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RESEARCH ARTICLE

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Evaluation of global laboratory methods and establishing on-therapy ranges for monitoring apixaban and rivaroxaban: Experience at a single institution

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Background: Apixaban and rivaroxaban are approved for the prevention and treatment of deep vein thrombosis (DVT), pulmonary embolism (PE), and embolic stroke in atrial fibrillation (AF) patients. The aim of this study was to find appropriate methods of monitoring the anticoagulant effects of are direct oral anticoagulants (DOACs) and establish on-therapy ranges using conventional tests.

Methods: A total of 184 samples were collected from 91 patients receiving DOACs. Concentrations of apixaban and rivaroxaban in plasma were accessed by an anti-factor Xa chromogenic assay. PT, APTT, antithrombin, D-dimer, dRVVT screen/confirm, FDP, and fibrinogen levels were measured. On-therapy ranges were calculated by substituting previously reported trough plasma concentrations of DOACs.

Results: Anti-factor Xa chromogenic assay-based DOACs levels were 26.0-279.5 (115.9 ± 56.5) ng/mL for apixaban at 2.5 mg BID, 19.9-565.1 (205.3 ± 162.4) ng/mL for apixaban at 5 mg BID, 2.3-395.3 (205.3 ± 162.4) ng/mL for rivaroxaban at 15 mg OD, 3.6-494.8 (119.6 ± 95.1) ng/mL for rivaroxaban at 20 mg OD, and 9.6-431.4 (140.8 ± 113.6) ng/mL for rivaroxaban at 15 mg BID. PT (%), antithrombin, and dRVVT confirm tests showed good correlation with plasma apixaban levels. Plasma rivaroxaban concentrations were correlated well with PT (sec), PT (%),and dRVVT confirm results. On-therapy ranges established for dRVVT confirm test by linear regression were as follows: 1.32-1.52 for apixaban 2.5 mg BID, 1.12-1.75 for apixaban 5 mg BID, 1.11-1.78 for rivaroxaban 15 mg OD, 1.09-1.64 for rivaroxaban 20 mg OD, and 1.22-1.81 for rivaroxaban 20 mg BID.

Conclusions: Apixaban concentrations were well correlated with PT (%), antithrombin, and dRVVT confirm test. Rivaroxaban concentrations showed good correlation with PT (sec), PT (%), and dRVVT confirm test.

KEYWORDS

anti-factor Xa activity, antithrombin, apixaban, dilute Russell viper venom time, prothrombin time, rivaroxaban

1 | INTRODUCTION

Direct oral anticoagulants (DOACs), such as apixaban and rivaroxaban, are designed to reduce the inconvenience of frequent monitoring during heparin and warfarin therapy. Apixaban and rivaroxaban are approved for the prevention and treatment of deep vein thrombosis (DVT), pulmonary embolism (PE), and embolic stroke in atrial fibrillation (AF) patients.[1,2] Apixaban and rivaroxaban are direct factor Xa inhibitors. Peak plasma apixaban concentration is reached approximately three hours post-dose and its average elimination half-life is 12 hours. Apixaban has been reported to be safe and well tolerated following when administered as single doses of up to 50 mg in healthy subjects without a history of clinically relevant bleeding events.[4] Rivaroxaban is also absorbed rapidly and maximum plasma concentration is reached only 2-4 hours after single doses up to 80 mg, and in healthy young subjects, it is eliminated from plasma with a half-life of 5-9 hours.[5,6] Both apixaban and rivaroxaban are eliminated in liver and kidneys.[4,7] DOACs generally do not require routine monitoring because they have predictable pharmacokinetics and pharmacodynamics, but measurement of plasma DOAC level may be desired in special circumstances, such as, after a suspected overdose, in cases of renal or hepatic insufficiency, in the elderly, and in those with a low body weight.[4,6,8] Recently, the clinical administration of DOACs has increased rapidly in the contexts of stroke prevention in patients with AF and for the treatment of DVT and PE. Nonetheless, intracranial hemorrhage (0.5%) and fatal bleeding (0.2%) can occur in patients receiving DOACs.[9]

No therapeutic ranges for DOACs have been published based on the results of clinical trials and potential interferences by clinical and laboratory parameters have not been well characterized.[10,11] Manufacturers and previous reports provide only peak and/or trough plasma concentrations as determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS), and thus, term of on-therapy range was used in the current study, as has been previously described.[10,12]

