CLINICAL STUDY

Analysis of bioMARKer Distribution and Individual Reproducibility Under Rivaroxaban Treatment in Japanese Patients with Non-Valvular Atrial Fibrillation (R-MARK Study, CVI ARO2)

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Summary

The “on-therapy range” of direct oral anticoagulants is the 90% interval of drug concentration. Previously, we reported the on-therapy range of rivaroxaban in a single-center cohort. The present study aimed to confirm the range and intraindividual reproducibility in a multicenter cohort.

Eligible patients with non-valvular atrial fibrillation under rivaroxaban treatment for prevention of ischemic stroke were enrolled from nine institutes in Tokyo, Japan, between June 2016 and May 2017 (\(n = 324\)). The first and second (three months later) blood samples both taken within 1-5 hours after rivaroxaban intake were analyzed (\(n = 219\)). Plasma concentration of rivaroxaban (PC-Riv) and prothrombin time (PT) with five reagents were measured.

The 90% interval of PC-Riv was 47.3-532.9 ng/mL. The 90% interval of PT measured with RecombiPlasTin 2G was 11.8-22.3 seconds, the widest range among the five reagents examined. PC-Riv reproducibility within a 90% interval was evaluated bidirectionally (first-to-second and second-to-first), and 92.4% of samples were reproducible. The change rate (CR) of PC-Riv between two samplings ranged widely, and high CR (\(\geq 54.3\%\), cutoff for predicting non-reproducibility) was predicted by concomitant drugs (non-dihydropyridine calcium antagonist and thiazide) and mitral regurgitation.

We reported the on-therapy range of rivaroxaban in a multicenter cohort. This range was consistent with that of a single-center cohort and was highly reproducible within three months in daily clinical practice. However, caution is necessary regarding several factors that may affect the intraindividual variation of PC-Riv.

Key words: Oral anticoagulation, Plasma concentration, On-therapy range

The direct oral anticoagulant (DOAC), rivaroxaban, directly inhibits factor Xa activity.\(^1\) Due to its predictable pharmacokinetic and pharmacodynamic profiles, primary monitoring is not recommended. That might be one of the reasons for patient satisfaction under DOAC therapy comparing to warfarin therapy.\(^2\) However, in some situations, including severe bleeding or emergent surgery, measurement of anticoagulant markers may be required to judge an excessive anticoagulant effect of rivaroxaban.\(^3,4\)

Although data on the relationship between the plasma levels of DOACs and clinical outcomes have been reported,\(^5-7\) a definite therapeutic range for DOACs has not been identified. In the absence of therapeutic ranges, Cukier, et al. proposed the “on-therapy range”\(^8\) that is defined as the interval between the 5th and the 95th percentile concentration (90% interval) for a given dose of DOACs. The on-therapy range is useful because it indicates the drug level at which most patients in a steady state will fall.

However, there are uncertainties in the use of the on-therapy range in daily clinical practice. DOAC pharmacokinetics, as well as the risks of thromboembolism or bleeding under DOAC treatment, may vary according to...
ethnicity. This is the case for rivaroxaban. Therefore, for Japanese patients receiving a different dose compared to other countries, the specific adequate plasma concentration of rivaroxaban (PC-Riv) should be investigated. Second, although we generally believe that if patients once obtain a good PC-Riv (i.e., on-therapy range), they would get similar PC-Riv ranges in the clinical course with steady state, the reproducibility of PC-Riv is not necessarily assured.

We previously investigated the 90% interval of PC-Riv using liquid chromatography-tandem mass spectrometry (LC-MS/MS), anti-Xa assay, and prothrombin time (PT) with five different reagents in Japanese non-valvular atrial fibrillation (NVAF) patients under treatment with rivaroxaban as a single-center study. The present study aimed to validate the range of 90% interval in a multicenter cohort involving various types of NVAF patients and to evaluate the reproducibility of PC-Riv within the on-therapy range in each individual.

**Methods**

This was a multicenter prospective observational study, registering Japanese NVAF patients under treatment with rivaroxaban (UMIN Clinical Trials Registry: UMIN 000016424). The objectives of this study were to investigate the range of 90% interval (on-therapy range) of PC-Riv in NVAF patients under rivaroxaban treatment in a multicenter cohort, including various types of NVAF patients. Through this, we validated the 90% interval of PC-Riv, which we reported previously in a similar population. The second objective was to evaluate the reproducibility of PC-Riv within the on-therapy range in each individual.

**Ethics and informed consent:** This study was performed following the ethical norms based on the Declaration of Helsinki (revised in 2013) and Ethical Guidelines for Medical and Health Research Involving Human Subjects (Public Notice of the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare, Japan, issued in 2017). All participants provided written informed consent. The study protocol was reviewed by the Institutional Review Board of The Cardiovascular Institute, Tokyo, Japan.

