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ORIGINAL ARTICLE

Comparison of anti-inflammatory effects of rivaroxaban vs. dabigatran in patients with non-valvular atrial fibrillation (RIVAL-AF study): multicenter randomized study

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Abstract

Some experimental studies have shown that direct oral anticoagulants (DOACs) have anti-inflammatory effects. However, the interval changes in inflammatory markers in patients with non-valvular atrial fibrillation (AF) who receive DOACs remain unknown. Between July 2013 and April 2014, a total of 187 AF patients randomly assigned to receive rivaroxaban ($n=91$) or dabigatran ($n=96$) were assessed for eligibility. The levels of the following inflammatory markers were serially evaluated: high-sensitivity C-reactive protein, pentraxin-3, interleukin (IL)-1β, IL-6, IL-18, tumor necrosis factor-α, monocyte chemotactic protein-1, growth and differentiation factor-15, and soluble thrombomodulin (sTM). The aim in this study was to evaluate the anti-inflammatory effects of rivaroxaban and dabigatran in patients with AF, in addition to the impact of markers on bleeding events. Finally, 117 patients (rivaroxaban: $n=55$, dabigatran: $n=62$) were included in the analysis at 12 months. Although the interval changes in sTM levels tended to be greater in the dabigatran group [0.3 (0–0.7) vs. 0.5 (0–1.0) FU/ml, $p=0.061$], there were no significant differences in the interval changes in any inflammatory marker between 2 groups. There were no significant differences in bleeding events between 2 groups. The interval changes in sTM levels were significantly greater in patients with bleeding compared with those without [0.8 (0.5–1.3) vs. 0.4 (−0.1–0.8) FU/ml, $p=0.017$]. There were no significant differences in the interval changes in any inflammatory marker between rivaroxaban and dabigatran treatments in patients with AF. The increased levels of sTM after DOACs treatment might be related to bleeding events.

Keywords Inflammation · Direct oral anticoagulation · Atrial fibrillation · Bleeding · Thrombomodulin

Introduction

Inflammation is related to the development and maintenance of atrial fibrillation (AF) and the risk of adverse events such as stroke in AF patients [1, 2]. Direct oral anticoagulants (DOACs) have been developed to prevent stroke and systemic embolism in patients with non-valvular AF. Both factor Xa and thrombin are targets of DOACs. Factor Xa represents the point of convergence of the extrinsic and intrinsic pathways of the coagulation cascade and converts prothrombin into thrombin. Thrombin is the central protease of the coagulation cascade, involved in the formation of a hemostatic plug to avoid severe bleeding. In addition, molecular biological studies have shown that both factor Xa and thrombin are involved in inflammatory processes and vascular endothelial dysfunction [3, 4]. Some experimental studies have shown that factor Xa inhibitors and direct thrombin inhibitors have anti-inflammatory effects.

DOACs, particularly factor Xa inhibitors, have been expected to prevent the progression of atherosclerotic lesions [4–7]. The inhibition of inflammation by DOACs may play an important role in the suppression of atherosclerosis. In clinical practice, the ROCKET AF trial (Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared

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with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation) showed that the rate of myocardial infarction tended to be lower in patients with AF who received rivaroxaban than in those who received warfarin [8]. On the other hand, in the randomized evaluation of long-term anticoagulation therapy (RE-LY) trial, the rate of myocardial infarction tended to be higher in patients with AF who received dabigatran than in those who received warfarin [9]. A meta-analysis showed that the risk of coronary events was significantly higher for dabigatran, but significantly lower for rivaroxaban [10]. Moreover, in the Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome–Thrombolysis in Myocardial Infarction 51 (ATLAS ACS2-TIMI51) trial, a low dose of rivaroxaban reduced the risk of the composite endpoint of death from cardiovascular causes, myocardial infarction, or stroke in patients with recent acute coronary syndromes (ACS) [11]. Thus, factor Xa and thrombin may play different roles in mediating cellular signaling effects associated with the development of atherosclerosis. Inhibiting factor Xa or thrombin might provide additional therapeutic benefits and be an attractive target for therapeutic intervention in ACS [12]. However, to date, interval changes in inflammatory markers in patients with AF who receive DOACs remain unknown. The present study was designed to evaluate the anti-inflammatory effects of the factor Xa inhibitor rivaroxaban as compared with the thrombin inhibitor dabigatran in patients with non-valvular AF.

Materials and methods

Patients

We conducted a prospective, randomized, open-label study designed to serially assess the anti-inflammatory effects of DOACs in Japanese patients with non-valvular AF. Patients with a CHA_2DS_2-VASc score of 1 or more and no contraindication for anticoagulation were enrolled. Female patients with gender alone as the sole risk factor (a CHA_2DS_2-VASc score of 1) were not required to receive anticoagulants. The exclusion criteria were (1) stroke or systemic embolism within 6 months before enrollment; (2) ACS or peripheral artery disease within 6 months before enrollment; (3) acute heart failure; (4) severe chronic renal failure (creatinine clearance < 30 ml/min); (5) treatment with dual antiplatelet therapy; (6) a body weight of 50 kg or less; (7) uncontrolled hypertension; (8) active malignancy; (9) surgery within 6 months before enrollment; (10) collagen disease; (11) infectious disease; (12) patients scheduled to undergo catheter ablation for AF; (13) contraindications to rivaroxaban or dabigatran; and (14) patients considered unsuitable for participation in the trial by the treating physician.

Patients were assigned to receive a Japanese standard dose of rivaroxaban (15 mg once daily) or dabigatran (150 mg twice daily) according to a computer-generated randomization sequence at the central registration center. All patients were required to receive rivaroxaban or dabigatran during this study. Patients with a creatinine clearance of 30–49 ml/min received rivaroxaban 10 mg once daily. Patients at high risk for bleeding received dabigatran 110 mg twice daily. If the patient was receiving warfarin prior to randomization, warfarin was discontinued and serial international normalized ratio (INR) tests were performed to ensure that the INR dropped to below 2.0 before taking baseline blood samples and initiating the study drug.