Liquid chromatography/tandem mass spectrometry is the gold standard method for measuring plasma DOAC levels, but DOAC levels vary widely from trough to peak among individualpatients.[13,14] The anti-factor Xa chromogenic assay shows excellent linearity with plasma DOAC levels and is more rapid and much less expensive than LC-MS/MS[15,16] Measurement of anti-factor Xa activity is also useful in high-risk patients, such as the elderly, and those with low body weight or low renal function.[18] However, neither LC-MS/MS nor the anti-factor Xa chromogenic assay are widely available for routine clinical testing in many laboratories.

The aims of the present study were as follows: to find an appropriate method for monitoring the anticoagulant effects of DOACs, to evaluate the correlation between anti-factor Xa activity-based plasma DOAC concentrations and the results of

TABLE 1 Clinical characteristic of the study subjects receiving apixaban

	Apixaban 2.5 mg BID	Apixaban 5 mg BID	*P*-value
Samples	71	28	
Male/Female	26/45	17/11	0.042
Apixaban level (ng/mL, mean ±SD)	26.0-279.5 (115.9 ± 56.5)	19.9-565.1 (205.3 ± 162.4)	0.025
Age (y, mean ±SD)	56-89 (78.5 ± 8.5)	44-88 (68.7 ± 13.3)	0.000
Body weight (kg, mean ±SD)	35-75 (52.6 ± 11.2)	40-94 (64.0 ± 12.5)	0.000
BMI (kg/m^2, mean ±SD)	15.5-29.4 (21.4 ± 3.8)	14.7-31.6 (23.8 ± 3.9)	0.008
Creatinine (mg/dL, mean ±SD)	0.18-2.7 (0.91 ± 0.51)	0.40-1.20 (0.77 ± 0.21)	0.552
CrCl[a] (mL/min, mean ±SD)	18.4-137.7 (62.4 ± 28.8)	42.9-156.7 (90.0 ± 36.7)	0.000
Impression			
AF (%)	68 (95.8)	28 (100)	0.556
DVT or PE (%)	3 (4.2)	0 (0.0)	
Medication			
Heparin (%)	1 (1.4)	0 (0.0)	1.000
Warfarin (%)	0 (0.0)	0 (0.0)	
Aspirin (%)	9 (12.7)	5 (17.9)	0.530
Clopidogrel (%)	8 (11.3)	1 (3.6)	0.439

AF, atrial fibrillation; BMI, body mass index; CrCl, creatinine clearance; DOAC, direct oral anticoagulant; DVT, deep vein thrombosis; PE, pulmonary embolism.

[a]Creatinine clearance (CrCl) was calculated using the Cockcroft-Gault formula.

conventional coagulation assays, such as, prothrombin time (PT), activated partial thromboplastin time (APTT), dilute Russell viper venom time (dRVVT) screen and dRVVT confirm results, and antithrombin, D-dimer, fibrin degradation product (FDP), and fibrinogen levels. We also tried to establish on-therapy ranges using conventional tests that showed strong correlations with anti-factor Xa activity-based plasma DOAC concentrations by ex vivo plasma samples.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

One hundred eighty-four blood samples were collected in 3.2% sodium citrate tubes (Becton Dickenson, Franklin Lakes, NJ) from 91 patients receiving DOACs at Gachon University Gil Medical Center from January to August 2017. Blood samples of patients with creatinine clearance rate <15 mL/min or BMI ≥40 kg/m^2 were excluded. Blood samples were centrifuged at 3000 *g* for 5 minutes and plasma was then frozen at −70°C in aliquots of 1 mL. APTT was measured before freezing and after thawing plasma to determine sample quality. Ninety-nine samples from patients taking apixaban and 85 samples from patients taking ribaroxan were obtained at trough concentrations. Thirteen patients were excluded because plasma DOAC was not detected or samples were insufficient. The study was approved by the Institutional Review Board (IRB) of Gachon University Gil Medical Center (No. GAIRB2017-261).

