Anticoagulant monitoring of rivaroxaban by chromogenic anti-Xa assays and UHPLC-MS/MS in patients with atrial fibrillation

Xiao-qin Liu
Huashan Hospital
Yi Gu
Renji Hospital
Yu-fei Zhang
Huashan Hospital
Wei Shen
Renji Hospital
Zhi-chun Gu
Renji Hospital
Ming-kang Zhong
Huashan Hospital
Hong-yan Ding
Huashan Hospital
Chun-lai Ma (chunlaima@126.com)
Huashan Hospital

Research Article

Keywords: rivaroxaban, chromogenic anti-Xa assay, UHPLC-MS/MS, drug monitoring

Posted Date: April 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1521812/v1

Abstract

Background: Monitoring the level of anticoagulation by rivaroxaban in patients with non-valvular atrial fibrillation (NVAF) is valuable in clinical practice. Our study aimed to measure the plasma levels of rivaroxaban both by chromogenic anti-Xa assays and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) in Chinese patients with NVAF, and compare with the expected drug levels.

Materials and methods: A prospective clinical study was conducted to include NVAF patients taking rivaroxaban. Rivaroxaban levels were determined using UHPLC-MS/MS and chromogenic anti-Xa assays (Biophen DiXal and Zhenyuan anti-Xa). The correlation and agreement among measurements by different assays were evaluated. The rivaroxaban levels in Chinese patients were also compared with the expected drug levels, which were estimated from previous clinical trials and widely accepted.

Results: A total of 243 plasma samples were collected from 182 patients. Results measured using the two chromogenic anti-Xa assays were both linearly correlated with those measured using UHPLC-MS/MS, especially in the range of 50–200 ng/mL, but the concentrations determined using anti-Xa assays were systematically underestimated. Rivaroxaban levels measured by either UHPLC-MS/MS or chromogenic anti-Xa assays were both larger than the expected ranges. There was no difference in the distribution of concentration whether the patients took an appropriate dose or not. However, for Chinese patients with an inappropriate lower dose, trough concentrations were less likely to exceed the expected concentration ranges.

Conclusion: Rivaroxaban concentration in patients with NVAF were highly variable compared to the expected ranges estimated from clinical trials, whether measured by UHPLC-MS/MS or chromogenic anti-Xa assays. Chinese patients taking an appropriate rivaroxaban dose are more likely to obtain a rivaroxaban concentration level higher than expected, independent on the assay used.

1. Introduction

Rivaroxaban, a direct factor Xa inhibitor, is a novel oral anticoagulant (NOAC) widely used for stroke prevention in patients with non-valvular atrial fibrillation (NVAF) [1, 2]. Rivaroxaban has predictable pharmacokinetic and pharmacodynamic profiles, and a fixed dose can be applied in most patients [3]. Regular therapeutic drug monitoring of rivaroxaban is not necessary [4]. However, in some specific clinical situations, quick quantitative measurements of drug levels could aid medical decisions. These clinical situations include bleeding, accidental or intentional overdose, determination of treatment interruption before an elective surgery, judgement about medication adherence, accumulation of drugs in kidney failure, and so on [5–7].

The most precise quantitative method for the measurement of rivaroxaban plasma concentration is liquid chromatography with tandem mass spectrometry (LC-MS/MS) and is considered as the gold standard [8, 9]. However, this method is usually not available in most clinical settings; moreover, it is not fast enough to address clinical emergencies [10]. The chromogenic anti-Xa assay is recommended as a simpler and quicker quantitative method to assess the level of rivaroxaban in human plasma [11].

Few studies have assessed the correlation of some commercial chromogenic anti-Xa assay kits with the high performance LC-MS/MS (HPLC-MS/MS) method, such as Biophen® DiXal, and it is widely believed that the chromogenic anti-Xa assays are comparable to HPLC-MS/MS [12–21]. A homebred chromogenic anti-Xa assay, Zhenyuan anti-FXa, was also approved by the National Medical Products Administration (NMPA) of China [22]. However, its accuracy and sensitivity have not been evaluated and reported to date.

Currently, expected ranges of rivaroxaban plasma trough concentration (C_{trough}, 12–137 μg/L) and peak concentration (C_{max}, 184–343 μg/L) had been estimated from clinical trials [2], which also has been widely accepted [10, 11, 23]. Considering the special ethnic characteristics of Asian population compared to the Caucasian population, it`s believed that the pharmacokinetics (PK) of rivaroxaban was different, and some studies had reported a higher-than-expected NOAC levels in Asian population [24, 25]. However, limited data had been reported in Chinese patients from real-world clinical setting.

The aims of this study were to (i) determine the rivaroxaban concentration of Chinese patients with NVAF by ultra-high performance LC-MS/MS (UHPLC-MS/MS) and two anti-Xa factor assays (Biophen® DiXal and Zhenyuan anti-Xa) and assess the correlation and agreement between different measurements; (ii) compare the concentration measurements from Chinese patients to the reported expected ranges.

2. Materials And Methods

2.1 Patients

A prospective single-centre study was conducted at Huashan Hospital, Fudan University, from 1 September 2020 to 30 June 2021. The study enrolled patients who were diagnosed with NVAF and treated with rivaroxaban to reach a steady state. For each participant, demographic characteristics, laboratorial tests, medication information were collected at enrollment. CrCl was calculated using Cockcroft-Gault method.

Moreover, CHA2DS2-VASc scores and HAS-BLED scores for each patient were calculated to assess the risk of thromboembolism and bleeding. The study was approved by the Ethics Committee of Huashan Hospital (KY2020-016). Informed consent was obtained from each participant.

Patients would be grouped into two groups: “appropriate dose group” or “inappropriate dose group” according to CrCl. As stated in package insert, 20 mg for patients with CrCl ≥ 50 mL/min and 15 mg for patients with CrCl 15–49 mL/min would be regarded as “appropriate dose”. Other cases would be considered as “inappropriate dose”.

2.2 Sampling

For inpatients, three blood samples were collected at different time points: before dosing, 3 ± 0.5 h and 7 ± 0.5 h after dosing. For outpatients, blood samples were collected opportunistically. The timing for sampling was recorded. For inpatients, the timing of rivaroxaban intake was recorded by nurses; for outpatients, it was recorded by the patients themselves.