Blood samples and inflammatory markers

We evaluated the levels of the following inflammatory markers: high-sensitivity C-reactive protein (hsCRP), pentraxin-3 (PTX-3), interleukin (IL)-1β, IL-6, IL-18, tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and growth and differentiation factor-15 (GDF-15). In addition, soluble thrombomodulin (sTM) levels were measured. During the 6 months of follow-up, the levels of inflammatory markers, including hsCRP, PTX-3, IL-6, and IL-18, and sTM were serially assessed at baseline and 1 month, 3 months, and 6 months after randomization. There may be inter-seasonal variability in the levels of the inflammatory markers because of occult viral infections or seasonal allergies. Therefore, we decided to additionally measure the above inflammatory markers and sTM 12 months after randomization for patients from whom written informed consent was obtained at 6 months. Blood samples, each consisting of 12 ml of blood in a citrate tube and 2 ml of blood in an EDTA-containing tube, were drawn from an antecubital vein. The choice of rhythm control or rate control therapy was left to the discretion of the treating physician; however, the protocol did not permit blood sampling within 7 days after cardioversion.

The levels of serum hsCRP were measured by nephelometry (N-Latex CRPII; Date Behring, Tokyo, Japan). The levels of plasma PTX-3 (Human PTX3 ELISA System; Perseus Proteomics Inc., Tokyo, Japan), serum IL-1β (IL-1β EASIA; Biosource Europe S.A., Fleurus, Belgium), serum IL-18 (Human IL-18 ELISA kit; MBL, Nagoya, Japan), serum TNF-α (Quantikine Human TNF-α/TNFSF1A Immunoassay kit; R&D Systems, Inc., Minneapolis, MN, USA), serum MCP-1 (Quantikine Human CCL2/MCP-1 Immunoassay kit; R&D Systems, Inc., Minneapolis, MN, USA), and serum GDF-15 (Quantikine Human GDF-15 Immunoassay kit; R&D Systems, Inc., Minneapolis, MN, USA) were assessed by enzyme-linked immunosorbent assay (ELISA). The level of serum IL-6 was determined by chemiluminescent enzyme immunoassay (CLEIA) (Human IL-6 CLEIA

Fujirebio; Fujirebio, Tokyo, Japan). The level of serum TM was measured by enzyme immunoassay (EIA) (TM Panasera; FujiRebio, Tokyo, Japan).

Endpoints

The primary outcome measure was the between-group difference in the interval changes in the inflammatory markers (including hsCRP, PTX-3, IL-6, IL-18, IL-1β, TNF-α, MCP-1, and GDF-15) and sTM from baseline to 12 months later, as compared between the treatment groups. The secondary outcome measures were the time courses of inflammatory markers and sTM during the 12-month follow-up period in each treatment group. Other pre-specified outcome measures were the frequencies of adverse cardiac and cerebrovascular events (including cardiovascular death, myocardial infarction, revascularization, ischemic stroke, and systemic embolism) and the frequency of major bleeding at 12 months. Major bleeding was defined according to the International Society on Thrombosis and Haemostasis (ISTH) criteria and included fatal bleeding; symptomatic bleeding; and bleeding causing a fall in the hemoglobin level of ≥ 2 g/dl or leading to transfusion of ≥ 2 units of whole blood or red blood cells [13]. Minor bleeding was defined as clinically relevant bleeding, but did not meet the criteria for major bleeding. Myocardial infarction was defined according to the third universal definition proposed in 2012 [14]. Ischemic stroke was a combined outcome, including transient ischemic attack as well as cerebrovascular infarction.

The study protocol was approved by the institutional review boards of all participating centers and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. Clinical, laboratory, and outcome data were prospectively collected by independent research personnel unaware of the study aims and were entered into a central database (ClinicalTrials.gov number NCT02331602). Follow-up visits were conducted at our hospitals.

Statistical analysis

Categorical variables are reported as frequencies and percentages and were compared with the use of Chi square tests. Continuous variables are reported as mean ± SD and were compared using Student's *t* tests. The levels of inflammatory markers and sTM are reported as medians (interquartile range), and nonparametric tests were used for comparisons (the Wilcoxon signed-rank test for within-group comparisons, and the Wilcoxon rank sum test for between-group comparisons). The interval changes in the inflammatory markers and sTM [the change amounts from baseline to 12 months (Δ(ba-12)) = (levels of markers at 12 months) – (levels of markers level at baseline)] were analyzed with the use of Wilcoxon rank sum test to assess differences between groups. Multiplex analysis was not performed. Relationships between bleeding events and various clinical characteristics including the interval changes in sTM were analyzed by univariable and multivariable regression analyses. Multivariate regression model was designed to study independent predictors of subsequent bleeding events using variables with p value of < 0.10 in univariate analysis. Spearman's rank correlation coefficients were used for the calculation of correlation between inflammatory markers and sTM. P values of < 0.05 were considered to indicate statistical significance. In this study, no power calculation was performed, because useful reference data from the previous study was absent. Data were analyzed with JMP Pro12 (SAS Institute Inc., Cary, NC, USA). The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Between July 2013 and April 2014, a total of 193 patients with AF (140 men; mean age, 71 years) were randomly assigned to receive rivaroxaban ($n = 94$) or dabigatran ($n = 99$) in 16 Japanese centers (Fig. 1). Among them, 187 patients who received rivaroxaban ($n = 91$) or dabigatran ($n = 96$) were assessed for eligibility. Finally, 117 patients (rivaroxaban: $n = 55$, dabigatran: $n = 62$) were included in the analysis at 12 months.