2.2 | Quantification of plasma apixaban and rivaroxaban levels using the anti-factor Xa chromogenic assay

Thawed plasma samples were used. Concentrations of apixaban and rivaroxaban were determined using an anti-factor Xa chromogenic assay performed using HemosIL® liquid anti-Xa kit (Instrumentation Laboratory, Bedford, MA), HemosIL® apixaban calibrators and controls (Instrumentation Laboratory), and HemosIL® rivaroxaban calibrators and controls (Instrumentation Laboratory) on an ACL TOP 700 CTS (Instrumentation Laboratory). The anti-factor Xa chromogenic assay was performed according to the manufacturer's instructions.

2.3 | Conventional coagulation assays

Prothrombin time (sec) (reference range 9.5-13.0 sec), PT (%) (reference range 70%-130%), APTT (reference range 27.0-395 sec), antithrombin (reference range 85%-135%), D-dimer (reference range 0.-0.22 μg/mL), dRVVT screen (reference range <1.16), dRVVT confirm (reference range <1.22), FDP (reference range 0-2.3 μg/mL), and fibrinogen (reference range 220-480 mg/dL) (HemosIL®, Instrumentation Laboratory) levels were measured on the ACL TOP 700 CTS using thawed plasma.

2.4 | Establishing on-therapy ranges

We referred to the Clinical and Laboratory Standards Institute (CLSI) guideline H47-A2 that has been used for establishing the therapeutic

TABLE 2 Clinical characteristics of the study subjects receiving rivaroxaban

	Rivaroxaban 15 mg OD	Rivaroxaban 20 mg OD	Rivaroxaban 15 mg BID	*P*-value
Samples	13	36	36	
Male/Female	6/7	24/12	16/20	0.137
Rivaroxaban level (ng/mL, mean ± SD)	2.3-395.3 (70.0 ± 106.3)	3.6-494.8 (119.6 ± 95.1)	9.6-431.4 (140.8 ± 113.6)	0.010
Age (y, mean ± SD)	56-88 (80.2 ± 8.1)	60-96 (76.0 ± 9.8)	22-88 (65.7 ± 17.6)	0.003
Body weight (kg, mean ± SD)	45-70 (60.1 ± 7.4)	42-70 (55.5 ± 7.5)	38-100 (57.9 ± 13.9)	0.290
BMI (kg/m^2, mean ± SD)	20.0-27.3 (23.8 ± 3.0)	18.4-33.7 (21.7 ± 3.8)	15.8-28.9 (21.4 ± 3.4)	0.088
Creatinine (mg/dL, mean±SD)	0.60-1.60 (0.90 ± 0.32)	0.36-1.87 (0.80 ± 0.32)	0.17-2.10 (0.54 ± 0.38)	0.000
CrCl[a] (mL/min, mean ± SD)	27.7-97.8 (62.7 ± 25.0)	26.5-210.7 (70.7 ± 35.3)	22.3-301.0 (144.8 ± 69.5)	0.000
Impression				
AF (%)	12 (92.3)	26 (72.2)	0 (0.0)	0.000
DVT or PE (%)	1 (7.7)	10 (27.8)	36 (100)	
Medication				
Heparin (%)	0 (0.0)	11 (30.6)	7 (19.4)	0.065
Warfarin (%)	0 (0.0)	0 (0.0)	0 (0.0)	
Aspirin (%)	3 (23.1)	8 (22.2)	0 (0.0)	0.010
Clopidogrel (%)	1 (7.7)	7 (19.4)	0 (0.0)	0.018

AF, atrial fibrillation; BMI, body mass index; CrCl, creatinine clearance; DOAC, direct oral anticoagulant; DVT, deep vein thrombosis; PE, pulmonary embolism.

[a]Creatinine clearance (CrCl) was calculated using the Cockcroft-Gault formula.

TABLE 3 Associations between apixaban concentrations and conventional coagulation test results as determined by univariate linear regression

	Apixaban 2.5 mg BID		Apixaban 5 mg BID	
	Correlation	*P*-value	Correlation	*P*-value
PT (sec)	0.199	0.096	0.817	0.000
PT (%)	−0.332	0.005	−0.783	0.000
APTT	−0.032	0.789	0.358	0.061
Antithrombin	0.395	0.001	0.745	0.000
D-dimer	0.039	0.744	−0.124	0.528
dRVVT_Scr	0.204	0.088	0.853	0.000
dRVVT_Con	0.367	0.002	0.847	0.000
FDP	0.051	0.672	−0.027	0.892
Fibrinogen	0.042	0.725	−0.018	0.929

