The Influence of Assay Selection on Prothrombin Time Measured in Patients Treated With Rivaroxaban for Nonvalvular Atrial Fibrillation

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Background: Prothrombin time (PT) can provide a qualitative assessment of the relative intensity of anticoagulation by rivaroxaban. More than ten types of assay are available for the measurement of PT in clinical settings, but it is not yet fully understood whether their interactions with rivaroxaban are uniform or inconsistent. **Methods:** We examined 139 blood samples from patients taking rivaroxaban. We measured PT using five different commercially available assays. We also evaluated the estimated rivaroxaban concentration using a chromogenic anti-factor Xa assay. **Results:** The median estimated concentration of rivaroxaban was 192 ng/ml (interquartile range 85–284 ng/ml). The correlation coefficient (r)

between PT and the estimated concentrations of rivaroxaban was as follows: Thromborel S, $r = 0.768$; Thrombocheck PT, $r = 0.861$; Coagpia PT-N, $r = 0.909$; Neoplastin Plus, $r = 0.882$; and Triniclot PT Excel S, $r = 0.870$. The gradients of the regression plots differed more than fourfold, and the standard deviation of the regression line ranged from 1.001 to 2.980, which tended to be higher for the assays with the higher regression slope gradients. **Conclusion:** The estimated concentration of rivaroxaban varied greatly depending on the assay, so the PT measured in patients taking rivaroxaban should be interpreted with caution. J. Clin. Lab. Anal. 30:941–946, 2016. © 2016 Wiley Periodicals, Inc.

Key words: rivaroxaban; anticoagulants; prothrombin time; atrial fibrillation; monitoring

INTRODUCTION

New oral anticoagulants have emerged and commercialized as an alternative for vitamin K antagonists for thromboembolic prevention in patients with nonvalvular atrial fibrillation (NVAf). There are concerns about their coagulation monitoring to minimize the risks of bleeding and lack of effectiveness (1, 2). Rivaroxaban is one of the new oral anticoagulants, which acts by selectively inhibiting the enzymatic activity of coagulation factor Xa (3). The ROCKET AF trial compared rivaroxaban (20 mg/day; 15 mg/day in patients with creatinine clearance [CLCr] 30–49 ml/min) with dose-adjusted warfarin in patients with NVAf, and demonstrated the noninferiority of rivaroxaban compared with warfarin for the prevention of stroke and systemic embolism (4, 5). The

J-ROCKET AF study compared the safety of rivaroxaban with warfarin in Japanese patients with atrial NVAf and found that it was noninferior; (6, 7) consequently, rivaroxaban was approved in Japan at a lower dose (15 mg/day; 10 mg/day in patients with CLCr 30–49 ml/min). At the time of introduction of rivaroxaban routine coagulation monitoring was considered unnecessary, as its pharmacology was predictable and the dose was fixed. Nonetheless,

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cases of substantial hemorrhage with a markedly prolonged coagulation time have been reported in patients taking rivaroxaban (8). Additionally, in certain circumstances, it is important to monitor the plasma concentration of rivaroxaban or its anticoagulant action to manage the risk of bleeding (8–10). The prothrombin time (PT) provides a qualitative assessment of anticoagulant activity in patients treated with rivaroxaban (11–13). More than ten different proprietary assays are commercially available, which employ a variety of reagents to measure PT in clinical laboratories. It is not yet fully understood whether there are differences in interactions between these assays (11,12). Furthermore, the lot-to-lot variation of the assays has not been reported. We measured PT in blood samples obtained from patients taking rivaroxaban using five different PT assays to assess whether PT is indeed a useful and reliable means of monitoring the anticoagulant activity of rivaroxaban. We also examined the estimated blood concentration of rivaroxaban using a chromogenic anti-factor Xa assay, and evaluated the strength of correlation between the estimated concentration and PT.