Primary endpoint (the between-group difference) and secondary endpoint (the within-group difference: time course)

On the analysis at 12 months ($N = 117$), there were no significant differences in baseline characteristics, including age, smoking, chronic heart failure, hypertension, diabetes, and chronic kidney disease, between 2 groups (Table 1). The mean age was 71.2 ± 8.2 years, and approximately 30% of the subjects were female. Only 5% of the subjects had prior myocardial infarction. The mean CHA_2DS_2-VASc score was 3.2, and 44% of the patients had paroxysmal AF, with no significant difference between the 2 groups. There were no significant differences in medication at baseline, except for the proportion of patients who received a reduced dose of the assigned study drug (38% in the rivaroxaban group vs. 71% in the dabigatran group).

There were no significant differences in the interval changes from baseline to 12 months in any inflammatory marker between the groups (Table 2). The interval changes in sTM levels from baseline to 12 months tended to be greater in the dabigatran group than in the rivaroxaban group [0.3 (0–0.7) vs. 0.5 (0–1.0) FU/ml, $p = 0.061$]. There

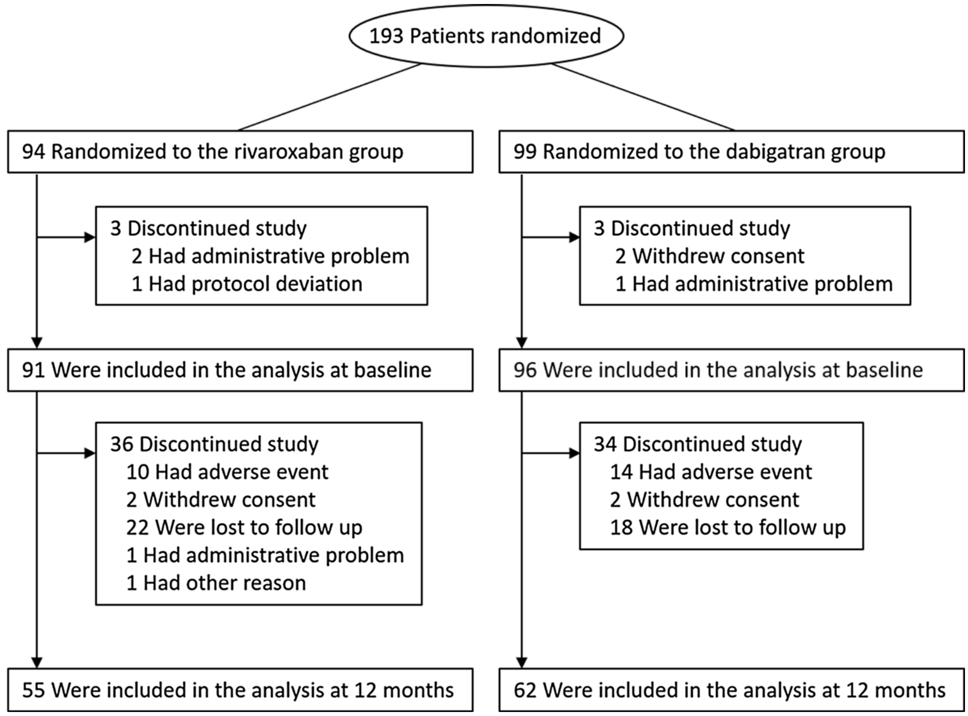

Fig. 1 Patient flow diagram

were no significant differences in the interval changes in any marker between patients treated with a reduced dose and those with a standard dose. Among each group, there were no significant differences in the interval changes in any marker according to dosage of DOACs (data not shown).

The time courses of inflammatory markers (hsCRP, PTX-3, IL-6, IL-18) and sTM during the 12 months of follow-up (at baseline, 1 month, 3 months, 6 months, and 12 months) in each treatment group are shown in Fig. 2. There was no consistent trend in the time course of any inflammatory marker in each group (Table 2). The levels of GDF-15 increased significantly from baseline to 12 months in both the rivaroxaban group [1260 (929–1650) vs. 1350 (980–1850) pg/ml, $p<0.01$] and the dabigatran group [1220 (955–1650) vs. 1345 (931–1913) pg/ml, $p=0.045$]. Although the interval changes in IL-6 and IL-18 levels were similar between 2 groups, the levels of IL-6 [2.8 (2.0–3.8) vs. 2.3 (1.6–3.3) pg/ml, $p=0.049$] and IL-18 [213 (186–259) vs. 210 (173–259) pg/ml, $p<0.01$] significantly decreased from baseline to 12 months in the dabigatran group. Furthermore, the levels of sTM increased significantly from baseline to 12 months in both the rivaroxaban groups [2.8 (2.4–3.4) vs. 3.2 (2.6–3.7) FU/ml, $p<0.001$] and the dabigatran group [2.8 (2.5–3.2) vs. 3.3 (2.6–4.0) FU/ml, $p<0.001$].

Adverse events and inflammatory markers

Among all subjects ($N=187$), 17 patients (18.7%) in the rivaroxaban group and 20 patients (20.8%) in the dabigatran group had adverse events (Table S1). In the rivaroxaban group, 11 patients (12.1%) had bleeding events, whereas 11 patients (11.5%) had bleeding events in the dabigatran group. There were no significant differences in bleeding events between the groups. The rate of patients with major bleeding was 3.3% ($n=3$) in the rivaroxaban group and 5.2% ($n=5$) in the dabigatran group. No patient had intracranial bleeding during the study. Although there were no thromboembolic events, 2 coronary events occurred during follow-up: 1 patient in the rivaroxaban group had acute coronary syndrome and 1 patient in the dabigatran group had congestive heart failure due to ischemic heart disease and underwent percutaneous coronary intervention.