APTT, activated partial thromboplastin time; Con, confirm; dRVVT, dilute Russell viper venom time; FDP, fibrin degradation product; PT, prothrombin time, Scr, screen.

range of APTT for unfractionated heparin therapy using anti-factor Xa chromogenic assay in the clinical laboratories.[19] Relationships between plasma DOAC levels and conventional coagulation assay results significant at the $P < 0.01$ level were evaluated using regression models. Linear relationship (y = slope (x) + intercept)with adjusted R-square values and correlation (r) were also determined. Conventional coagulation assays with r of 0.7-1 were considered to have a "strong linear relationship" with plasma DOAC levels. The established on-therapy ranges were calculated by substituting previously reported trough concentrations of DOACs using the fitted line [1,20,21]

2.5 | Statistical analysis

The analysis was performed using SPSS statistics 24 (IBM Corporation, Armonk, NY). Fisher's Exact tests for categorical variables and Mann-Whitney tests and Kruskal-Wallis tests for continuous variables were used determine the significances of differences between clinical characteristics. Pearson's correlation coefficients were used to determine levels of correlation between DOAC levels and conventional coagulation tests. Linear regression was used to establish the on-therapeutic ranges of variables. Statistical significance was accepted for *P*-value of <0.01.

3 | RESULTS

3.1 | Clinical characteristics of the study subjects

The clinical characteristics are summarized in Tables 1 and 2. Of the 184 samples, 71 were from patients taking apixaban 2.5 mg twice a day (BID), 28 were from patients taking apixaban 5 mg BID, 13 were from patients taking rivaroxaban 15 mg once daily (OD), 36 were from patients taking rivaroxaban 20 mg OD, and 36 were from patients taking rivaroxaban 15 mg BID. Anti-factor Xa chromogenic assay-based plasma DOAC levels were 26.0-279.5 (115.9 ± 56.5) ng/mL for apixaban 2.5 mg BID, 19.9-565.1 (205.3 ± 162.4) ng/mL on apixaban 5 mg BID, 2.3-395.3 (205.3 ± 162.4) ng/mL for rivaroxaban 15 mg OD, 3.6-494.8 (119.6 ± 95.1) ng/mL for rivaroxaban 20 mg OD, and 9.6-431.4 (140.8 ± 113.6) ng/mL for rivaroxaban 15 mg BID. Plasma concentrations of apixaban (*P*-value 0.025) and rivaroxaban (*P*-value 0.010) tended to increase with increasing dosages (Tables 1 and 2). Furthermore, the proportions of older patients and patients with higher creatinine clearance were lower among those receiving lower daily dosages of apixaban and rivaroxaban. Plasma apixaban levels were similar in patients treated or not with heparin, aspirin, and clopidogrel. The plasma concentrations of rivaroxaban showed significant difference between AF and DVT or PE patients (Table 2).

3.2 | Subgroup analyses of patients administered apixaban 2.5 mg BID

Prpthrombin time (%), antithrombin, and dRVVT confirm levels were significantly correlated with plasma apixaban levels (Table 3).

TABLE 4 Associations between rivaroxaban concentrations and conventional coagulation test results as determined by univariate linear regression

	Rivaroxaban 15 mg OD		Rivaroxaban 20 mg OD		Rivaroxaban 15 mg BID	
	Correlation	*P*-value	Correlation	*P*-value	Correlation	*P*-value
PT (sec)	0.986	0.000	0.881	0.000	0.847	0.000
PT (%)	−0.861	0.000	−0.781	0.000	−0.858	0.000
APTT	0.801	0.001	0.161	0.349	0.177	0.301
Antithrombin	0.470	0.105	0.431	0.009	0.069	0.688
D-dimer	0.198	0.517	0.151	0.380	−0.120	0.487
dRVVT_Scr	0.680	0.011	0.736	0.000	0.713	0.000
dRVVT_Con	0.970	0.000	0.875	0.000	0.871	0.000
FDP	0.260	0.391	0.234	0.169	−0.082	0.636
Fibrinogen	−0.641	0.018	0.065	0.706	−0.367	0.028

APTT, activated partial thromboplastin time; Con, confirm; dRVVT, dilute Russell viper venom time; FDP, fibrin degradation product; PT, prothrombin time, Scr, screen.