MATERIALS AND METHODS

Data Collection

Venous blood samples were collected from patients with NVAf treated with rivaroxaban without concomitant use of other anticoagulants between February 2013 and January 2015 at Tenri Hospital, Japan. We measured PT using five different PT assays: Thromborel S (Siemens Healthcare Diagnostics, Marburg, Germany), Thrombocheck PT (Sysmex, Kobe, Japan), Coagpia PT-N (Sekisui Medical Co., Tokyo, Japan), Neoplastin Plus (Diagnostica Stago, Asnieres, France), and Triniclot PT Excel S (Trinity Biotech, Bray, Ireland). The estimated concentrations of rivaroxaban were calculated from a chromogenic anti-factor Xa assay (STA-Liquid Anti-Xa, STA-Rivaroxaban Calibrator, STA-Rivaroxaban Control, Diagnostica Stago). All assays were conducted using a Coapresta 2000 machine (Sekisui Medical). We did not measure the PT-international normalized ratio (PT-INR) as the International Sensitivity Index is determined individually for each assay and is specific for warfarin, so it is not appropriate to apply PT-INR to rivaroxaban. The correlation and the regression line between PT (plotted on the *y*-axis) and estimated concentration of rivaroxaban (*x*-axis) were calculated for each PT assay. We also examined whether there was lot-to-lot variation for each of the five assays, using two lots of each assay to measure PT in 34 samples obtained at the end of the study period. The lots used were as follows: Thromborel S, lot numbers 545551 and 546808; Thrombocheck PT, lots 060 and 063; Coagpia PT-N, lots 901REL and 907RJL; Neoplastin

Plus, lots 110927 and 112083; and Triniclot PT Excel S, lots 132 and 133. The study was conducted in accordance with the Declaration of Helsinki. The institutional ethics committee approved the protocol, and written informed consent was obtained from each patient.

Statistical Analysis

Continuous variables are presented as the mean $\pm$ standard deviation (SD) or median and interquartile range (IQR), depending on the distribution of the data. Comparisons were made with the χ^2 test for categorical variables and with the Mann–Whitney *U* test for continuous variables. The extent of the relationship between PT and the estimated concentration of rivaroxaban was examined using Pearson's correlation coefficient method. A *P*-value <0.05 was considered statistically significant. All analyses were performed using Stat Flex (Artech, v. 6, Osaka, Japan).

RESULTS

Baseline Characteristics

We collected 139 venous blood samples from patients taking rivaroxaban: their mean age was 66.5 ± 8.5 years (range 42–82 years) and 77.8% were men (Table 1). The dose of rivaroxaban was 15 mg once daily in 68.3% of patients and 10 mg once daily in 31.7%. CLCr measured using the Cockcroft–Gault formula was 81.3 ± 29.6 ml/min, and a CLCr <50 mL/min was observed in 21 samples (15.1%). A Pglycoprotein inhibitor was coadministered to the donors of 15 samples (10.8%), verapamil was coadministered to the donors of eight samples, diltiazem to three, amiodarone to three, and amiodarone and atorvastatin were coadministered to the donor of one sample. Patients receiving 10 mg of rivaroxaban were significantly older than those taking 15 mg and had lower CLCr, lower estimated glomerular filtration rate, and lower weight than those receiving the 15 mg dose; however, no significant difference was observed in the estimated concentrations of rivaroxaban between the groups.

Estimated Rivaroxaban Concentration

The median estimated concentration of rivaroxaban was 192 ng/ml (IQR 85–284 ng/mL): 39 samples (28.1%) had an estimated concentration in the range 0–99 ng/ml, 32 samples (23.0%) in the range 100–199 ng/ml, 46 samples (33.1%) in the range 200–299 ng/ml, 18 samples (12.9%) in the range 300–399 ng/ml, and four samples (2.9%) in the range 400–499 ng/ml. Higher rivaroxaban concentrations were observed in samples from patients with reduced renal function (CLCr < 50 ml/min).