For each sampling, 2.7 mL blood samples were collected into 0.109 mol/L trisodium citrate-containing tubes. Within 4 h of sample collection, the collected samples were temporarily stored at 4°C. Platelet-poor plasma was prepared by centrifugation at 3000 × *g* for 10 min at 20°C. The processed samples were transferred into coded plastic tubes: 100 µL for the measurement of rivaroxaban plasma concentration, 200 µL for the chromogenic assay by Biophen DiXal, and 500 µL for the chromogenic assay by Zhenyuan anti-Xa. All samples were stored at − 80°C immediately after processing and were analysed within 3 months.

The study was approved by the Ethics Committee of Huashan Hospital and was conducted in accordance with the Declaration of Helsinki (2013). All participants signed an informed consent before enrollment.

2.3 Bioanalysis

2.3.1 Ultra-high performance liquid chromatography with tandem mass spectrometry

The rivaroxaban plasma concentrations were measured by UHPLC-MS/MS, as described previously [26]. Briefly, 100 µL human plasma samples were mixed with 890 µL of methanol and 10 µL of internal standard (IS, rivaroxaban-d_4) and were centrifuged. The residuals were dried with nitrogen gas at room temperature and were treated with 100 µL of 1% (v/v) formic acid (FA). After centrifugation, 50 µL of the supernatant was mixed with 450 µL of water, and 2 µL of the resulting mixture was injected into the UHPLC-MS/MS system.

Liquid chromatography was performed using the Agilent 1290 infinity series UHPLC system (Agilent Technologies, Santa Clara, CA, USA) and the Agilent ZORBAX Eclipse XDB-C18 (3.5 µm, 2.1 × 100 mm) column, with mobile phase A (0.1% FA in water) and phase B (0.1% FA in acetonitrile). Mass spectrometric analysis was conducted using a 6500 QTRAP®System (SCIEX, USA) triple quadrupole mass spectrometer detector coupled to an electrospray ion (ESI) source.

The calibration curve was linear over the range of 1–1000 ng/mL. The accuracy ranged from 85.9–105%, and the precision was lower than 6.18% variable coefficient at the lower limit of detection (0.5 ng/mL) and at three quality control levels (4, 40, and 400 ng/mL).

2.3.2 Chromogenic anti-Xa assays

Chromogenic anti-Xa assays were performed using two commercial assays, Biophen® DiXal or Biophen® DiXal Low (HYPHEN BioMed, Neuville-sur-Oise, France) and Zhenyuan anti-Xa (Vascutech Diagnosis, Shanghai, China). These two assays are both chromogenic assays for the quantitative measurement of direct FXa inhibitors in human citrated blood plasma. Raw results from the two anti-Xa assays, which were both reported as optical density (OD) value/min by the analysers, were transformed into concentrations based on the calibration curves.

The Biophen® DiXal test was performed according to the manufacturer’s instructions on a CN6000 coagulometer (TOA Medical Electronics Co., Kobe, Japan). Biophen® rivaroxaban control and calibrators for normal and low plasma concentrations were purchased from HYPHEN Biomed Inc. (Neuville-sur-Oise, France). The calibration ranges of Biophen® DiXal and Biophen® DiXal Low were 0–500 and 0–100 ng/mL, respectively. In each working session, two quantity control (QC) samples were tested separately before determination of the plasma samples. Samples lower than 80 ng/mL detected by UHPLC-MS/MS were analysed with Biophen® DiXal Low. Concentrations below 14 ng/mL were not directly reported by the analyser. Based on the original calibration curve and linear relationship between the OD/min and concentration established by the calibrated samples, we manually calculated the estimated concentration with the individual values.

The Zhenyuan anti-Xa test was performed according to the manufacturer’s instructions on an ACL TOP 700 CTS coagulometer (Werfen Medical Device Trading Company, Spain). Matched rivaroxaban controls and calibrators were used in this study. The calibration range of Zhenyuan anti-Xa was 0–450 ng/mL. The Zhenyuan anti-Xa assay included five levels of rivaroxaban calibrators, which were approximately 0,

60, 120, 320, and 480 ng/mL, with specificity within batches. Two QC samples, approximately 100 and 200 ng/mL, were tested separately before the determination of the plasma samples.

2.4 Statistical analysis

Statistical analyses were performed using R software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics of patients were presented as mean ± SD (for continuous variables) or count (for categorical variables).

Comparisons of the rivaroxaban plasma concentrations by Biophen® DiXal and UHPLC-MS/MS method, Zhenyuan anti-Xa and UHPLC-MS/MS were done using Spearman correlation analysis, Deming regression and Bland-Altman analyses. Referring to the previous literatures[15], samples were also grouped for subgroup analysis according to the measured concentration (< 50 μg/L, 50–200 μg/L and > 200 μg/L). Moreover, rivaroxaban concentrations measured in our study were compared with the reported expected ranges (C_{max} 184–343 μg/L, C_{trough} 12–137 μg/L)[2].

3. Results

3.1 Patients and samples

A total of 243 samples from 182 patients taking rivaroxaban were collected. The demographics of patients were shown in Table 1. Of the 182 patients, the proportion of patients with CrCl 15–49, 50–79 and > 80 mL/min were 19.8%, 44.0% and 36.2%. 97 patients (53.5%) were prescribed inappropriate rivaroxaban doses, of which 17.5%, 50.5% and 32.0% were with CrCl 15–49, 50–79 and > 80 mL/min, respectively. Most prescribed inappropriate doses were lower than the appropriate doses. Among patients with CrCl < 50 mL/min, 4 patients (11.1%) were prescribed an inappropriate higher dose.