Relationship between bleeding events and the levels of each marker at baseline and at 12 months (Tables 3, 4). Patients with bleeding had higher hsCRP levels both at baseline [1150 (455–3905) vs. 613 (294–1393) ng/ml, $p=0.085$] and at 12 months [1825 (979–2515) vs. 567 (348–1350) ng/ml, $p=0.025$] compared with those without. IL-6 at baseline tended be higher in patients with bleeding than in those without [3.5 (1.9–6.5) vs. 2.5 (1.6–3.5) pg/ml, $p=0.068$] and GDF-15 levels at 12 months tended to be higher in patients with bleeding than in those without [2125 (1056–3098) vs. 1330 (975–1843) pg/ml, $p=0.077$].

Relationship between bleeding events and interval changes in markers from baseline to 12 months is shown in Table 5. There was no significant difference in the interval changes in any inflammatory markers between patients with bleeding and those without, except IL-1β, which seemed to be not clinically significant, although it was statistically significant [0 (− 1.5–0) vs. 0 (0–1.3) pg/ml, $p=0.03$]. Interval

Table 1 Baseline characteristics

	Rivaroxaban ($n=55$)	Dabigatran ($n=62$)	*P* value
Age (years)	72.3 ± 6.3	70.2 ± 9.5	0.16
> 75 years	21 (38)	24 (39)	0.95
Female	16 (29)	19 (31)	0.86
Height (cm)	161.6 ± 9.0	163.2 ± 8.6	0.32
Weight (kg)	64.6 ± 11.6	64.6 ± 11.7	0.98
Smoking	28 (52)	31 (51)	0.91
Alcohol drinking	37 (69)	31 (51)	0.054
Anemia	8 (15)	5 (8)	0.27
CHF	13 (24)	15 (24)	0.94
Hypertension	46 (84)	57 (92)	0.17
Diabetes	12 (22)	21 (34)	0.15
Hyperlipidemia	35 (64)	47 (76)	0.15
CKD	22 (40)	29 (47)	0.46
Prior stroke	6 (11)	7 (11)	0.95
Prior MI	2 (4)	4 (7)	0.68
PAD	1 (2)	2 (3)	1
CHA2D2-VASc score			0.73
Score 1	4 (7)	4 (7)	
Score 2	16 (29)	14 (23)	
Score 3	13 (24)	18 (29)	
Score 4	16 (29)	14 (23)	
Score 5	4 (7)	9 (15)	
Score 6	2 (4)	3 (5)	
Type of AF			0.31
Paroxysmal	22 (40)	30 (48)	
Persistent	6 (11)	10 (16)	
Permanent	27 (49)	22 (36)	

Data given as mean ± SD or *n* (%)

Anemia was defined as a hemoglobin concentration below 12 g/dl in females and below 13 g/dl in males. CKD was defined as an estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73 m^2

CHF chronic heart failure, *CKD* chronic kidney disease, *MI* myocardial infarction, *PAD* peripheral arterial disease, *AF* atrial fibrillation

changes in sTM levels in patients with bleeding significantly increased compared with those without [0.8 (0.5–1.3) vs. 0.4 (−0.1–0.8) FU/ml, $p=0.017$]. On the 1-month landmark sub-analysis ($N=173$, the details of patients' characteristics are shown in Table S2), interval changes in sTM levels from baseline to 1 month [ΔTM(ba-1)] were greater in patients with subsequent bleeding events from 1 month to 12 months than in those without [0.5 (0.1–0.8) vs. 0.2 (0–0.5) FU/ml, $p=0.039$]. On multivariate logistic regression analysis, ΔTM(ba-1) ≥ 0.4FU/ml, cut-off value determined by receiver operating characteristic curve, was an independent predictor of subsequent bleeding events (OR 2.92, 95% CI 1.05–8.58; $p=0.039$), as well as prior use of warfarin (OR 3.85, 95% CI 1.28–14.27; $p=0.016$) (Table 6).

Table 2 Interval changes between the groups from baseline to 12 months

	Rivaroxaban ($n=55$)					Dabigatran ($n=62$)					*P* value of the interval changes between the groups
	Baseline		12 months		Δ(ba-12)	Baseline		12 months		Δ(ba-12)	
	Median (IQR)	CV (%)	Median (IQR)	CV (%)		Median (IQR)	CV (%)	Median (IQR)	CV (%)		
hsCRP (ng/ml)	667 (323–1520)	200.5	668 (422–1820)	184.9	−18 (−466–299)	748 (375–2173)	154.2	605 (319–1773)	245.4	−60 (−532–312)	0.64
PTX-3 (ng/ml)	2.0 (1.5–2.5)	58	1.8 (1.3–2.5)	59.7	−0.1 (−0.6–0.3)	1.9 (1.5–2.8)	54.9	1.8 (1.3–3.1)	65.3	−0.2 (−0.6–0.3)	1
IL-1β (pg/ml)	10 (10–12)	43.8	10 (10–14)	66.9	0 (0–1.0)	10 (10–12)	49.8	10 (10–13)	40	0 (0–1.3)	0.64
IL-6 (pg/ml)	2.6 (1.6–3.5)	80.5	1.9 (1.4–3.1)	166.9	−0.4 (−1.4–0.7)	2.8 (2.0–3.8)	99.4	2.3 (1.6–3.3)†	81.8	−0.6 (−1.4–0.5)	0.97
IL-18 (pg/ml)	198 (158–260)	39.2	194 (157–260)	37.9	−8 (−37–25)	213 (186–259)	36.5	210 (173–259)††	36.2	−16 (−42–13)	0.41
TNF-α (pg/ml)	1.1 (0.8–1.3)	35.1	1.1 (0.8–1.6)	40.7	0.1 (−0.1–0.3)	1.2 (0.8–1.5)	39.9	1.1 (0.8–1.5)	136.2	0 (−0.2–0.3)	0.41
MCP-1 (pg/ml)	282 (238–352)	33.6	304 (242–360)	25.9	13 (−22–43)	256 (220–292)	44.4	256 (215–315)	43.6	−5 (−40–45)	0.28
GDF-15 (pg/ml)	1260 (929–1650)	40.2	1350 (980–1850)**	46	91 (−25–265)	1220 (955–1650)	50	1345 (931–1913)*	62.6	65 (−83–243)	0.4
sTM (FU/ml)	2.8 (2.4–3.4)	24.4	3.2 (2.6–3.7)***	25.9	0.3 (0–0.7)	2.8 (2.5–3.2)	20.5	3.3 (2.6–4.0)***	25.5	0.5 (0–1.0)	0.061