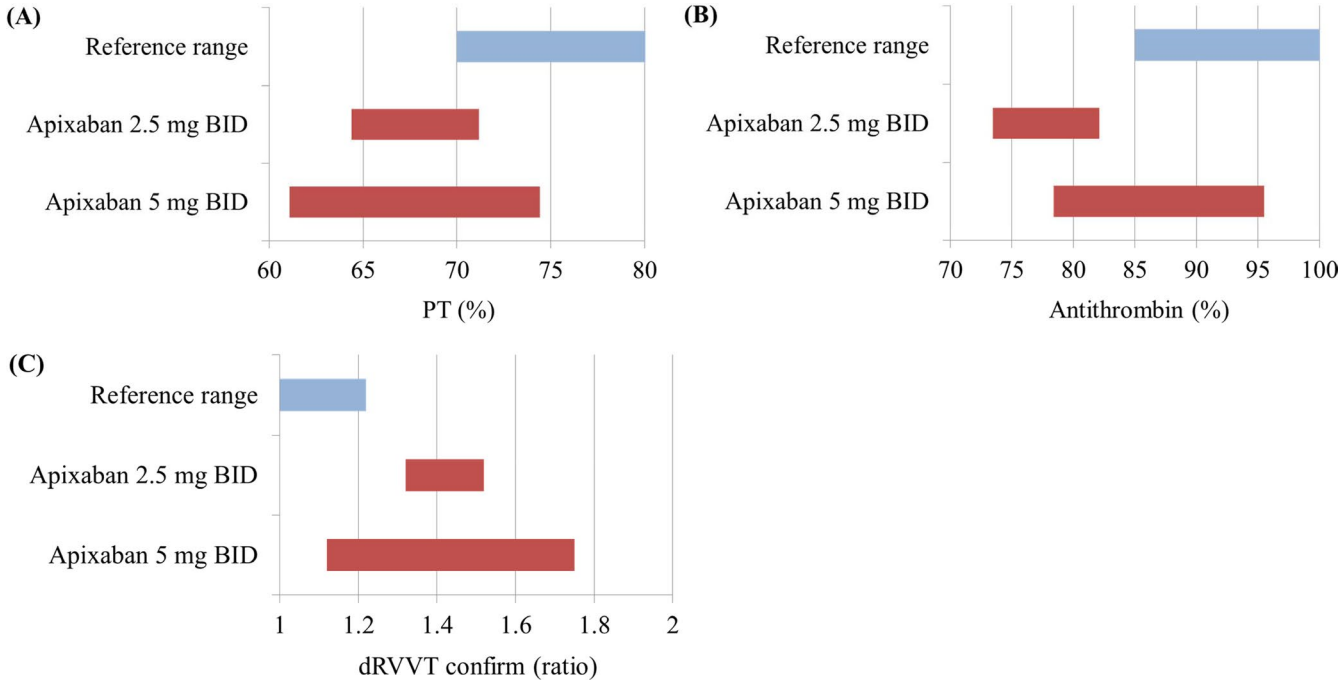

FIGURE 1 Established on-therapy ranges of (A) PT (%), (B) antithrombin, and (C) dRVVT confirm for patients taking apixaban