**Study population:** Eligible patients with NVAF under rivaroxaban treatment for prevention of ischemic stroke were enrolled in this study from nine institutes in Tokyo, Japan, between June 2016 and May 2017. The appropriate dose of rivaroxaban was consistent with the Japanese pharmaceutical reference. Patients with any of the following during the enrollment period were excluded: (1) receiving dual antiplatelet therapy; (2) inadequate dosage of rivaroxaban at enrollment; (3) rivaroxaban hypersensitivity; (4) liver dysfunction with clotting disorder; (5) moderate or high liver dysfunction (Child-Pugh classification B or C); (6) renal dysfunction (creatinine clearance < 30 mL/minute); (7) women who are pregnant or may be pregnant; (8) patients taking HIV protease inhibitors (ritonavir, atazanavir, indinavir, etc.); (9) patients taking azole antifungal agents (itraconazole, voriconazole, ketoconazole, etc., excluding fluconazole); (10) patients taking drugs containing cobicistat; (11) patients with acute bacterial endocarditis; (12) patients who did not provide written informed consent to participation in this study; and (13) patients who were judged by the researchers to be inappropriate for this study (i.e., incapable of understanding the study protocol due to dementia, intellectual disturbance, and/or psychiatric/psychosomatic disorder).

**Data acquisition:** After obtaining informed consent, plasma samples were collected from patients fulfilling the inclusion criteria twice at the time of the first visit, and 3 (± 1) months later. At each time point, PC-Riv was determined by LC-MS/MS, anti-factor Xa assay, and PT with five reagents under conditions of rivaroxaban treatment. Patients were requested to visit the hospital to keep the time of blood sampling within 2-4 hours after rivaroxaban intake. However, the actual timing of blood sampling could not be controlled strictly in daily clinical practice, and we accepted samples collected within 1-5 hours of drug intake. Samples collected < 1 hour or > 5 hours after drug intake were excluded from the analysis.

Baseline data were collected and included: patient profiles (age, sex, height, weight, rivaroxaban dose, and types of atrial fibrillation); risk factors (history of smoking and drinking alcohol, hypertension, diabetes mellitus, dyslipidemia, history of cerebral infarction or transient ischemic attack, chronic obstructive pulmonary disease, aortic disease, history of cerebral bleeding, bleeding disorders, history of significant bleeding, active cancer, and use of other medications); and structural heart diseases (heart failure, mitral regurgitation, aortic regurgitation, aortic stenosis, tricuspid regurgitation, history of myocardial infarction, history of percutaneous intervention, history of the coronary artery bypass graft, hypertrophic cardiomyopathy, and dilated cardiomyopathy). The patients were followed for a maximum of 6 months.

**Plasma concentration and PD measurement:**

**Direct plasma rivaroxaban concentration (LC-MS/MS)** Direct PC-Riv was measured using a validated, selected chromatographic assay with LC-MS/MS. Isope-labeled rivaroxaban was used as an internal standard. The calibration range of the procedure was from 1 ng/mL (lower limit of quantification, LLOQ) to 1000 ng/mL. Quality control samples at concentrations of 2, 50, and 800 ng/mL were determined with accuracies of 95.9%-106.4%, 100.3%-105.5%, and 96.9%-100.6%, respectively. All samples were stored at −80°C and analyzed within 12 months after sampling. The analyte was confirmed to be stable for this period under these storage conditions.

**Indirect plasma rivaroxaban concentration (anti-Xa assay)** Indirect measurement of PC-Riv was performed using a commercially available anti-factor Xa activity assay (STA-Liquid Anti-Xa; Diagnostica Stago, Asnières sur Seine, France) at LSI Medience Corporation (Tokyo, Japan). The lower limit of detection for this assay was 15 ng/mL, whereas the upper limit of quantification for this assay was 450 ng/mL.

**PT with five different reagents** PT was measured by mixing platelet-poor plasma at 37°C with calcium thromboplastin and determined as the time needed for the sample to form a clot using the photometric method. The different thromboplastin reagents used were Neoplastin Plus.
Patients with non-valvular atrial fibrillation under rivaroxaban (324 patients)

Blood sampling at the next visit after providing informed consent (Data A)

Blood sampling 3 months later (Data B)

Eligible patients (219 patients)

Figure 1. Flowchart of the study. Among 324 patients, 219 eligible patients were included in this study. A total of 438 samples were collected from these 219 patients and analyzed. In the present study, we analyzed the two samples bidirectionally. Therefore, we named the samples at the first visit and three months later, as Data A and B, respectively. We copied the dataset interchanging Data A and B, and then the dataset and the copied dataset were combined. In the new, doubled dataset, first and second samples were derived from Data A and B in half and conversely derived from Data B and A, respectively, in the remaining half.