Data given as medians (25th–75th percentiles) or mean ± SD

IQR interquartile range, *CV* coefficient of variation

*$P<0.05$ and ***$P<0.001$, increase in the levels of markers from baseline to 12 months within each group

†$P<0.05$ decrease in the levels of markers from baseline to 12 months within each group

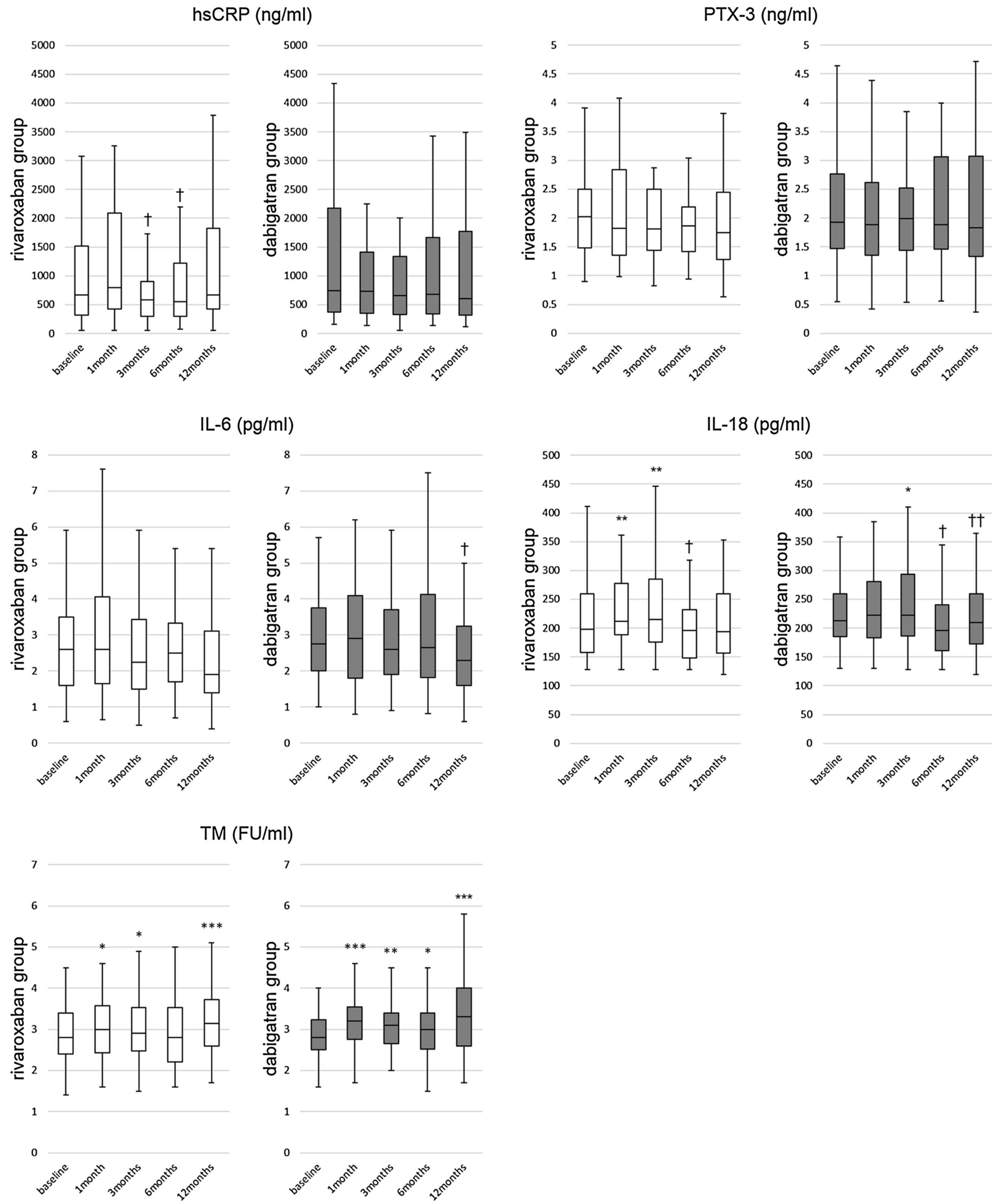

Fig. 2 Changes in inflammatory markers over time in 117 patients with 12-month follow-up. Box plots show the 25th and 75th percentiles, and the medians are indicated by the horizontal black lines. Whiskers indicate the maximum and the minimum values, excluding outliners, set using the interquartile range. *$P<0.05$, **$P<0.01$, and ***$P<0.001$, increase in the levels of inflammatory markers from baseline within each group. †$P<0.05$, ††$P<0.01$, decrease in the levels of inflammatory markers from baseline within each group

Table 3 Relationship between bleeding events and the levels of markers at baseline