Table 1
Baseline characteristics of patients

Variables	Median (range)/count	Mean ± SD
Male/female	**102/80**	/
Age (years)	68 [28–96]	66.84 ± 11.74
Body weight (kg)	67.5 [36.5–106]	68.46 ± 12.49
Daily dose (mg)	15 [5–20]	15.8 ± 3.9
CHA2SDS2-VASc score	3 [0–7]	2.71 ± 1.66
HAS-BLED score	2 [0–7]	2.41 ± 1.2
Hemoglobin (g/L)	133 [62–191]	133.92 ± 20.86
Alanine aminotransferase (U/L)	21 [3-204]	27.04 ± 22.56
Aspartate aminotransferase (U/L)	21 [8–75]	23.37 ± 11.13
Total bilirubin (μmol/L)	11.6 [3.3–63.3]	14.62 ± 9.43
Albumin (g/L)	40 [30–50]	39.79 ± 4.09
Serum creatinine (mg/dL)	0.92 [0.25–2.85]	0.94 ± 0.33
Creatinine clearance (mL/min)	71.0 [19.1-163.5]	75.7 ± 29.7

Of the 243 samples, 166, 40, and 37 were taken at 19.5–26 h after the previous dose, 1.5–4 h after dosing, and 4.5–13 h after dosing, respectively. The rivaroxaban plasma concentration ranged from 1.06 to 632.9 ng/mL by UHPLC-MS/MS; from 0 to 460.44 ng/mL by Biophen® DiXal; and from 0 to 334.20 ng/mL by Zhenyuan anti-Xa. Scatterplots of rivaroxaban plasma concentration along with the time after dose are shown in Fig. 1.

In general, variation of peak samples was greater than that of the trough samples. For trough samples, rivaroxaban concentration determined by the UHPLC-MS/MS, Biophen® DiXal and Zhenyuan anti-Xa ranged from 1.06 to 261.00 ng/mL, 0 to 243.97 ng/mL, and 0 to 194.87 ng/mL, respectively.

Thirty-six samples determined by Biophen® DiXal were < 14 ng/mL. Therefore, the concentration was estimated using raw OD ratios. Three samples were measured as 0 ng/mL, and their corresponding concentrations determined by UHPLC-MS/MS were all < 4 ng/mL. The

measurement of 63 samples was 0 ng/mL determined by Zhenyuan anti-Xa, and their corresponding concentration by UHPLC-MS/MS ranged from 1.06 to 66.1 ng/mL.

3.2 Correlation between anti-Xa factor assays and UHPLC-MS/MS

Results from Biophen DiXal and UHPLC-MS/MS, Zhenyuan and UHPLC-MS/MS analyses both showed a strong linear relationship (Fig. 2). The Spearman correlation coefficient and slope in the Deming regression between Biophen DiXal and UHPLC-MS/MS were closer to 1, compared to that between Zhenyuan and UHPLC-MS/MS (Table 2).

The bias from the Bland–Altman analysis was 15.10 ng/mL and 41.87 ng/mL for Biophen DiXal and Zhenyuan, respectively. Overall underestimated measurements were detected by anti-Xa factor assays than by UHPLC-MS/MS. In addition, the extent of underestimation by the Zhenyuan anti-Xa was greater than that by Biophen® DiXal (Fig. 3). Subgroup analysis showed that samples 50–200 ng/mL measured by both anti-Xa factor assays had the best correlation and agreement with the results from UHPLC-MS/MS (Table 2). Zhenyuan could not determine most samples with concentration < 50 ng/mL, manifesting as a linear relationship in the Bland-Altman plot. For samples > 200 ng/mL, both anti-Xa factor assays showed an obvious underestimation (Supplementary Fig. 1).

Table 2
Correlation statistics between coagulation assays and rivaroxaban plasma concentration measured by UHPLC-MS/MS in patients with non-valvular atrial fibrillation

	< 50 µg/L (n = 104)	50–200 µg/L (n = 93)	> 200 µg/L (n = 46)	Total
Biophen DiXal				
Pearson`s correlation coefficient (95% CI)	0.87 (0.83, 0.90)	0.98 (0.95, 0.98)	0.89 (0.53, 0.96)	0.987 (0.978, 0.990)
Deming regression				
Slope (95% CI)	0.93 (0.81, 1.05)	0.96 (0.86, 1.05)	0.72 (0.64, 0.79)	0.83 (0.79, 0.87)
Intercept (95% CI)	-0.26 (-2.88, 2.37)	-8.62 (-17.51, 0.27)	39.8 (19.27, 60.33)	3.87 (0.74, 7.01)
Zhenyuan anti-Xa				
Pearson`s correlation coefficient (95% CI)	0.71 (0.55, 0.79)	0.94 (0.91, 0.96)	0.85 (0.61, 0.93)	0.976 (0.965, 0.983)
Deming regression				
Slope (95% CI)	0.52 (0.40, 0.64)	0.92 (0.81, 1.03)	0.58 (0.49, 0.67)	0.68 (0.65, 0.72)
Intercept (95% CI)	-6.80 (-9.20, -4.40)	-27.80 (-38.85, -16.76)	22.83 (-3.85, 49.52)	-6.90 (-10.07, -3.73)
Prothrombin time (Thromborel S)				
Pearson`s correlation coefficient (95% CI)	0.15 (-0.06, 0.34)	0.27 (0.02, 0.46)	0.24 (-0.06, 0.53)	0.57 (0.47, 0.67)
Deming regression				
Slope (95% CI)	0.0181 (-0.006, 0.0420)	0.0147 (0.0002, 0.0274)	0.0073 (-0.0015, 0.0162)	0.0133 (0.0104, 0.0163)
Intercept (95% CI)	12.75 (12.08, 13.43)	13.20 (11.84, 14.56)	15.07 (12.02, 18.12)	13.11 (12.77, 13.45)

3.3 Anticoagulant monitoring for patients by different assay methods

The distribution of rivaroxaban concentration according to CrCl and the appropriateness of prescribed dose was shown in Fig. 4. Considering different assay used, the proportion of concentration falling within the expected ranges differed greatly. Of all the samples collected 1.5-4 h after dosing (n = 40), the concentrations falling within the expected ranges were 60%, 57.5% and 37.5% by UHPLC-MS/MS, Biophen DiXal and Zhenyuan, respectively. Of all samples collected 19.5–26 h after dosing (n = 166), the concentrations falling within the expected ranges were 74.1%, 75.3% and 46.4% by UHPLC-MS/MS, Biophen DiXal and Zhenyuan, respectively.