(Roche Diagnostics, Mannheim, Germany), Thromborel S®, Thrombocheck PT®, and Thrombocheck PT Plus® (Siemens Healthcare Diagnostics, Marburg, Germany), and RecombiPlasTin 2G® (Instrumentation Laboratory, Bedford, MA). These reagents were selected because they are currently the most commonly used reagents in Japan. Neoplastin Plus, Thrombocheck PT, and Thrombocheck PT Plus are derived from rabbit brain. RecombiPlasTin 2G contains a recombinant human tissue factor, whereas Thromborel S is derived from the human placenta. The clotting time of Neoplastin Plus was measured using an STA-compact® coagulometer (Diagnostica Stago). Thromborel S, Thrombocheck PT, and Thrombocheck PT Plus were measured using a CS-2000i® coagulometer (Siemens Healthcare Diagnostics). RecombiPlasTin 2G was measured using an ACL-TOP coagulometer (Instrumentation Laboratory). All samples were stored at −80°C and analyzed within 12 months after sampling.

Adverse events: During the 6-month follow-up period, adverse events were monitored, including all-cause death, stroke, systemic thromboembolism, bleeding requiring hospital admission, and cardiovascular events requiring hospital admission (acute myocardial infarction, unstable angina pectoris, percutaneous coronary intervention, and heart failure).

Evaluation and statistical analysis: Categorical and serialized data are presented as number (%) and mean ± standard deviation, respectively. Statistical analyses were performed using SPSS for Windows version 19.0 software (IBM, Armonk, NY). Statistical significance was set at two-sided P < 0.05.

In the present study, we planned to analyze the two samples bidirectionally. Therefore, the samples from the first visit and three months later were named Data A and B, respectively. We copied the dataset interchanging Data A and B, and then the dataset and the copied dataset were combined. In the new, doubled dataset, first and second samples were derived from Data A and B in half and conversely derived from Data B and A, respectively, in the remaining half (Figure 1). Using this doubled dataset, we performed three patterns of analyses, as outlined below.

Analysis 1 We described the 90% interval (values of 5th and 95th percentiles), minimum value (min), and maximal value (max) for direct and indirect PC-Riv, and PT with five reagents.

Definition of on-therapy range In the present study, the 90% interval of direct and indirect PC-Riv, and PT with five reagents in the doubled dataset was defined as the on-therapy range.

Analysis 2 The reproducibility of direct PC-Riv was evaluated by two methods. First, the correlation between PC-Riv in Data A and Data B was assessed by Pearson’s correlation coefficient. This analysis was done in the non-copied (non-doubled) dataset because it did not require bidirectional analysis; overestimation using a doubled dataset should be avoided. Second, samples within the on-
therapy range were determined in first and second samples. A “reproducible PC-Riv within the on-therapy range” occurs when the PC-Riv in the first sample and the second sample are within the on-therapy range. We calculated the proportion of the samples of reproducible PC-Riv among those in which the first sample was within the on-therapy range.

Analysis 3 The relative factors of the non-reproducibility of PC-Riv within the on-therapy range were investigated. When PC-Riv in the first sample was within the on-therapy range, but that in the second sample was out of the on-therapy range, it was defined as “non-reproducible PC-Riv within the on-therapy range.” The analysis was performed as follows: (1) the change rate (CR) of PC-Riv was calculated: \[ \text{CR of PC-Riv} = \frac{\text{PC-Riv in the second sample} - \text{PC-Riv in the first sample}}{\text{PC-Riv in the first sample}} \times 100 \text{ (%).} \] (2) With the assumption that elevated CR of PC-Riv is associated with non-reproducibility of PC-Riv within the on-therapy range, the cutoff value of the CR for non-reproducibility of PC-Riv was determined as the Youden index in receiver operating characteristic (ROC) curve analysis. (3) The samples within the on-therapy range of PC-Riv in the first sample were divided into two groups according to the PC-Riv’s CR cutoff value. (4) The relative factors for high PC-Riv CR were determined by univariate and multivariate logistic regression analyses. In logistic regression analysis, the clinical factors, shown in Table I and Table II, were used as covariables, except for those with less than five samples in either category. Variables with \( P < 0.05 \) in the univariate models were used in the multivariate model with the stepwise method.

Results

Study population: A total of 324 patients were registered initially in this study, and the distribution of PC-Riv by the timing of blood sampling is shown in Figure 2. Paired blood samples were obtained within 1-5 hours after rivaroxaban intake from 219 patients (Figure 1).