	Bleeding ($n=21$)	Non-bleeding ($n=166$)	P value
hsCRP(ba) (ng/ml)	1150 (455–3905)	613 (294–1393)	0.085
PTX-3(ba) (ng/ml)	2.1 (1.4–3.2)	2.0 (1.5–2.8)	0.66
IL-1β(ba) (pg/ml)	10 (10–14)	10 (10–12)	0.58
IL-6(ba) (pg/ml)	3.5 (1.9–6.5)	2.5 (1.6–3.5)	0.068
IL-18(ba) (pg/ml)	219 (180–269)	207 (173–260)	0.43
TNF-α(ba) (pg/ml)	1.2 (0.8–1.3)	1.1 (0.8–1.4)	0.85
MCP-1(ba) (pg/ml)	275 (247–343)	264 (225–327)	0.6
GDF-15(ba) (pg/ml)	1575 (684–2298)	1200 (936–1620)	0.36
sTM(ba) (FU/ml)	2.7 (2.3–3.8)	2.8 (2.4–3.3)	0.77

Data given as medians (25th–75th percentiles)

Table 4 Relationship between bleeding events and the levels of markers at 12 months

	Bleeding ($n=10$)	Non-bleeding ($n=107$)	P value
hsCRP(12) (ng/ml)	1825 (979–2515)	567 (348–1350)	0.025
PTX-3(12) (ng/ml)	1.8 (1.2–3.6)	1.8 (1.3–2.5)	0.7
IL-1β(12) (pg/ml)	10 (10–11)	10 (10–14)	0.25
IL-6(12) (pg/ml)	2.6 (1.9–3.7)	2.0 (1.4–3.2)	0.17
IL-18(12) (pg/ml)	211 (164–271)	198 (167–259)	1
TNF-α(12) (pg/ml)	1.3 (1.0–1.8)	1.1 (0.8–1.5)	0.22
MCP-1(12) (pg/ml)	314 (230–355)	274 (231–334)	0.48
GDF-15(12) (pg/ml)	2125 (1056–3098)	1330 (975–1843)	0.077
sTM(12) (FU/ml)	4.0 (2.8–4.5)	3.2 (2.6–3.8)	0.11

Data given as medians (25th–75th percentiles)

Influence of prior warfarin use

Although there were no significant differences in the levels at baseline and the interval changes in any inflammatory marker from baseline to 12 months between patients with prior warfarin use and those without, the rate of bleeding events was higher [$n=14$ (16%) vs. $n=7$ (7%), $p=0.043$] and the increased levels of sTM from baseline to 12 months tended to be greater in patients with prior warfarin compared with those without [0.5 (0.1–1.0) vs. 0.3 (−0.1–0.8) FU/ml, $p=0.066$].

Associations between inflammatory markers and soluble thrombomodulin

On the analysis at 12 months ($N=117$), there were positive correlations between markers, as shown in Fig. 3. The levels of sTM were positively correlated with PTX-3 ($\rho=0.32$, $p<0.001$), IL-6 ($\rho=0.30$, $p=0.001$), TNF-α ($\rho=0.29$, $p=0.002$), and GDF-15 ($\rho=0.52$, $p<0.001$).

Discussion

Cross-talk between coagulation and inflammatory pathways has been well documented [15]. Experimental studies have reported that factor Xa and thrombin participate in inflammatory processes involved in the development of atherosclerosis and vascular endothelial dysfunction [3, 16]. Factor Xa might be a preferred target because of its upstream position in the coagulation cascade and its role in cellular functions mediated by proteinase-activated receptors (PARs) [4]. Factor Xa enhances the secretion of pro-inflammatory cytokines, such as IL-6, IL-8, and MCP-1 [3, 17]. Moreover, treatment with rivaroxaban reduces the expression of pro-inflammatory markers such as IL-6, TNF-α, and MCP-1 in apolipoprotein E-deficient mice, indicating atherosclerotic plaque stabilization [5]. In contrast to factor Xa inhibitors, a previous study reported that ximelagatran, another thrombin inhibitor, increases inflammatory markers in patients with myocardial infarction [18]. Factor Xa is more thrombogenic than thrombin and plays important roles in cellular proliferation [19] and the inflammatory process [20]. A previous

Table 5 Relationship between bleeding events and interval changes in markers from baseline to 12 months

	Bleeding ($n=10$)	Non-bleeding ($n=107$)	P value
ΔhsCRP(ba-12) (ng/ml)	−132 (−1628–1814)	−27 (−466–299)	0.96
ΔPTX-3(ba-12) (ng/ml)	0 (−0.3–0.8)	−0.2 (−0.7–0.3)	0.13
ΔIL-1β(ba-12) (pg/ml)	0 (−1.5–0)	0 (0–1.3)	0.03
ΔIL-6(ba-12) (pg/ml)	−1.2 (−1.6–0.9)	−0.4 (−1.4–0.5)	0.5
ΔIL-18(ba-12) (pg/ml)	14 (−53–35)	−12 (−38–12)	0.36
ΔTNF-α(ba-12) (pg/ml)	0.3 (0–0.5)	0 (−0.2–0.3)	0.12
ΔMCP-1(ba-12) (pg/ml)	7 (−37–56)	3 (−30–43)	0.77
ΔGDF-15(ba-12) (pg/ml)	245 (0–818)	77 (−51–231)	0.13
ΔsTM(ba-12) (FU/ml)	0.8 (0.5–1.3)	0.4 (−0.1–0.8)	0.017

Data given as medians (25th–75th percentiles)

Table 6 Univariable and multivariable analyses for determinants of subsequent bleeding events in patients with 1-month treatment with DOACs