There was no significant difference in the distribution of concentration for patients with different renal function, no matter whether took an appropriate dose or not. However, for patients with CrCl > 80 mL/min, those with trough concentrations higher than expected range were generally taking appropriate doses. Similarly, more than half of patients with CrCl 50–79 mL/min were prescribed inappropriate lower dose,

and the trough concentration were less likely to exceed the expected range, even though some samples collected 4 h after dosing were still higher than the expected range of C_{max}.

4. Discussion

In this study, rivaroxaban levels were measured by two chromogenic anti-Xa assays and UHPLC-MS/MS method in 243 samples from 182 rivaroxaban-treated Chinese patients with NVAF. Results showed that a wider range of rivaroxaban concentration from Chinese patients in real-world clinical setting compared to the expected range from clinical trial, no matter what kind of method was used. Moreover, chromogenic anti-Xa assays showed a good correlation with the UHPLC-MS/MS over the range of 50–200 ng/mL, but with a trend of underestimation over the whole range.

A large inter-individual variability of rivaroxaban plasma concentration was observed, and the variability was greatest by UHPLC-MS /MS, followed by Biophen DiXal and Zhenyuan. This range is consistent with previous reports in patients or healthy volunteers [15, 18, 19, 27]. Our studies showed a larger inter-individual variability in both peak levels (115.8–600 ng/mL) and trough levels (1.06–261 ng/mL) measured by UHPLC-MS/MS, compared to previously expected ranges for C_{max} (178–343 ng/mL) and C_{trough} (12–137 ng/mL) for NVAF patients taking 20 mg qd with CrCl ≥ 50 mL/min [2]. The variability of trough and peak concentrations measured in our study was greater, which could be explained by the fact that some patients took a relatively low dose (5 mg qd) and the higher inter-individual variability in patients from real-world clinical practice than in the clinical trial [14].

We observed a high proportion of patients who were not prescribed an inappropriate dose, especially for those with CrCl 50–79 mL/min. Meanwhile, these patients with CrCl 50–79 mL/min had a lower probability of trough concentrations exceeding the expected range, compared to patients with CrCl 15–49 or > 80 mL/min. Lower prescribed NOAC doses in Asian population have been widely reported previously, perhaps due to the consideration of lower body weight, higher proportion of elderly, higher risk of bleeding of Asians[28–31]. Although there was a gap between the rivaroxaban exposure and clinical outcomes till now, some studies showed that a lower rivaroxaban dose did not reduce drug effectiveness and improve the safety in some patients [29, 31, 32].

Chromogenic anti-Xa assays showed a good linear correlation with UHPLC-MS/MS over the quantitative range. However, results showed that there is a systematic underestimation by the chromogenic anti-Xa assays, and the median bias in Biophen DiXal (15.1 ng/mL) was less than in Zhenyuan anti-Xa (41.9 ng/mL). This could be explained by the fact that anti-Xa assays did not directly measure the physical quantity of rivaroxaban in plasma samples, but measured the OD value of chromogenic products hydrolysed from residual FXa based on the assumption that the amount of chromogenic product was proportional to the level of factor activity in the samples[33, 34]. Therefore, anti-Xa assays may not be appropriate in scenarios requiring the detection of low concentrations, such as treatment interruption before an elective surgery, accumulation of drugs in kidney failure, judgement about medication adherence, and so on.

A few studies have reported the correlation between Biophen DiXal and HPLC-MS/MS in plasma samples [13–15, 18–20]. In general, Biophen DiXal showed a good correlation with HPLC-MS/MS, especially for concentrations < 200 ng/mL. The overall bias for Biophen DiXal was at 15.1 ng/mL, which was within the range reported in previous studies (-11.3 to 28.7 ng/mL) [13–15, 18–20]. Due to the limited sample size, the majority of previous studies could only assess correlation in samples with concentrations < 200 ng/mL, or only included several samples with high levels of concentration. Our study included 46 samples > 200 ng/mL and demonstrated that the anti-Xa assays may not be accurate enough for high-level samples.

Zhenyuan anti-Xa has been approved for several years by the NMPA; however, its correlation with HPLC-MS/MS has not yet been reported. Only a few studies have assessed its precision, accuracy, and linearity in healthy volunteers [35], or measured peak and trough concentrations in NVAF patients [36]. Our study demonstrated that Zhenyuan anti-Xa may be used to measure concentrations between 50–200 ng/mL. It should be recognised that Zhenyuan anti-Xa was only approved for the quantitative analysis of low molecular weight heparin and unfractionated heparin in clinical settings. Even though we used the rivaroxaban-specific control and calibrator samples, the accuracy and sensitivity of Zhenyuan anti-Xa were still inferior to Biophen DiXal overall, which indicates need for further optimisation of this assay.

In clinical practice, trough samples are frequently used to assess the degree of anticoagulation for patients in non-emergent situations [5]. In the subgroup analysis of trough concentrations at different doses, we found that Biophen DiXal exhibited better accuracy and precision than Zhenyuan anti-Xa. The application of Zhenyuan anti-Xa in the measurement of trough concentration may be limited, especially for patients taking rivaroxaban lower than 10 mg qd. It should be noted that a trough concentration of 0 ng/mL by Zhenyuan anti-Xa does not exclude the absence of rivaroxaban.

Our study has several limitations. First, we did not compare the correlation of the Biophen DiXal standard calibrator and low calibrator in the same sample. The Biophen DiXal Low calibrator was only used for samples < 80 ng/mL, and other samples were measured with a standard calibrator. Second, we only included 46 samples > 200 ng/mL, which may limit the interpretation of the correlation at high concentrations.

Further studies may focus on the comparison of samples with high concentrations, which are common in clinical situations, such as major bleeding, overdose, and accumulation of rivaroxaban in renal failure [5–7]. Third, the lack of data about clinical outcome prevented us from analyzing dose-response relationship in Chinese patients. The results could only focus on the concentration level till now.

5. Conclusion

In conclusion, compared with the expected ranges of rivaroxaban concentration estimated from clinical trials, the measured concentration in patients with NVAF from real-world clinical setting were with higher inter-individual variability. The results were independent on the assays used, including UHPLC-MS/MS and chromogenic anti-Xa assays. When prescribed an appropriate rivaroxaban dose, the rivaroxaban concentration in some Chinese patients tended to exceed the expected ranges.