Patient characteristics are shown in Table I and Table II. The study population included 168 men (76.7%), and the subjects had a mean age of 70.1 ± 9.2 years. Among the 219 patients, the standard dose (15 mg) and reduced dose (10 mg) were prescribed for 153 (69.9%) and 66 (30.1%) patients, respectively.

On-therapy range of plasma rivaroxaban concentration: A total of 438 paired blood samples were collected from 219 eligible patients. The on-therapy range of direct PC-Riv was 47.3-532.9 ng/mL, and those of indirect PC-Riv and PT with five reagents corresponding to direct PC-Riv were identified (Table III). When we separated the patients into those with 10 mg and 15 mg dose once daily, the on-therapy range of direct PC-Riv was 97.8-489.2 and 105.8-483.7 ng/mL, respectively (Table IV), which were comparable between 2 doses.

Reproducibility of on-therapy range with plasma rivaroxaban concentration: The correlation of PC-Riv between the first and second samples was moderate \( (r = 0.425, P < 0.001; \text{Figure 3}) \). PC-Riv was within the on-therapy range in 396 of the 438 blood samples in the first sample. The second sample was within the on-therapy range in 366 of these 396 samples (92.4%).

| Table I. Patient Characteristics |
|----------------------------------|
| n = 219                          |
| Age, years                       | 70.1 ± 9.2 |
| Height, cm                       | 164.7 ± 8.9 |
| Weight, kg                       | 67.0 ± 13.9 |
| Male, n (%)                      | 168 (76.7) |
| Type of atrial fibrillation, n (%)| Paroxysmal 88 (40.2) |
|                                  | Persistent 131 (59.8) |
| CHADS: score, n (%)              | 0 25 (11.4) |
|                                  | 1 86 (39.3) |
|                                  | 2 70 (32.0) |
|                                  | 3 31 (14.2) |
|                                  | 4 5 (2.3)   |
|                                  | 5 1 (0.5)   |
|                                  | 6 1 (0.5)   |
| CHAsDS2-VASc score, n (%)        | 0 5 (2.3)   |
|                                  | 1 51 (23.3) |
|                                  | 2 51 (23.3) |
|                                  | 3 55 (25.1) |
|                                  | 4 38 (17.4) |
|                                  | 5 16 (7.3)  |
|                                  | 6 2 (0.9)   |
|                                  | 7 0 (0.0)   |
|                                  | 8 1 (0.5)   |
| Rivaroxaban dose, n (%)          | 15 mg once daily 153 (69.9) |
|                                  | 10 mg once daily 66 (30.1) |
| New administration of rivaroxaban, n (%) | 19 (8.7) |
| After meal dosing, n (%)         | First sampling 175 (79.9) |
|                                  | Second sampling 171 (78.1) |
| History of smoking, n (%)        | 11 (5.0)   |
| Habitual drinking alcohol, n (%) | 54 (24.7)  |
| Medical history                  | Hypertension, n (%) 197 (90.0) |
|                                  | Diabetes mellitus, n (%) 54 (24.7) |
|                                  | Heart failure, n (%) 42 (19.2) |
|                                  | Valvular heart disease, n (%) 10 (4.6) |
|                                  | Cardiomyopathy, n (%) 7 (3.2) |
|                                  | Angina pectoris, n (%) 19 (8.7) |
|                                  | Myocardial infarction, n (%) 8 (3.7) |
|                                  | History of cerebral infarction or transient ischemic attack, n (%) 15 (6.8) |
|                                  | Aortic disease, n (%) 3 (1.4) |
|                                  | History of major bleeding, n (%) 2 (0.9) |
|                                  | Chronic obstructive pulmonary disease, n (%) 5 (2.3) |
|                                  | History of cancer, n (%) 19 (8.7) |
|                                  | Liver disease, n (%) 5 (2.3) |
|                                  | Thyroid disease, n (%) 7 (3.2) |
|                                  | Cognitive disorder, n (%) 2 (0.9) |