Characteristics	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
ΔsTM(ba-1) ≥ 0.4FU/ml	3.61 (1.34–10.36)	0.011	2.92 (1.05–8.58)	0.039
Rivaroxaban	0.85 (0.31–2.28)	0.75		
CHF	0.71 (0.16–2.31)	0.6		
Hypertension	1.12 (0.29–7.42)	0.89		
Age > 75 years	0.64 (0.20–1.80)	0.41		
Female	0.50 (0.11–1.62)	0.27		
Prior use of warfarin	4.60 (1.57–16.80)	0.0046	3.85 (1.28–14.27)	0.016
CHA2D2-VASc score ≥ 4	0.84 (0.28–2.28)	0.73		
HASBLED score ≥ 3	1.00 (0.37–2.70)	1		

OR odds ratio, *CI* confidence interval

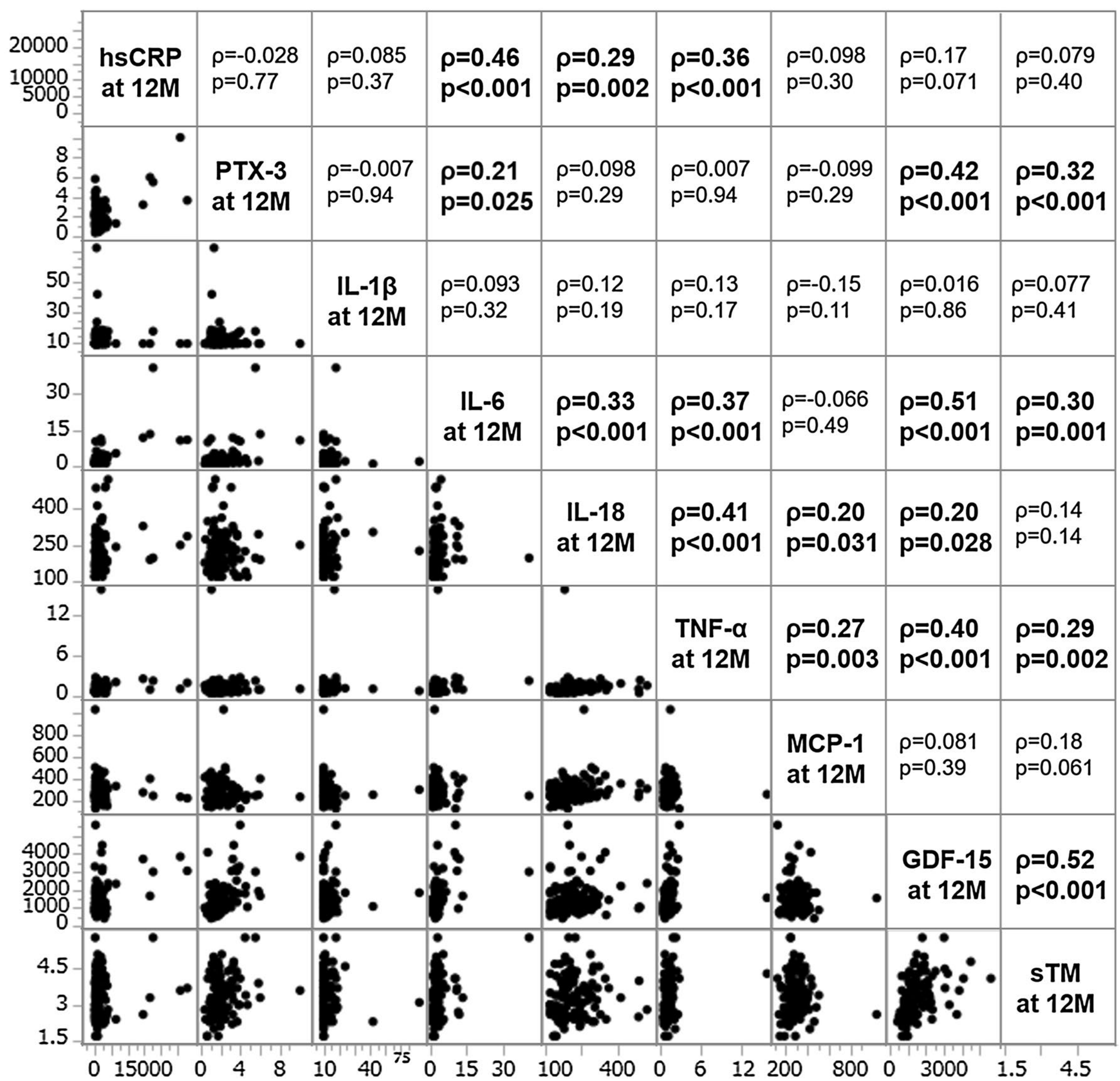

Fig. 3 Associations between each marker using Spearman's rank correlation coefficients

study suggested that the modulation of factor Xa activity via inhibition of PAR-2 expression might be more important than the modulation of thrombin [3]. Rivaroxaban inhibits the generation of reactive oxygen species and suppresses MCP-1 gene expression in advanced glycation end product-exposed tubular cells by blocking thrombin/PAR-2 system [21]. Currently available data suggest that factor Xa inhibitors (e.g., rivaroxaban) have not only anticoagulant effects but also anti-inflammatory effects.