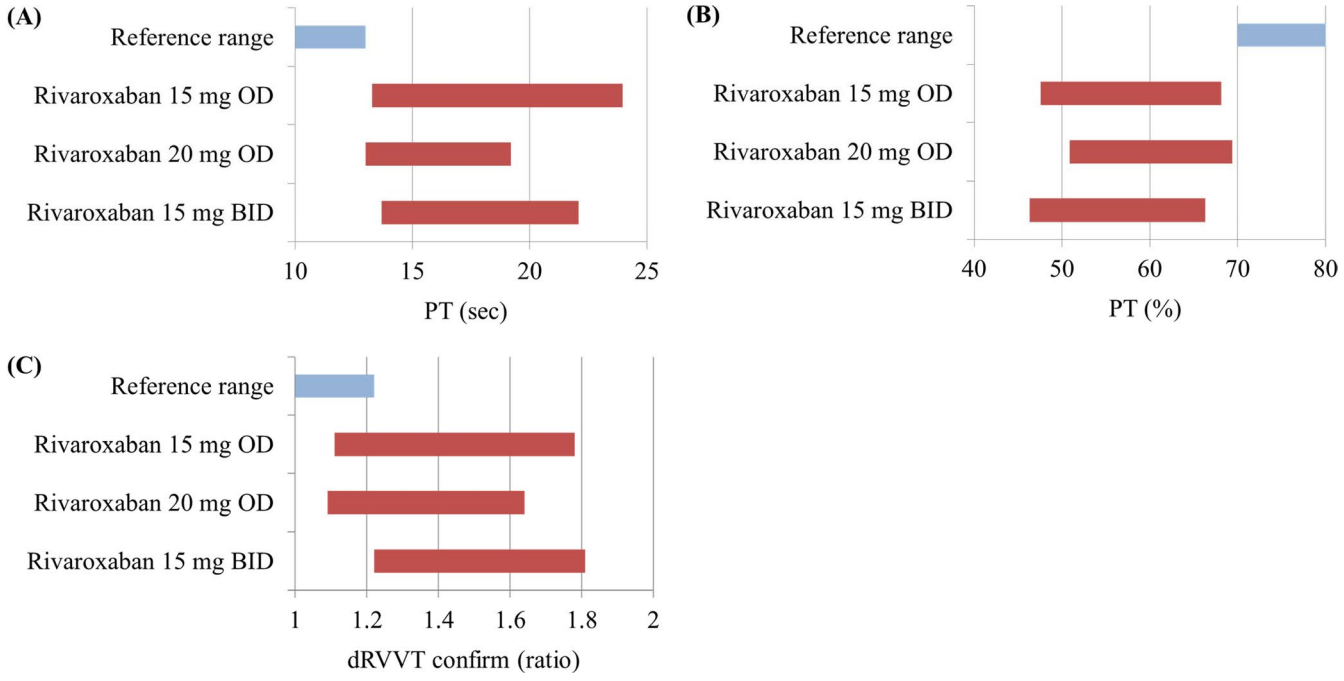

FIGURE 2 Established on-therapy ranges of (A) PT (sec), (B) PT (%), (C) dRVVT confirm for patients taking Rivaroxaban

Weakly negative or positive linear relationships with correlations of −0.332, 0.395, and 0.367 were observed, respectively (Table S1). The trough apixaban level was previously reported to be 15-83 ng/mL.[20] In the present study, established on-therapy ranges for apixaban 2.5 mg BID were 64.37%-71.17% for PT (%), 73.46%-82.09% for antithrombin, and a ratio between 1.32 and 1.52 for the dRVVT confirm test.

3.3 | Subgroup analyses of patients administered apixaban 5 mg BID

Strong correlations were observed between PT (sec), PT (%), antithrombin, dRVVT screen, and dRVVT confirm levels and plasma apixaban levels (Table 3). Strongly positive or negative linear relationships were observed (0.7 to 1 or −0.7 to −1)

TABLE 5 Summary of established on-therapy ranges

	Correlation	Minimum	Maximum
Apixaban 2.5 mg BID			
PT (%)	−0.332	64.37	71.17
Antithrombin	0.395	73.46	82.09
dRVVT confirm	0.367	1.32	1.52
Apixaban 5.0 mg BID			
PT (sec)	0.817	13.57	16.40
PT (%)	−0.783	61.07	74.42
Antithrombin	0.745	78.38	95.49
dRVVT screen	0.853	0.92	1.39
dRVVT confirm	0.847	1.12	1.75
Rivaroxaban 15 mg OD			
PT (sec)	0.986	13.28	23.96
PT (%)	−0.861	47.57	68.14
APTT (sec)	0.801	38.00	45.21
dRVVT confirm	0.970	1.11	1.78
Rivaroxaban 20mg OD			
PT (sec)	0.881	13.00	19.21
PT (%)	−0.781	50.89	69.37
Antithrombin	0.431	82.96	91.65
dRVVT screen	0.736	1.34	2.17
dRVVT confirm	0.875	1.09	1.64
Rivaroxaban 15mg BID			
PT (sec)	0.847	13.70	22.09
PT (%)	−0.858	46.32	66.33
dRVVT screen	0.713	1.60	2.34
dRVVT confirm	0.871	1.22	1.81

dRVVT, dilute Russell viper venom time; PT, Prothrombin time.