Consecutive variables are presented as mean ± standard deviation. Categorical variables are presented as number (%).
Table II. Laboratory Data and Medication Profile at Baseline

| Laboratory data                                  | 0.90 ± 0.2 | 73.7 ± 25.3 | 3.9 ± 1.0 | 14.1 ± 1.9 | 42.2 ± 5.2 | 0.7 ± 0.5 | 26.3 ± 12.9 | 24.2 ± 15.8 |
|------------------------------------------------|------------|-------------|-----------|------------|------------|-----------|------------|------------|
| Serum creatinine, mg/mL                          | 1.913-6.376| 0.82)       |           |            |            |           |            |            |
| Creatinine clearance, mL/minute                  |            |            |           |            |            |           |            |            |
| Albumin, g/dL                                    |            |            |           |            |            |           |            |            |
| Hemoglobin, g/dL                                 |            |            |           |            |            |           |            |            |
| Hematocrit, %                                    |            |            |           |            |            |           |            |            |
| Total bilirubin, mg/dL                           |            |            |           |            |            |           |            |            |
| AST, U/L                                         |            |            |           |            |            |           |            |            |
| ALT, U/L                                         |            |            |           |            |            |           |            |            |
| Medications                                      |            |            |           |            |            |           |            |            |
| ARB, n (%)                                       | 101 (46.1) | 22 (10.0)   | 31 (14.2) | 92 (42.0)  | 111 (50.7) | 24 (11.0) | 25 (11.4)  | 69 (31.5)  |
| ACE inhibitor, n (%)                             |            |            |           |            |            |           |            |            |
| Non-dihydropyridine calcium channel antagonist, n (%) | 92 (42.0) | 111 (50.7) | 24 (11.0) | 25 (11.4)  | 69 (31.5)  | 25 (11.4) | 2 (0.9)    | 3 (1.4)    |
| Dihydropyridine calcium channel antagonist, n (%) | 92 (42.0) | 111 (50.7) | 24 (11.0) | 25 (11.4)  | 69 (31.5)  | 2 (0.9)   | 3 (1.4)    | 2 (0.9)    |
| Beta blocker, n (%)                              | 101 (46.1) | 22 (10.0)   | 31 (14.2) | 92 (42.0)  | 111 (50.7) | 24 (11.0) | 25 (11.4)  | 69 (31.5)  |
| Digitalis, n (%)                                 | 14 (6.4)   | 10 (4.6)    | 3 (1.4)   | 15 (6.8)   | 29 (13.2)  | 5 (2.3)   | 18 (8.2)   | 14 (6.4)   |
| Antiarrhythmic drug, n (%)                       | 10 (4.6)   | 3 (1.4)     | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Statin, n (%)                                    | 2 (0.9)    | 3 (1.4)     | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Insulin, n (%)                                   | 10 (4.6)   | 3 (1.4)     | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Sulfonylurea drug, n (%)                         | 2 (0.9)    | 3 (1.4)     | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Thiazolidine, n (%)                              | 10 (4.6)   | 3 (1.4)     | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Biguanide, n (%)                                 | 15 (6.8)   | 29 (13.2)   | 5 (2.3)   | 18 (8.2)   | 14 (6.4)   | 10 (4.6)  | 15 (6.8)   | 29 (13.2)  |
| DPP4 inhibitor, n (%)                            |            |            |           |            |            |           |            |            |
| SGLT2 inhibitor, n (%)                           | 5 (2.3)    | 2 (0.9)     | 15 (6.8)  | 29 (13.2)  | 5 (2.3)    | 2 (0.9)   | 15 (6.8)   | 29 (13.2)  |
| Loop diuretic, n (%)                             | 18 (8.2)   | 14 (6.4)    | 10 (4.6)  | 15 (6.8)   | 29 (13.2)  | 5 (2.3)   | 18 (8.2)   | 14 (6.4)   |
| Mineralocorticoid receptor inhibitor, n (%)      | 14 (6.4)   | 10 (4.6)    | 2 (0.9)   | 15 (6.8)   | 29 (13.2)  | 5 (2.3)   | 18 (8.2)   | 14 (6.4)   |
| Thiazide, n (%)                                   | 2 (0.9)    | 10 (4.6)    | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Torvaptan, n (%)                                 | 2 (0.9)    | 10 (4.6)    | 2 (0.9)   | 14 (6.4)   | 10 (4.6)   | 2 (0.9)   | 10 (4.6)   | 2 (0.9)    |
| Number of antiplatelet, n (%)                    | 205 (94.0) | 13 (5.9)    | 1 (0.5)   | 27 ± 1.7   | 11 (5.0)   | 11 (5.0)  | 11 (5.0)   | 11 (5.0)   |
| Number of baseline concomitant medications, n (%)|            |            |           |            |            |           |            |            |
| 0                                                | 205 (94.0) | 13 (5.9)    | 1 (0.5)   | 27 ± 1.7   | 11 (5.0)   | 11 (5.0)  | 11 (5.0)   | 11 (5.0)   |
| Taking over 5 medications, n (%)                 |            |            |           |            |            |           |            |            |

Consecutive variables are presented as mean ± standard deviation. Categorical variables are presented as number (%). AST indicates aspartate transaminase; ALT, alanine transaminase; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; DPP4, dipeptidyl peptidase-4; and SGLT2, sodium glucose cotransporter 2.