Declarations

Acknowledgement

This project was supported by the National Science Foundation for Youth Scientists of China (Grant No. 81703613), the Shanghai "Rising Stars of Medical Talent" Youth Development Program (Youth Medical Talents–Clinical Pharmacist Program) and Shanghai Key Clinical Specialty Projects-Clinical Pharmacy to MZ (shslczdzk06502). We would thank Editage (www.editage.cn) for English language editing.

Availability of data and materials

The data supporting the findings of the study are included within the article. Raw data are available from the corresponding authors on request.

Authors' contribution

XQL, YG, HYD and CLM conceived and planned the research; XQL, YG and YFZ performed the sample analysis; XQL, YG, WS and ZCG performed the statistical analysis; XQL and YG created the initial draft of the manuscript; MKZ and CLM funded the research. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Ethics approval and consent to participate

The protocol was approved by the Ethics Committee of Huashan Hospital, Fudan University. Informed consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

There are no conflicts of interest.

References

1. Vimalesvaran K, Dockrill SJ and Gorog DA Role of rivaroxaban in the management of atrial fibrillation: insights from clinical practice. Vasc Health Risk Manag. 2018; 14:13–21. https://doi.org/10.2147/VHRM.S134394.
2. Mueck W, Stampfuss J, Kubitza D and Becka M Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet. 2014; 53(1):1–16. https://doi.org/10.1007/s40262-013-0100-7.
3. Ingrasciotta Y, Crisafulli S, Pizzimenti V, Marciano I, Mancuso A, Ando G, et al. Pharmacokinetics of new oral anticoagulants: implications for use in routine care. Expert Opin Drug Metab Toxicol. 2018; 14(10):1057–69. https://doi.org/10.1080/17425255.2018.1530213.
4. Testa S, Paoletti O, Legnani C, Dellanoce C, Antonucci E, Cosmi B, et al. Low drug levels and thrombotic complications in high-risk atrial fibrillation patients treated with direct oral anticoagulants. J Thromb Haemost. 2018; 16(5):842–48. https://doi.org/10.1111/jth.14001.

5. Gosselin RC, Adcock DM and Douxfils J An update on laboratory assessment for direct oral anticoagulants (DOACs). Int J Lab Hematol. 2019; 41 Suppl 1:33–39. https://doi.org/10.1111/ijlh.12992.
6. Douxfils J, Ageno W, Samama CM, Lessire S, Ten Cate H, Verhamme P, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost. 2018; 16(2):209–19. https://doi.org/10.1111/jth.13912.
7. Dale BJ, Chan NC and Eikelboom JW Laboratory measurement of the direct oral anticoagulants. Br J Haematol. 2016; 172(3):315–36. https://doi.org/10.1111/bjh.13810.
8. Douxfils J, Mani H, Minet V, Devalet B, Chatelain B, Dogne JM, et al. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. Biomed Res Int. 2015; 2015:345138. https://doi.org/10.1155/2015/345138.
9. Derogis PB, Sanches LR, de Aranda VF, Colombini MP, Mangueira CL, Katz M, et al. Determination of rivaroxaban in patient's plasma samples by anti-Xa chromogenic test associated to High Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS). PLoS One. 2017; 12(2):e0171272. https://doi.org/10.1371/journal.pone.0171272.
10. Douxfils J, Adcock DM, Bates SM, Favaloro EJ, Gouin-Thibault I, Guillermo C, et al. 2021 Update of the International Council for Standardization in Haematology Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2021; 121(8):1008–20. https://doi.org/10.1055/a-1450-8178.
11. Gosselin RC, Adcock DM, Bates SM, Douxfils J, Favaloro EJ, Gouin-Thibault I, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2018; 118(3):437–50. https://doi.org/10.1055/s-0038-1627480.
12. Steffel J, Collins R, Antz M, Cornu P, Desteghe L, Haeusler KG, et al. 2021 European Heart Rhythm Association practical guide on the use of non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. Europace. 2021; 23(10):1612–76. https://doi.org/10.1093/europace/euab065.
13. Schmitz EM, Boonen K, van den Heuvel DJ, van Dongen JL, Schellings MW, Emmen JM, et al. Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. J Thromb Haemost. 2014; 12(10):1636–46. https://doi.org/10.1111/jth.12702.
14. Königsbrügge O, Quehenberger P, Belik S, Weigel G, Seger C, Griesmacher A, et al. Anti-coagulation assessment with prothrombin time and anti-Xa assays in real-world patients on treatment with rivaroxaban. Ann Hematol. 2015; 94(9):1463–71. https://doi.org/10.1007/s00277-015-2407-y.
15. Studt JD, Alberio L, Angelillo-Scherrer A, Asmis LM, Fontana P, Korte W, et al. Accuracy and consistency of anti-Xa activity measurement for determination of rivaroxaban plasma levels. J Thromb Haemost. 2017; 15(8):1576–83. https://doi.org/10.1111/jth.13747.
16. Cini M, Legnani C, Padrini R, Cosmi B, Dellanoce C, De Rosa G, et al. DOAC plasma levels measured by chromogenic anti-Xa assays and HPLC-UV in apixaban- and rivaroxaban-treated patients from the START-Register. Int J Lab Hematol. 2020; 42(2):214–22. https://doi.org/10.1111/ijlh.13159.
17. Zhang Y, Qian Q, Qian G and Sun G Laboratory monitoring of rivaroxaban and assessment of its bleeding risk. Br J Biomed Sci. 2016; 73(3):134–39. https://doi.org/10.1080/09674845.2016.1195151.
18. Douxfils J, Tamigniau A, Chatelain B, Chatelain C, Wallemacq P, Dogné JM, et al. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. Thromb Haemost. 2013; 110(4):723–31. https://doi.org/10.1160/th13-04-0274.
19. Jensen KOF, Hansen SH, Goetze JP, Jesting A, Stensballe J and Hansen H Preliminary report: Measurement of apixaban and rivaroxaban in plasma from bleeding patients. Eur J Haematol. 2017; 99(5):431–36. https://doi.org/10.1111/ejh.12942.
20. Lessire S, Douxfils J, Pochet L, Dincq AS, Larock AS, Gourdin M, et al. Estimation of Rivaroxaban Plasma Concentrations in the Perioperative Setting in Patients With or Without Heparin Bridging. Clin Appl Thromb Hemost. 2018; 24(1):129–38. https://doi.org/10.1177/1076029616675968.
21. Henskens YMC, Gulpen AJW, van Oerle R, Wetzels R, Verhezen P, Spronk H, et al. Detecting clinically relevant rivaroxaban or dabigatran levels by routine coagulation tests or thromboelastography in a cohort of patients with atrial fibrillation. Thromb J. 2018; 16:3. https://doi.org/10.1186/s12959-017-0160-2.
22. National Medical Products Administration. Domestic medical equipment products 2021 [Available from: http://app1.nmpa.gov.cn/data_nmpa/face3/base.jsp?tableId=26&tableName=TABLE26&title=%B9%FA%B2%FA%D2%BD%C1%C6%C6%F7%D0%B5%B2%FA%C6%B7%A3%A8%D7%A2%B2%E1%A3%A9&bcId=152904417281669781044048234789.
23. Samuelson BT and Cuker A Measurement and reversal of the direct oral anticoagulants. Blood Rev. 2017; 31(1):77–84. https://doi.org/10.1016/j.blre.2016.08.006.