(Table S2). Skeppholm et al reported a trough apixaban concentration range of 29-186 ng/mL for patients administered 5 mg BID.[20] Established on-therapy ranges for apixaban 5 mg BID were 13.57-16.40 s for PT (sec), 61.07%-74.42% for PT (%), 78.38%-95.49% for antithrombin, a ratio between 0.92 and 1.39 for the dRVVT screen test, and a ratio between 1.12 and 1.75 for the dRVVT confirm test.

3.4 | Subgroup analyses of patients administered rivaroxaban 15 mg OD

Prothrombin time (sec), PT (%), APTT, and dRVVT confirm levels were strongly linearly correlated with plasma rivaroxaban levels (Table 3, Table S3). On-therapy ranges for rivaroxaban 15 mg OD were calculated using a previously reported trough rivaroxaban concentration range (9.42-143 ng/mL).[15] PT (sec), PT (%), APTT, and dRVVT confirm test ranges were 13.28-23.96 seconds, 47.57%-68.14%, 1.17-2.11, 38.00-45.21 seconds, and 1.11-1.78, respectively.

3.5 | Subgroup analyses of patients administered rivaroxaban 20 mg OD

Strong correlations were also observed between PT (sec), PT (%), antithrombin, dRVVT screen, and dRVVT confirm levels and plasma rivaroxaban levels (Table 4). Strongly positive or negative linear relationships with the exception of antithrombin were observed (Table S4). Mueck et al reported trough rivaroxaban concentrations for patients receiving 20 mg once daily of 9.02-147 ng/mL.[21] Established on-therapy ranges for rivaroxaban 20 mg OD were 13.00-19.21 seconds for PT (sec), 50.89%-69.37% for PT (%), 82.96%-91.65% for antithrombin, 1.34-2.17 for the dRVVT screen test, and 1.09-1.64 for the dRVVT confirm test.

3.6 | Subgroup analyses of patients administered rivaroxaban 15 mg BID

Prothrombin time (sec), PT (%), dRVVT screen, and dRVVT confirm levels were strongly and linearly correlated with plasma rivaroxaban levels (Table 4, Table S5). The on-therapy range for rivaroxaban 15 mg BID was calculated using a previously reported trough rivaroxaban concentration ranges (42.90-143 ng/mL).[21] Established on-therapy ranges for rivaroxaban 15 mg BID were 13.70-22.09 seconds for PT (sec), 46.32%-66.33% for PT (%), 1.60-2.34 and 1.22-1.81 for the dRVVT screen and dRVVT confirm tests.

4 | DISCUSSION

The present study was conducted at a single institution to establish on-therapy ranges for conventional coagulation assays using anti-factor Xa chromogenic assay results. PT was less sensitive to apixaban than rivaroxaban and could be within the reference range in patients with clinically relevant situations.[22,23] Only PT (%) results were well correlated with plasma apixaban levels, whereas both PT (sec) and PT (%) results were well correlated with plasma rivaroxaban levels. However, PT does not always predictably reflect the anticoagulant activity of rivaroxaban, and the sensitivity of PT to rivaroxaban depends on the laboratory reagents used.[16,27,28] APTT was less sensitive to apixaban and rivaroxaban than PT, which concurs with previous reports.[24,25] In the present study, APTT was well correlated with plasma rivaroxaban levels in patients treated with rivaroxaban at 15 mg OD. Previous studies have shown the effects of plasma apixaban and rivaroxaban on antithrombin assay results are dependent on reagent type. Hillarp et al reported that the antithrombin activity increased with DOAC concentration, whereas in the present study, antithrombin activity decreased in patients receiving apixaban and slightly decreased in patients receiving rivaroxaban 20 mg OD.[27,29] Furthermore, in a previous study, fibrinogen levels were almost unaffected by rivaroxaban concentration, whereas in the present study, *P*-values of fibrinogen were 0.018 and 0.028 in rivaroxaban 15 mg OD and rivaroxaban 15 mg BID groups, respectively

(Table 4).[27] Neither D-dimer nor FDP exhibited correlations with apixaban or rivaroxaban concentration.