Figure 4. The proportion of samples showing non-reproducibility of PC-Riv within the on-therapy range (samples out of the on-therapy range in the second sample) increased with increasing CR of PC-Riv. The cutoff value of the CR of PC-Riv for non-reproducibility of on-therapy range determined by the Youden index of ROC curve analysis was 54.3% (sensitivity 0.87, specificity 0.82).

Samples were divided into two groups according to the cutoff value of 54.3% with the CR of PC-Riv (≥ 54.3%, n = 92; < 54.3%, n = 304), and the patient characteristics were compared between the two groups (Table V). In the univariate models on logistic regression analysis, hematocrit, mitral regurgitation, non-dihydropyridine calcium channel antagonist, digitalis, beta-blocker, statin, and thiazide use were significantly associated with high CR of PC-Riv (Table VI). In the multivariate model, non-dihydropyridine calcium channel antagonist (odds ratio: 3.493, 95% CI: 1.913-6.376, P < 0.001), thiazide (odds ratio: 4.561, 95% CI: 1.630-12.757, P = 0.004), and mi-
Figure 2. Distribution of plasma concentration of rivaroxaban by different sampling timing. Distributions of plasma rivaroxaban concentration by the timing of blood sampling after rivaroxaban intake.

Table III. The 90% Interval (On-therapy Range) of Rivaroxaban Plasma Concentration (LC-MS/MS and Anti-factor Xa Assay) and PT with Five Reagents

|                        | Multicenter study (This study) | Single-center study<sup>10</sup> |
|------------------------|-------------------------------|----------------------------------|
|                        | 5th percentile | 95th percentile | 5th percentile | 95th percentile |
| Direct Riv-PC (LC-MS/MS), ng/mL | 47.3          | 532.9          | 78.9           | 585.1           |
| Indirect Riv-PC (Anti-factor Xa assay), ng/mL | 45.9          | 493.0          | 63.7           | 441.2           |
| Prothrombin time, seconds |                        |                                |                |                |
| Thromborel S           | 12.6            | 25.5            | 13.1           | 22.6            |
| ThromboCheck PT        | 12.4            | 16.9            | 13.3           | 25.0            |
| STA Neoplastin Plus    | 13.9            | 21.6            | 16.1           | 33.8            |
| Hemos IL RecombiPlasTin 2G | 11.8          | 22.3            | 13.8           | 29.9            |
| ThromboCheck PT Plus   | 13.4            | 19.1            | 14.9           | 28.9            |

Consecutive variables are presented as mean ± standard deviation. Riv-PC indicates rivaroxaban plasma concentration.

Table IV. The 90% Interval (On-therapy Range) of Rivaroxaban Plasma Concentration (LC-MS/MS and Anti-factor Xa Assay) According to the Dosage of Rivaroxaban

|                        | 10 mg (n = 117) | 15 mg (n = 279) |
|------------------------|-----------------|-----------------|
|                        | 5th percentile | 95th percentile | median | 5th percentile | 95th percentile | median |
| Direct PC-Riv (LC-MS/MS), ng/mL | 97.8           | 489.2           | 274.3  | 105.8          | 483.7           | 307.5  |
| Indirect PC-Riv (Anti-factor Xa assay), ng/mL | 86.5           | 434.7           | 251.3  | 85.9           | 432.2           | 282.1  |
| Prothrombin time, seconds |                        |                |        |                |                |        |
| Thromborel S           | 13.6            | 21.2            | 17.0   | 13.4           | 20.5            | 16.6   |
| ThromboCheck PT        | 14.1            | 22.1            | 17.9   | 13.5           | 22.2            | 17.9   |
| STA Neoplastin Plus    | 16.4            | 27.9            | 21.8   | 15.9           | 27.5            | 21.8   |
| Hemos IL RecombiPlasTin 2G | 13.3           | 25.7            | 18.7   | 13.1           | 23.7            | 18.7   |
| ThromboCheck PT Plus   | 14.9            | 24.5            | 19.8   | 15             | 24.8            | 19.7   |

Consecutive variables are presented as mean ± standard deviation. Categorical variables are presented as number (%).

fluctuation of PC-Riv and the PC-Riv value itself (when a sample has a PC-Riv close to the borderline of the on-therapy range, the PC-Riv in the next sample could easily fall out of the range with a small degree of fluctuation) would affect reproducibility. Concerning the fluctuation of PC-Riv, the timing of blood sampling after rivaroxaban intake had the most significant impact. Therefore, in the present study, we restricted the sampling time to within 1-5 hours after rivaroxaban intake.