24. Lin SY, Kuo CH, Yeh SJ, Tsai LK, Liu YB, Huang CF, et al. Real-World Rivaroxaban and Apixaban Levels in Asian Patients With Atrial Fibrillation. Clin Pharmacol Ther. 2020; 107(1):278–86. https://doi.org/10.1002/cpt.1601.
25. Ng Tsai HO, Goh JJN, Aw JWX, Lin Y, Fong AYY, Tiong LL, et al. Comparison of rivaroxaban concentrations between Asians and Caucasians and their correlation with PT/INR. J Thromb Thrombolysis. 2018; 46(4):541–48. https://doi.org/10.1007/s11239-018-1726-y.
26. Zhang YF, Liu XQ, Wang Y, Xu X, Zhong MK, Zhang P, et al. Development and validation of an ultra-high performance liquid chromatography with tandem mass spectrometry method for the simultaneous quantification of direct oral anticoagulants in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2021; 1182:122952. https://doi.org/10.1016/j.jchromb.2021.122952.
27. Francart SJ, Hawes EM, Deal AM, Adcock DM, Gosselin R, Jeanneret C, et al. Performance of coagulation tests in patients on therapeutic doses of rivaroxaban. A cross-sectional pharmacodynamic study based on peak and trough plasma levels. Thromb Haemost. 2014; 111(6):1133–40. https://doi.org/10.1160/TH13-10-0871.
28. Cho MS, Yun JE, Park JJ, Kim YJ, Lee J, Kim H, et al. Pattern and Impact of Off-label Underdosing of Non-Vitamin K Antagonist Oral Anticoagulants in Patients With Atrial Fibrillation Who are Indicated for Standard Dosing. Am J Cardiol. 2020; 125(9):1332–38. https://doi.org/10.1016/j.amjcard.2020.01.044.
29. Chan YH, Lee HF, Wang CL, Chang SH, Yeh CH, Chao TF, et al. Comparisons of rivaroxaban following different dosage criteria (ROCKET AF or J-ROCKET AF trials) in Asian patients with atrial fibrillation. J Am Heart Assoc. 2019; 8(21):e013053. https://doi.org/10.1161/JAHA.119.013053.
30. Lee SR, Lee YS, Park JS, Cha MJ, Kim TH, Park J, et al. Label Adherence for Non-Vitamin K Antagonist Oral Anticoagulants in a Prospective Cohort of Asian Patients with Atrial Fibrillation. Yonsei Med J. 2019; 60(3):277–84. https://doi.org/10.3349/ymj.2019.60.3.277.
31. Lee HF, Chan YH, Tu HT, Kuo CT, Yeh YH, Chang SH, et al. The effectiveness and safety of low-dose rivaroxaban in Asians with non-valvular atrial fibrillation. Int J Cardiol. 2018; 261:78–83. https://doi.org/10.1016/j.ijcard.2018.03.063.
32. Huang HY, Lin SY, Cheng SH and Wang CC Effectiveness and Safety of Different Rivaroxaban Dosage Regimens in Patients with Non-Valvular Atrial Fibrillation: A Nationwide, Population-Based Cohort Study. Sci Rep. 2018; 8(1):3451. https://doi.org/10.1038/s41598-018-21884-y.
33. Samama MM, Amiral J, Guinet C, Perzborn E and Depasse F An optimised, rapid chromogenic assay, specific for measuring direct factor Xa inhibitors (rivaroxaban) in plasma. Thromb Haemost. 2010; 104(5):1078–9. https://doi.org/10.1160/TH10-03-0204.
34. Newall F Anti-factor Xa (anti-Xa) assay. Methods in molecular biology (Clifton, N.J.). 2013; 992:265 – 72. https://doi.org/10.1007/978-1-62703-339-8_19.
35. Fu L, Li J, Dong LM, Bi L, Wang Y, Liu MM, et al. Determination of heparin, low molecular weight heparin and rivaroxaban by anti-Xa activity Int J Lab Med. 2020; 41(10):1218–21, 25 (in Chinese).
36. Wang Y, Meng XY, Yang CG, Lu Y, Ren J and Wang F Correlation between the rivaroxaban concentration and bleeding events in patients with non-valvular atrial fibrillation Chin J Geriatr 2020; 39(5):501–04 (in Chinese).

