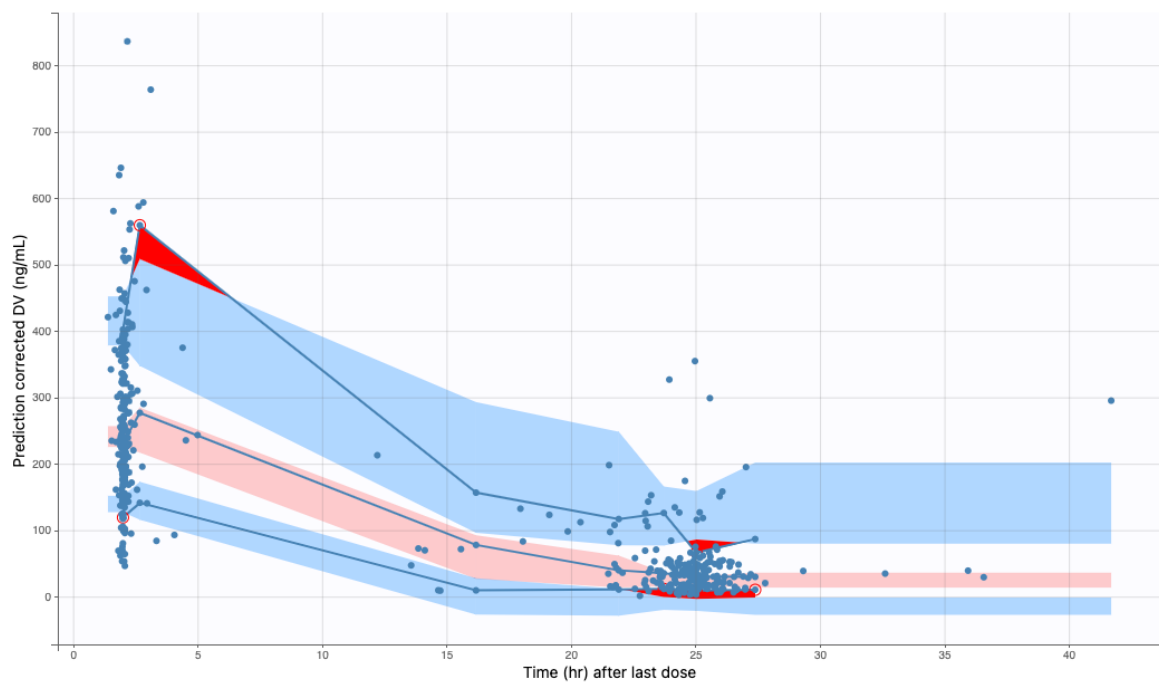


Figure S2. Visual predictive check for the final population pharmacokinetic model. Observed rivaroxaban concentrations are depicted as blue dots. The solid blue lines correspond to the 5th, 50th, and 95th percentiles of the observed data. The shaded regions represent the model-predicted 90% confidence intervals for these percentiles (light blue for the 5th and 95th percentiles; pink for the median).

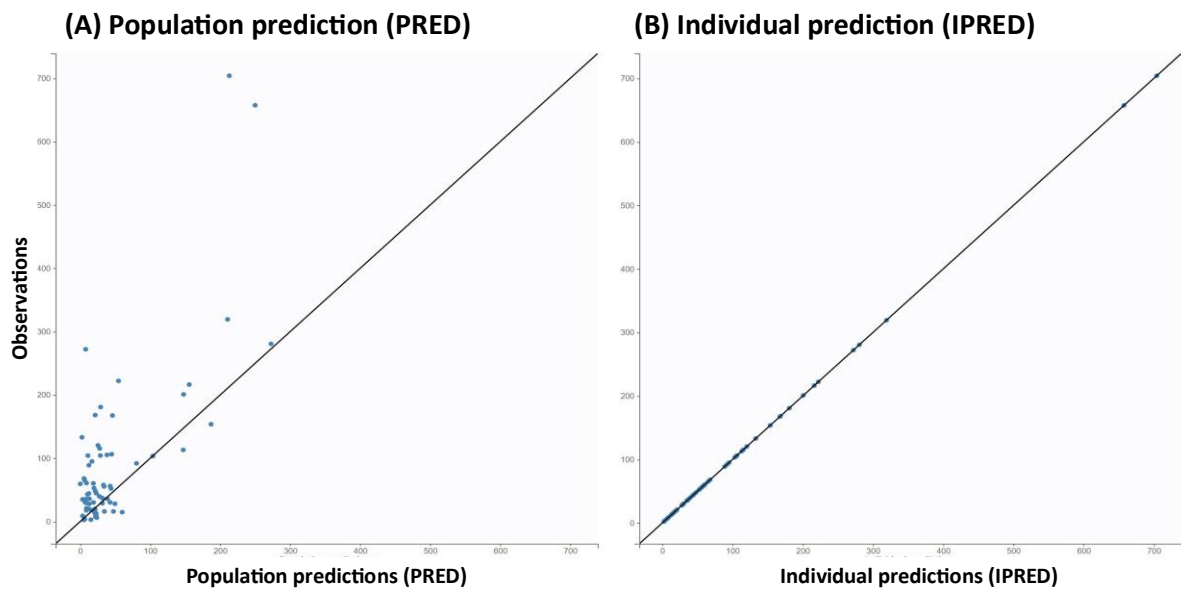


Figure S3. Goodness-of-fit plots of the previously established population pharmacokinetic (PPK) model, applied to the external validation cohort.