To our knowledge, this study is the first multicenter, randomized study to prospectively investigate whether rivaroxaban and dabigatran have anti-inflammatory effects in patients with AF. However, in the present study, there were no significant differences between the rivaroxaban group and dabigatran group in the interval changes in any inflammatory marker. GDF-15 levels increased significantly from baseline to 12 months in both groups. In addition, IL-6 and IL-18 levels significantly decreased from baseline to 12 months only in the dabigatran group, although these levels tended to decrease in the rivaroxaban group. There was no consistent trend in the time course of any inflammatory marker in each group. Several factors may account for the apparent lack of anti-inflammatory effects in patients who received rivaroxaban as compared with those who received dabigatran. First, the risk of coronary events in the subjects of the present study was relatively low. Only a few patients had a history of prior myocardial infarction. Second, dabigatran may also decrease the levels of inflammatory markers. Experimental studies have shown that dabigatran prevents the formation and decreases the size of atherosclerotic plaques and inhibits the expression of pro-inflammatory markers in apolipoprotein E-deficient mice [22, 23]. In a practical clinical trial among Asians with non-valvular AF, both rivaroxaban and dabigatran were associated with reduced risks of ischemic stroke or systemic embolism, intracranial hemorrhage, and all-cause mortality without a significantly increased risk of acute myocardial infarction as compared with warfarin [24]. In fact, in this study, IL-6 and IL-18 levels significantly decreased in the dabigatran group. It has been reported that IL-6 and IL-18 levels are the predictor of cardiovascular mortality in patients with metabolic syndrome [25, 26]. Third, the uncertainties of the results of the present study may suggest complex interactions between the coagulation systems and inflammation in daily clinical practice. Occult infection and allergic reaction might exist in some patients, although the sample timepoints in our patients were in the same season (at baseline and 12 months after) to avoid possible effects of the seasonal variations in inflammatory markers. In this study, there was a great variability of sequential measures of inflammatory markers, especially hsCRP and IL-6. A recent study assessing the effects of DOACs on inflammatory markers revealed that treatment with rivaroxaban and dabigatran in patients with AF was also not associated with changes in the levels of hsCRP [27]. Although factor Xa inhibitors and direct thrombin inhibitors may provide additional therapeutic benefits, supporting data remain limited. It was difficult to reveal the anti-inflammatory effect of DOACs in this clinical research, though basic research showed that DOACs stabilize and attenuate atherosclerotic plaque in apolipoprotein E-deficient mice [3, 4]. Fourth, the optimal dosage of rivaroxaban to prevent the progression of atherosclerotic lesions is uncertain. Rivaroxaban was prescribed in a dose of 10–15 mg/day in the present study, which was the standard dose used to prevent the development of systemic embolism in Japanese patients with AF, not ACS. In the ATLAS ACS2-TIMI51 trial, a twice-daily 2.5 mg dose of rivaroxaban in addition to dual antiplatelet therapy (DAPT) reduced overall and cardiovascular mortality [11]. Moreover, both a twice-daily 2.5 mg dose of rivaroxaban plus DAPT reduced the rate of recurrent hospitalization resulting from a cardiovascular cause as compared with oral vitamin K antagonist plus DAPT in the PIONEER AF-PCI trial (Open-Label, Randomized, Controlled, Multicenter Study Exploring Two Treatment Strategies of Rivaroxaban and a Dose-Adjusted Oral Vitamin K Antagonist Treatment Strategy in Subjects with Atrial Fibrillation who Undergo Percutaneous Coronary Intervention) [28]. These results suggest that very low and reduced doses of rivaroxaban might have anti-inflammatory effects.

In the present study, the levels of sTM increased significantly from baseline to 12 months in both the rivaroxaban group and the dabigatran group, as well as GDF-15. The increased levels of sTM in the present study were in accordance with the results of a recent study, which showed that treatment with rivaroxaban for 24 weeks increased sTM levels in patients with AF [29]. Some studies have shown that anticoagulation increased sTM [29–31]. The increased levels of sTM reflect the effect of DOACs and upregulation by anticoagulation [29]. However, the meaning of the increase in sTM levels after DOAC treatment is controversial, because there are conflicting mechanisms of increased levels of sTM. TM on the endothelial cell surface plays an important role in coagulation and fibrinolysis. In healthy people, the elevated levels of sTM were associated with decreased risk of coronary heart disease, because plasma levels of sTM may reflect endothelial expression of thrombomodulin [32]. On the other hand, sTM is increased by damage to endothelial cells [33]. Several clinical studies have confirmed a significant association between the elevated levels of sTM and a greater severity of atherosclerosis in patients with coronary artery disease and peripheral vascular disease [34]. Moreover, the increased levels of sTM in patients receiving anticoagulant treatment are associated with vascular mortality, all-cause mortality [30], and bleeding complications [31]. GDF-15 also is a risk indicator for major bleeding and all-cause mortality in AF patients [35]. In this study, GDF-15 levels at

12 months tended to be associated with bleeding events, although it was not significant, and the increased levels of sTM was associated with bleeding events. Moreover, there was a positive correlation between sTM and GDF-15. From the results of this study, it is suggested that sTM is related to bleeding events, as well as GDF-15. From the result of the 1-month landmark sub-analysis, sTM might be not only a marker, but a predictor for bleeding events in AF patients receiving DOACs. Although the interval changes in sTM levels were greater in the dabigatran group, there were no significant differences in bleeding events between 2 groups.

Our study had several limitations. First, clinical outcomes were evaluated in a small group of patients. In addition, only a few patients with a history of coronary artery disease were included as subjects, because DOACs were not approved for use in patients with ACS in Japan during the study period. Further investigations are needed to fully understand the specific roles of targeting factor Xa and thrombin in the setting of ACS. Moreover, it is possible that available assays are not sensitive enough to detect differences in the anti-inflammatory effects of DOACs, although highly sensitive assays were used. Furthermore, approximately half of the subjects received warfarin prior to randomization. Although there were no significant differences in the levels at baseline and the interval changes in any inflammatory marker between patients with prior warfarin use and those without, the rate of bleeding events was higher and the increased levels of sTM was tended to be greater in patients with prior warfarin.

In conclusion, there were no significant differences in the interval changes in any inflammatory marker measured in this study between the rivaroxaban group and dabigatran group. The increased levels of sTM might be a predictor for bleeding complication in AF patients receiving DOACs though we should need careful interpretation of the meaning.

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Compliance with ethical standards

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