Figures

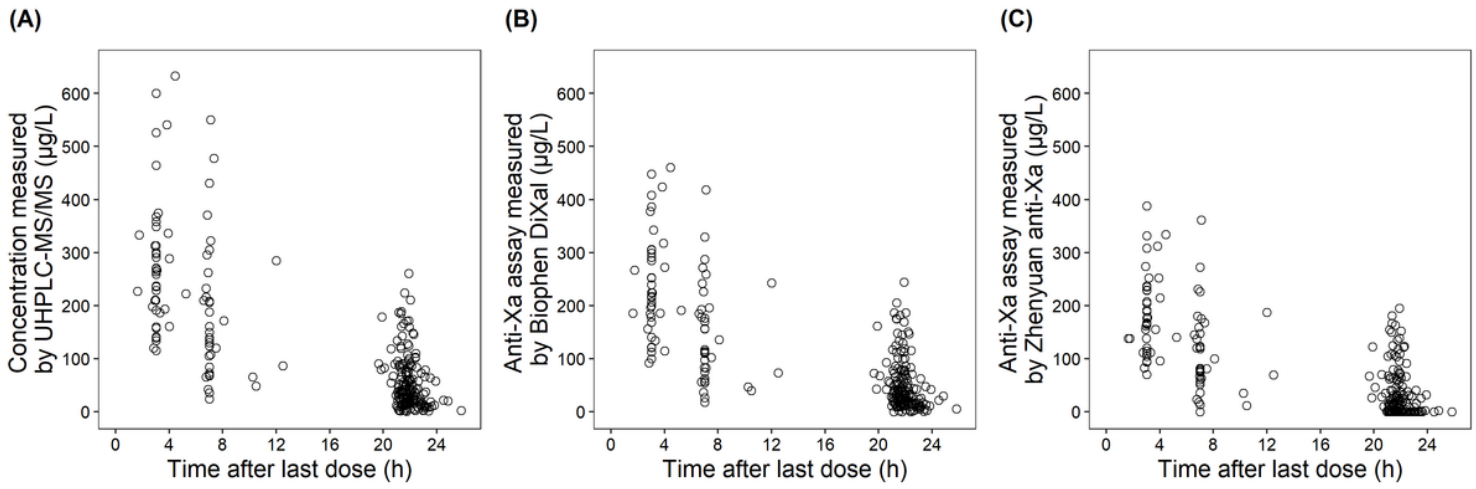

Figure 1

Scatterplots of rivaroxaban plasma concentration measured by (a) UHPLC-MS/MS; anti-Xa assays measured by (b) Biophen DiXal, (c) Zhenyuan anti-Xa with the time after last dose

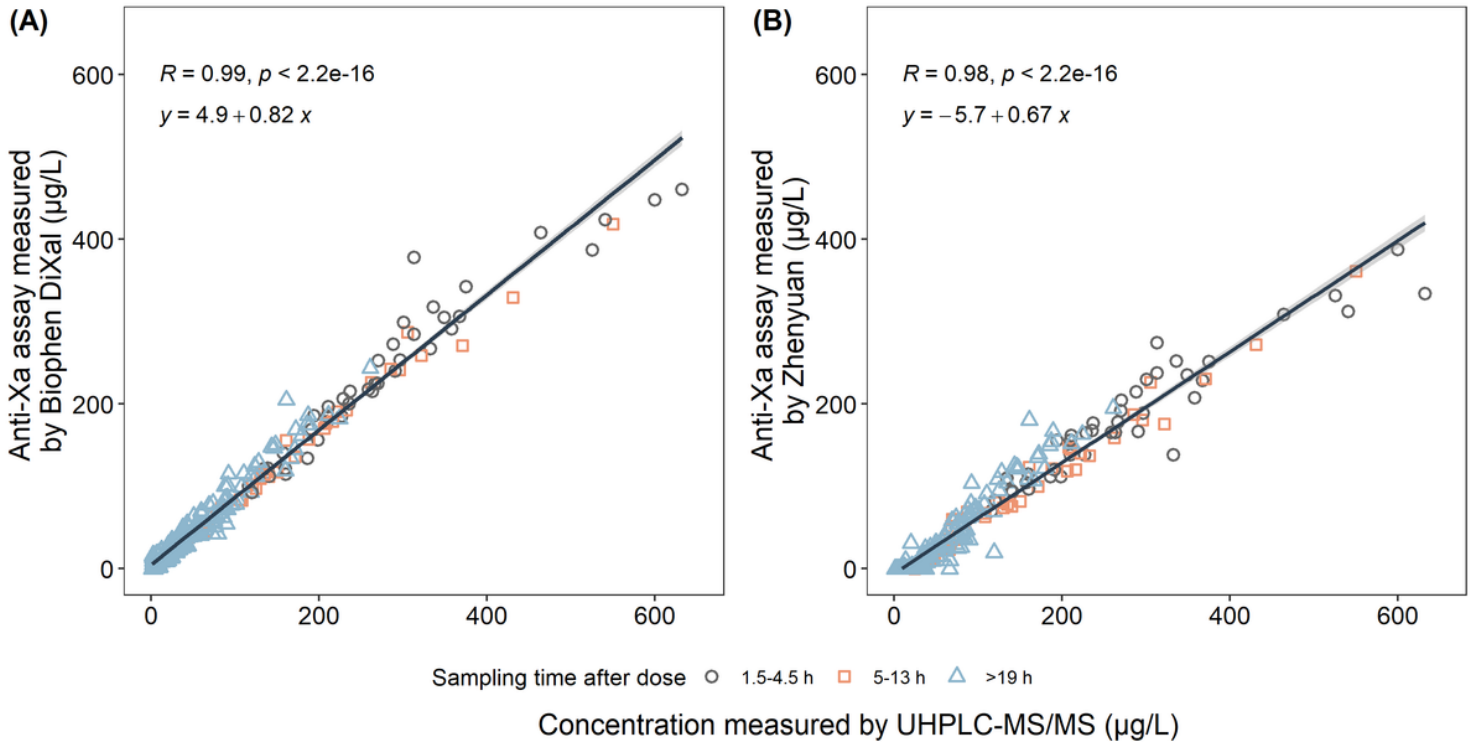

Figure 2

Linear regression between (a) Biophen DiXal, (b) Zhenyuan anti-Xa and UHPLC-MS/MS