In the present study, we focused on the CR of PC-
Riv between two samplings with a three-month interval and found a wide degree of variation. Among the 396 samples within the on-therapy range in the first sample, the subsequent second sample fell out of the range in 30 (7.6%) samples (non-reproducible PC-Riv within the on-therapy range). As the CR of PC-Riv increased, the pro-

Figure 3. Correlation of plasma concentration of rivaroxaban collected by different timing. There was a moderate correlation between plasma concentrations of rivaroxaban obtained on the first visit (Data A) and three months later (Data B).

Figure 4. Reproducibility of on-therapy range by deciles of the change rate of plasma rivaroxaban concentration. The numbers of second samples for which the first samples were within the on-therapy range (n = 296) are displayed by deciles of the change rate of plasma rivaroxaban concentration. The second samples that maintained the on-therapy range are indicated with light shading, while those that fell out of the on-therapy range are indicated with dark shading.
portion of “non-reproducible PC-Riv within the on-therapy range” increased (Figure 4), and the cutoff value for non-reproducibility by Youden index was 54.3%. The factors that were independently associated with high CR were non-dihydropyridine calcium channel antagonist, thiazide, and mitral regurgitation.

Notably, non-dihydropyridine calcium channel antagonist is both a P-glycoprotein (P-gp) inhibitor and a substrate of cytochrome P450 3A4 (CYP3A4), which could strongly affect the metabolism of rivaroxaban and thereby PC-Riv. Although approximately 35% of rivaroxaban is cleared by the kidneys, 50%-65% is metabolized to an inactive form in the liver with CYP3A4 or CYP212, and the rest is eliminated in the intestine by P-gp.\textsuperscript{13,14} When co-administered with a strong P-gp inhibitor, the eflux of rivaroxaban decreased to 45%-76% compared to control.\textsuperscript{15,16}

In healthy participants, co-administration of moderate CYP3A4 inhibitors or P-gp inhibitors has been reported to show no significant effect on rivaroxaban concentration.\textsuperscript{17} However, in sub-analysis of The Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) study, the incidences of major bleeding and intracranial hemorrhage under rivaroxaban treatment were higher when a non-dihydropyridine calcium channel antagonist was co-administered.\textsuperscript{18} Moreover, in patients with impaired renal function, co-administration of verapamil with rivaroxaban leads to elevation of PC-Riv and increases in the rate of hemorrhagic events.\textsuperscript{19,20} Accordingly, several lines of evidence supported that non-dihydropyridine calcium channel antagonist was co-administered.\textsuperscript{19} However, the effect of non-dihydropyridine calcium channel antagonist on the fluctuation of PC-Riv may require more thoughtful discussion, including the time of each drug absorption. When rivaroxaban is administered alone, the time to maximum drug plasma concentration

### Table V. Comparison of Patient Characteristics between High and Low Change Ratio of Plasma Rivaroxaban Concentration

| Body weight, kg | CR < 54.3% | CR ≥ 54.3% | P-value |
|----------------|------------|------------|---------|
| Hemoglobin, g/dL | 14.2 ± 1.6 | 13.9 ± 2.3 | 0.138   |
| Hematocrit, %   | 42.6 ± 4.3 | 41.4 ± 6.3 | 0.033   |
| Serum creatinine, mg/mL | 0.92 ± 0.22 | 0.88 ± 0.23 | 0.113   |
| Valvular disease, n (%) | 8 (2.6) | 7 (7.6) | 0.054   |
| Mitral regurgitation, n (%) | 6 (2.0) | 6 (6.5) | 0.037   |
| Angina, n (%)     | 20 (6.6) | 11 (12.0) | 0.119   |
| Heart failure, n (%) | 65 (21.4) | 13 (14.1) | 0.137   |
| Past history of major bleeding, n (%) | 0 (0.0) | 3 (3.3) | 0.012   |
| Non-dihydropyridine calcium channel antagonist, n (%) | 168 (55.3) | 39 (42.4) | 0.033   |
| Dihydropyridine calcium channel antagonist, n (%) | 136 (44.7) | 32 (34.8) | 0.094   |
| Antiarrhythmic agents, n (%) | 31 (10.2) | 14 (15.2) | 0.192   |
| Statin, n (%)      | 104 (34.2) | 19 (20.7) | 0.015   |
| Thiazide, n (%)    | 8 (2.6) | 8 (8.7) | 0.016   |
| Intake of rivaroxaban more than 3 months, n (%) | 273 (89.8) | 78 (84.8) | 0.192   |
| Malignant disease, n (%) | 20 (6.6) | 11 (12.0) | 0.119   |
| Meal before 2nd blood sampling, n (%) | 250 (82.2) | 70 (76.1) | 0.226   |

Consecutive variables are presented as mean ± standard deviation. Categorical variables are presented as number (%). CR indicates change ratio of plasma concentration of rivaroxaban.