Dilute Russell viper venom time confirm ratios were developed for the detection of lupus anticoagulant, and in the present study, they increased with plasma apixaban and rivaroxaban concentrations. In a previous study, apixaban, rivaroxaban, and dabigatran (a direct thrombin inhibitor) caused concentration-dependent prolongations of ratios of dRVVT screen and confirm tests using Diagnostica Stago® and Haematex Reserach™ dRVVT reagents, although DOACs sometimes caused false-positive lupus anticoagulation ratios because of the use of phospholipid-rich reagents.[29,30] In the present study, dRVVT confirm test showed strong correlations with the every group. In a previous cohort study, Diagnostica Stago® DRVV-confirm was found to provide a rapid estimation of the anticoagulation intensity by rivaroxaban without specific calibrators in the previous cohort study.[32,33] Recently, the DRVV-DOAC assay was developed by Haematex Reserach™. This assay uses a modified phospholipid-rich, liquid-stable, ready-to-use dRVVT reagent.[34] Sennesael et al reported that DRVV-DOAC results correlated well with plasma concentrations of apixaban, rivaroxaban, and dabigatran.[34] Therefore, the dRVVT confirm test could be useful conventional coagulation assay for monitoring the plasma DOAC concentrations in negative lupus anticoagulation patients even if further studies with more samples and variant reagents are conducted.

We tried to establish on-therapy ranges using variables that correlated well with plasma DOAC levels through linear regression. PT (%), antithrombin, and dRVVT confirm tests were well correlated with apixaban concentrations in both dosages groups. PT (sec), PT (%), and dRVVT confirm tests showed good correlations with rivaroxaban concentrations, regardless of dosage. Established on-therapy ranges were summarized in Table 5. Weak to moderate correlations were observed in patients receiving apixaban 2.5 mg than other patients (Table S1). Low apixaban concentration in apixaban 2.5 mg BID-treated patients might have been responsible for this observation because the anti-factor Xa chromogenic assay has poor sensitivity at low DOAC levels. A larger study using peak and/or trough concentration samples should be helpful to compensate low plasma DOAC levels because DOACs have rapid half-lives and exhibit individual differences. Only 13 samples were from rivaroxaban 15 mg OD treated patients because this regimen was not generally recommended. This small subject number could cause the wider on-therapy ranges than other patients.

Intracranial hemorrhage or fetal bleeding can occur in patients receiving DOACs, and it is widely recognized that these patients may experience severe or life-threatening bleeding during emergent surgery or after trauma. Therefore, it is clinically important to know the history of DOAC treatment or estimate plasma DOAC concentrations. Studies have been conducted on point-of-care coagulation tests for DOAC monitoring and reversal therapy of DOACs.[35,36] The dRVVT confirm assay could also be optimized for use as a point-of-care device. Idarucizumab, specific dabigatran reversal agent, has been approved, and reversal agents for apixaban and rivaroxaban are under development.

This study has limitations that deserve mention. Patients were not randomized to receive either apixaban or rivaroxaban because physicians decided on the drugs administered.Patients who received lower dosages were older and had lower creatinine clearance rates than patients who treated with higher dosages. Some patients were also concurrently treated with heparin, aspirin, and/or clopidogrel, and these treatments might have affected conventional coagulation assay results. Plasma samples were not tested for lupus anticoagulants that cause positive result in dRVVT tests. Administration periods were not considered and peak concentrations of DOACs were not measured. In this study, DOAC concentrations were measured using anti-factor Xa chromogenic assay in this study. However, quantification of low DOAC levels required the LC-MS/MS which was the gold standard method even though the linear correlation between anti-factor Xa chromogenic assay and LC-MS/MS[38] The study populations were small, and thus, we suggest a larger multi-institutional study be conducted.

In conclusion, we evaluated several conventional coagulation tests for monitoring the anticoagulant effects of DOACs. Apixaban concentrations as determined using anti-factor Xa chromogenic assay were well correlated with PT (%), antithrombin, and dRVVT confirm test in both 2.5 mg BID and 5 mg BID groups. Rivaroxaban concentrations commonly showed good correlation with PT (sec), PT (%), and dRVVT confirm test in 15 mg OD, 20 mg OD, and 15 mg BID groups. Established on-therapy ranges of each variable were summarized in Figures 1 and 2. The present study is the first to provide on-therapy range for apixaban and rivaroxaban at meaningful clinical doses in a single institution. Although these on-therapy ranges cannot be applied on clinical practice, they could be considered in emergent situations or future studies.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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