(A) Observed versus population predicted concentrations (PRED).

(B) Observed versus individual predicted concentrations (IPRED)

The solid diagonal lines indicate the line of identity. A closer alignment along this line suggests better predictive performance.

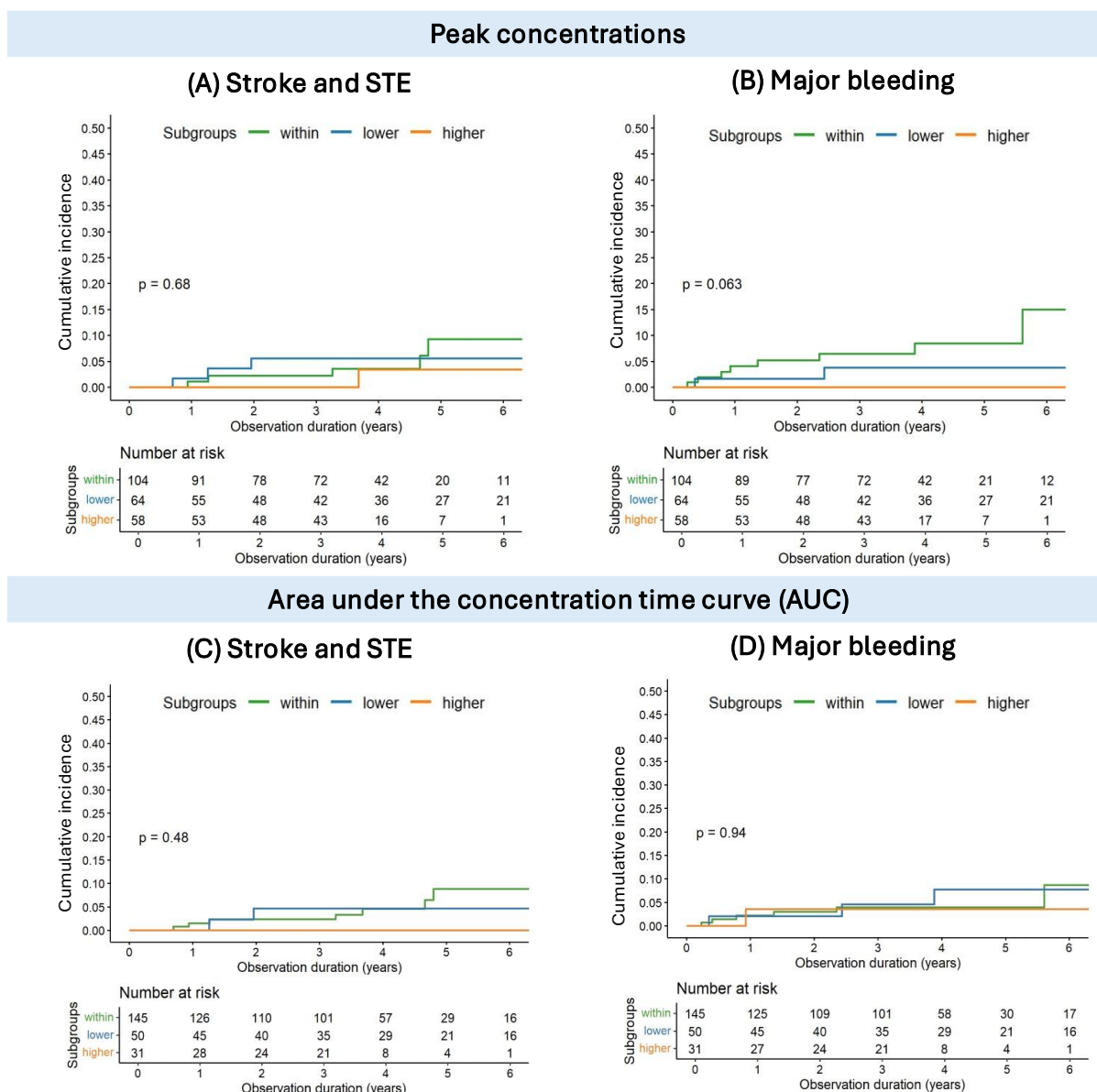


Figure S4. Kaplan-Meier survival curves for clinical outcomes based on rivaroxaban exposure metrics.

Top panel: (A) Stroke or systemic thromboembolism (STE) and (B) major bleeding stratified by peak concentration (C_{pk}) groups.

Bottom panel: (C) Stroke or STE and (D) major bleeding stratified by area under the concentration-time curve (AUC) groups.

In each plot, the green line represents patients with within-range exposure, the blue line represents those with lower-than-expected levels, and the orange line represents those with higher-than-expected levels. Log-rank test P-values are displayed in each subplot.