### Table VI. Factors Related to a High Change Ratio of Plasma Rivaroxaban Concentration

| Factors                  | Odds ratio | 95% CI        | P-value |
|--------------------------|------------|---------------|---------|
| Univariate models        |            |               |         |
| Hematocrit               | 0.951      | 0.905-0.998   | 0.042   |
| Mitral regurgitation      | 3.465      | 1.090-11.018  | 0.035   |
| Non-dihydropyridine calcium channel antagonist | 3.172 | 1.763-5.707 | <0.001 |
| Digitalis                | 2.075      | 1.068-4.034   | 0.031   |
| Beta blocker             | 0.596      | 0.372-0.954   | 0.031   |
| Statin                   | 0.501      | 0.287-0.874   | 0.015   |
| Thiazide                 | 3.524      | 1.284-9.670   | 0.014   |
| Multivariate models      |            |               |         |
| Non-dihydropyridine calcium channel antagonist | 3.494 | 1.913-6.376 | <0.001 |
| Thiazide                 | 4.561      | 1.630-12.757  | 0.004   |
| Mitral regurgitation      | 3.634      | 1.096-12.055  | 0.035   |
(T_{max}) is 1.4-4.0 hours, and T_{1/2} is 5.7-9 hours.21 On the other hand, the T_{max} of verapamil is 2.2 hours, and that of diltiazem is 3.2-3.3 hours, which increased to ~11 hours with its slow release agent. Given the potent effects of verapamil and diltiazem on the pharmacokinetics of rivaroxaban, an intraindividual day-to-day variation of the absorption of each drug may result in a complex interaction and may contribute to the variation of PC-Riv by measurement. Further studies are required to evaluate whether displacing the timing of drug intake may be beneficial to avoid the interaction of these drugs and maintain stable PC-Riv.

Thiazide and mitral regurgitation may contribute to the fluctuation of renal blood flow, leading to some variation of renal excretion of rivaroxaban. Mitral regurgitation would cause hemodynamic variation, which results in the variations in cardiac output and consequent renal blood flow. Wang, et al. reported that after they perform mitral clipping for the treatment of mitral regurgitation, significant improvement of renal function was observed.20 Reversely, the existence of mitral regurgitation would reduce the renal blood flow, and consequently impair the renal function. Although we do not have quantitative measurement, we speculate such hemodynamic variation by mitral regurgitation may affect the excretion of rivaroxaban and therefore affect the temporal variation in PC-Riv. However, given the small samples, caution is required in interpreting the results.

Limitations: This study had several limitations. First, this study was performed in daily clinical practice, and therefore unintended variations in the timing of administration of medications and blood sampling were unavoidable. We selected samples collected within 1-5 hours after rivaroxaban intake for analysis, excluding one-third of the samples collected with inappropriate timing. Second, polypharmacy may affect drug absorption, metabolism, and excretion. Although we examined the impact of non-dihydropyridine calcium channel antagonist, other P-gp antagonists and CYP3A antagonists were not thoroughly checked. Third, although non-dihydropyridine calcium channel antagonist and thiazide were determined as the predictors for the change rate of PC-Riv, we did not assess whether there is a dose-dependent effect. Unfortunately, lacking data for quantitative evaluation and a small number of patients prohibited further analyses. Finally, our data could not clarify the significance of the on-therapy range and the high CR of PC-Riv on thromboembolic and hemorrhagic events because no such adverse events were observed during the observation period in this study.

Conclusions

We reported the on-therapy range of rivaroxaban in a multicenter cohort, which was consistent with the previously reported data in a single-center cohort and was highly reproducible between first and second samplings in daily clinical practice. However, caution is necessary regarding several factors that may affect the intraindividual variation of PC-Riv.

Disclosure

Conflicts of interest: Dr. Suzuki received research funding from Daiichi-Sankyo and Mitsubishi-Tanabe. Dr. Yamasaki received lecture fees from Daiichi-Sankyo, Kowa-Soyaku, Bayer, Boehringer Ingelheim, MSD, Sanofi, Otsuka Pharmaceutical, Ono Pharmaceutical, Tanabe-Mitsubishi Pharmaceutical, and Toa Eiyo. Dr. Yamashita received lecture fees from Bristol Meyers Squibb, Daiichi-Sankyo, Bayer, Pfizer, Ono Pharmaceutical, and Toa Eiyo and research funding from Bayer and Daiichi-Sankyo.

There is no information to disclose for the other authors.

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