TABLE 1. Baseline Characteristics of Blood Samples

	<i>n</i> = 139	Dosage of rivaroxaban		<i>P</i> -value
		15 mg <i>n</i> = 95 (68%)	10 mg <i>n</i> = 44 (32%)	
Age (years)	66.5 ± 8.5	65.4 ± 7.7	69.0 ± 9.5	0.011
Males (<i>n</i> [%])	108 (78)	75 (79)	33 (75)	0.60
eGFR (ml/min/1.73 m ²)	68.5 ± 17.0	71.9 ± 16.0	61.1 ± 17.9	<0.001
Creatinine (mg/dl)	0.86 ± 0.23	0.82 ± 0.19	0.95 ± 0.29	0.014
CLCr < 50 ml/min (<i>n</i> [%])	21 (15)	5 (5)	16 (36)	<0.001
P-glycoprotein inhibitor (<i>n</i> [%])	15 (11)	7 (7)	8 (18)	0.06
Concentrations of plasma rivaroxaban (ng/ml)	192 (85–284)	204 (98–290)	177 (78–273)	0.30

Values are expressed as mean ± SDs or numbers and percentage.

CLCr measured using the Cockcroft–Gault formula.

eGFR, estimated glomerular filtration rate.

compared with those with normal renal function (CLCr ≥ 50 ml/min), but this difference was not significant (257 [85–287] vs. 190 [85–279] ng/ml, *P* = 0.29). The median estimated concentration of rivaroxaban in the 34 samples used for measuring lot-to-lot variation of PT assays was 177 ng/ml (IQR 101–247 ng/ml).

Relationship Between PT Measured by Each Assay and Estimated Concentration of Rivaroxaban

The relationships between PT (*y*-axis) and the estimated concentration of rivaroxaban (*x*-axis) measured using the five PT assays are shown in Figure 1. The gradients of the regression lines plotted for each assay were as follows: Thromborel S, $y = 0.010x + 11.9$; Thrombocheck PT, $y = 0.016x + 11.4$; Coagpia PT-N, $y = 0.032x + 12.1$; Neoplastin Plus, $y = 0.028x + 12.6$; and Triniclot PT Excel S, $y = 0.046x + 13.9$. A significant linear relationship (*P* < 0.001) was observed between PT and estimated concentration of rivaroxaban for each assay. In contrast, significant differences (*P* < 0.001 for all) were observed in PT when any one PT assay was compared with another. Furthermore, the gradients of the regression lines plotted varied from 0.010 to 0.046, a more than four-fold difference between the shallowest and steepest. The correlation coefficients (*r*) of each assay and SD of the regression line were: Thromborel S, *r* = 0.768, SD = 1.001; Thrombocheck PT, *r* = 0.861, SD = 1.063; Coagpia PT-N, *r* = 0.909, SD = 1.713; Neoplastin Plus, *r* = 0.882, SD = 1.705; and Triniclot PT Excel S, *r* = 0.870, SD = 2.980 (Fig. 1). The correlation coefficients varied from 0.768 to 0.909 depending on the PT assay used, and the SDs of the regression lines varied from 1.001 to 2.980, which tended to be higher for the assays with the steeper slopes. The gradients of regression lines of PT measured using two different lots of each PT assay are shown in Figure 2. The existence of lot-to-lot variation was observed with most assays.

DISCUSSION

Major Findings

Our main findings were that (a) there was substantial variation between different commercially available assays when PT was measured in the presence of rivaroxaban; (b) PT correlated significantly with the estimated concentration of rivaroxaban for all five assays, but again there were substantial interassay differences; and (c) there was substantial lot-to-lot variation with most assays.

Measurement of Rivaroxaban Concentration

The most accurate means of measuring blood rivaroxaban concentration requires liquid chromatography-tandem mass spectrometry (LC-MS/MS) (14, 15), but the technique is expensive and time consuming, and substantial pretreatment and preparation of the samples is needed, meaning that it is not suitable for routine clinical practice. Nonetheless, there appears to be a good correlation between the chromogenic anti-Xa coagulation assay and rivaroxaban concentration measured using LC-MS/MS (14–16), and consequently the anti-Xa coagulation assay is considered to represent an alternative and accurate method of estimating rivaroxaban concentration. We therefore elected to use the commercially available STA-Liquid Anti-Xa assay to estimate rivaroxaban concentration.