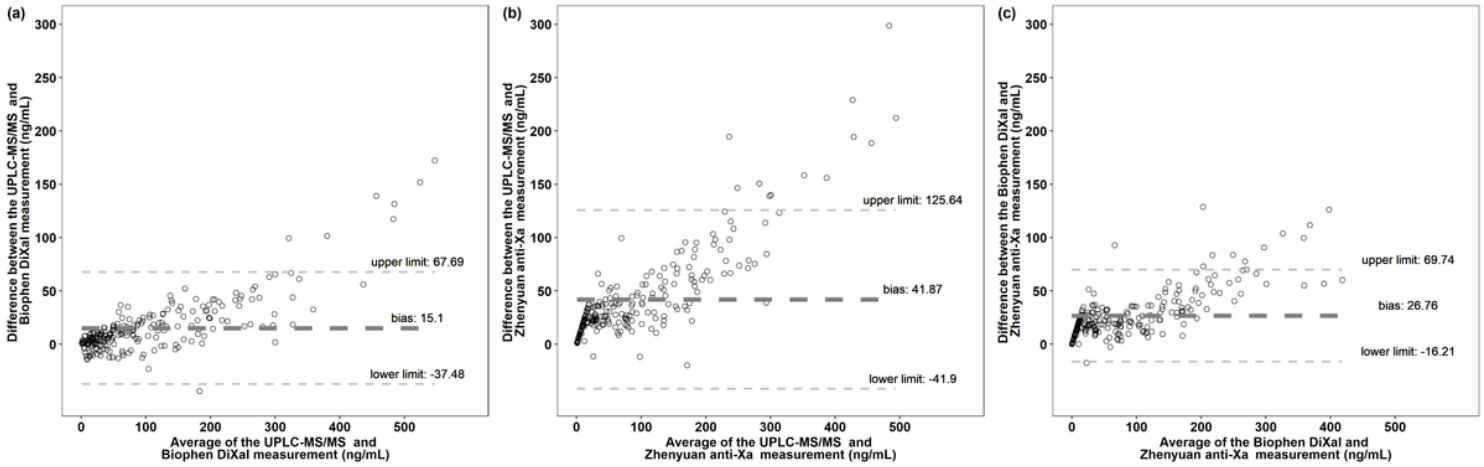

Figure 3

Bland–Altman analyses comparing UHPLC-MS/MS, Biophen DiXal, Zhenyuan anti-Xa

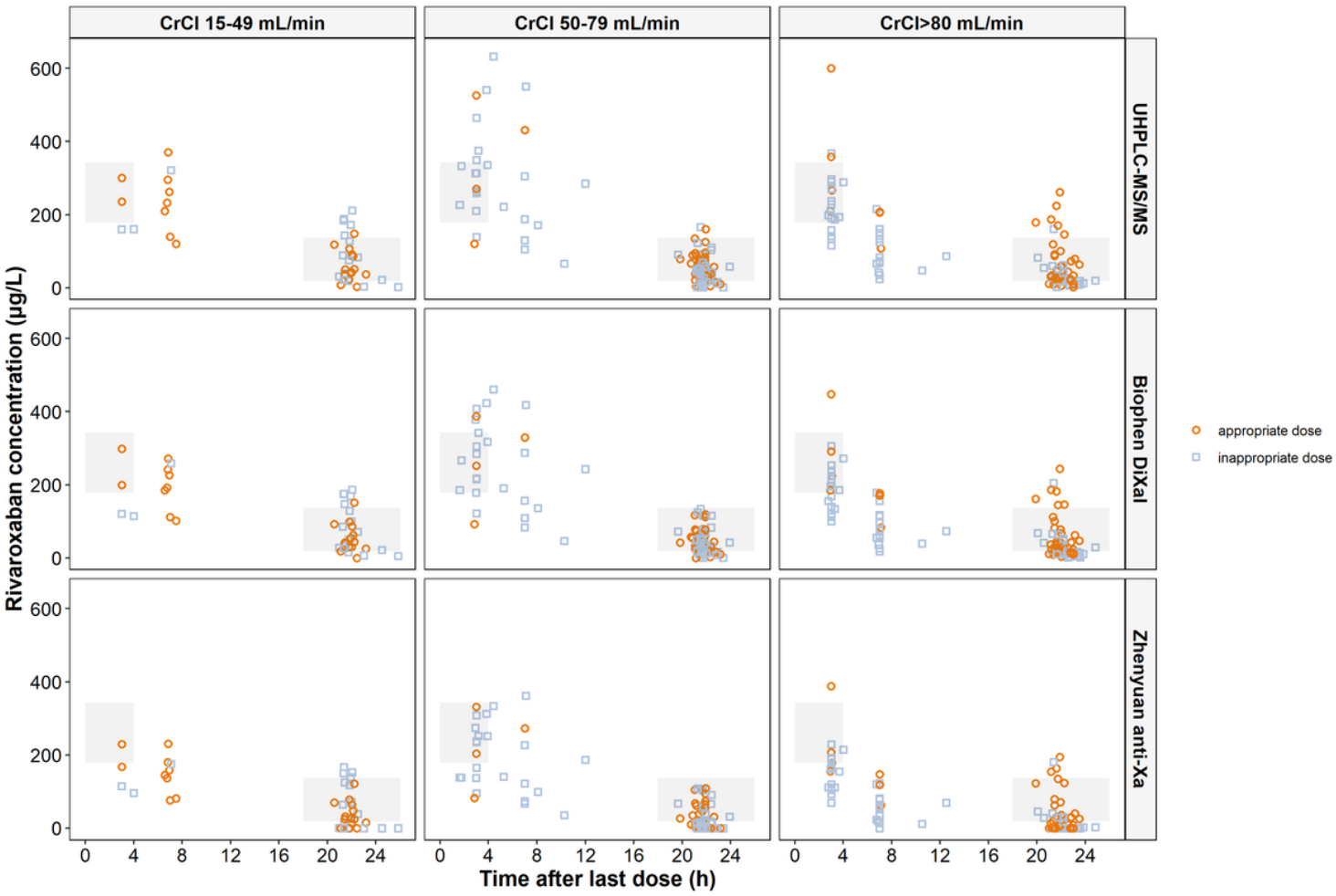

Figure 4

The distribution of rivaroxaban concentration according to creatinine clearance and the appropriateness of prescribed doses

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.tiff