Difference in Sensitivities of PT Assays to Rivaroxaban

In clinical settings, more than ten PT assays are commercially available. It is recognized that different PT assays are influenced differently by rivaroxaban (11, 12); however, our study is the first to compare five PT assays directly, and to establish the extent of lot-to-lot variability.

Fig. 1. Relationship between the estimated rivaroxaban concentration ($C_{\text{rivaroxan}}$) and PT measured by five different PT assays. The r value is the correlation coefficient and the SD value is the standard deviation of the regression line. PT reagent; A, T Thromborel S; B, Thrombocheck PT; C, Coagpia PT-N; D, Neoplastin Plus; E, Triniclot PT Excel S.

We found significant linear relationships between PT and estimated concentration of rivaroxaban for all assays, but PT does not always predictably reflect the anticoagulant activity of rivaroxaban. The highest correlation coefficient value of $r = 0.909$ was observed with the Coagpia PT-N assay; however, there was a wide range of regression slope gradients ranging more than fourfold from 0.010 for Thromborel S to 0.046 for Triniclot PT Excel S. This suggests that monitoring PT without a full understanding of the influence of rivaroxaban on each PT assay may lead to clinical errors and places patients at risk of bleeding or thrombosis. Furthermore, there was also substantial lot-to-lot variation with some assays. It is essential that when the pharmacological effect of rivaroxaban is evaluated using PT, clinicians should appreciate the difference in sensitivities of assays to rivaroxaban and lot-to-lot variation. Each routine laboratory must perform proper calibration with the PT assay that they use on a weekly basis. Furthermore, it is the responsibility to properly calibrate each new lot of PT assay.

Monitoring for the Safe Use of Rivaroxaban

The Official Communication from the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis recommended that “each laboratory should be aware of the sensitivity of their PT assay” (17), when coagulation activity of rivaroxaban is estimated using PT. A study investigating four assays used for measuring estimated concentrations of rivaroxaban from anti-Xa activity reported that each assay showed strong correlation with rivaroxaban concentration, and that the estimated concentrations of rivaroxaban calculated from the anti-Xa activity of the four assays were close (13). We used Liquid Anti-Xa in our study, and found it to be a suitable means of monitoring the risk of bleeding due to excess accumulation of rivaroxaban. A measurement of the estimated concentration of rivaroxaban using the anti-Xa activity is not currently performed in the daily clinical practice, but if this technique was adopted as routine, any differences between institutions would be eliminated and the safe use of rivaroxaban would be promoted.

Fig. 2. Comparison of the correlation of estimated rivaroxaban concentration ($C_{\text{rivaroxaban}}$) with PT of two lots (34 samples). PT reagent; A, T Thromborel S; B, Thrombocheck PT; C, Coagpia PT-N; D, Neoplastin Plus; E, Triniclot PT Excel S.

Study Limitations

First, our specimens were acquired from a relatively small cohort at a single hospital. Our findings should be interpreted with caution as the patients' demographic and clinical characteristics, the indications for rivaroxaban therapy and patient management strategies might not be generalizable to other institutions. Second, only two different PT assay lots were used to examine lot-to-lot variation; to understand the degree of lot-to-lot variation fully it will be necessary to compare several more lots. Third, none of our participants had experienced a hemorrhagic event, so we were not able to examine how high rivaroxaban concentration and concomitant high PT might predispose to bleeding. We were also unable to establish whether choice of PT assay might have led to insufficient sensitivity to detect an over-anticoagulated state that directly led to bleeding.

In summary, the results of this study showed that there are large differences in PT measured by different assays in the presence of rivaroxaban. When assessing PT to monitor rivaroxaban therapy, clinicians should be aware of interassay and lot-to-lot variation. As a result of this

variation, we recommend that an anti-Xa assay be used to estimate rivaroxaban concentration instead. Further studies are required to establish the best means of monitoring rivaroxaban treatment to avoid hemorrhagic adverse events.

CONFLICT OF INTEREST

None of the authors has a conflict of interest to declare.

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