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Cardiac valve calcification and use of anticoagulants: Preliminary observation of a potentially modifiable risk factor

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ABSTRACT

Aims: Direct oral anticoagulant (DOAC) has been recently introduced in the clinical practice. Rather than interfering with vitamin K-dependent posttranscriptional modification of various proteins, DOACs selectively inhibit factors involved in the coagulation cascade. In particular, in contrast with Warfarin, Rivaroxabn does not interfere with activation of matrix Gla Protein (MGP), a potent vascular calcification Inhibitor. We herein sought to investigate the impact of Rivaroxaban and Warfarin on cardiac valve calcifications in a cohort of moderate-to advanced CKD patients.

Methods and results: This is a multicenter, observational, retrospective, longitudinal study. Consecutive CKD stage 3b – 4 (according to KDIGO guidelines) patients from 8 cardiologic outpatient clinics were enrolled between May 2015 and October 2017. All patients received anticoagulation (100 Warfarin vs 247 Rivaroxaban) as part of their non-valvular atrial fibrillation management. Cardiac valve calcification was evaluated via standard trans-thoracic echocardiogram. 347 patients (mean age: 66 years; mean eGFR: 37 ml/min/1.73 m^2) were studied. Over a mean follow-up period of 16 months, Rivaroxaban compared to Warfarin reduced both mitral and aortic valve calcifications ($p < 0.001$) independently of the degree of calcifications at baseline and potential confounders. Notably, Rivaroxaban use was also associated with a significant reduction in C reactive protein (CRP) ($p < 0.001$) during follow-up.

Conclusion: This study generates the hypothesis that the use of Rivaroxaban associates with a reduction of cardiac valve calcification deposition and progression as compared to Warfarin, in a cohort of CKD stage 3b-4 patients. Future endeavors are needed to confirm and to establish the mechanisms responsible for these findings.

1. Introduction

Atrial fibrillation (AF) represents the most common type of cardiac arrhythmia in patients with chronic kidney disease (CKD) [1,2]. According to various published paper, its incidence ranges from 7 to 10%, with more advanced stages of CKD being the most affected [3]. As in the general population, AF portends an increased risk of thromboembolism and stroke [1]. Hence, anticoagulation is recommended as part of the AF management even though renal function impairment increases the risk of bleeding [4–6].

Oral anticoagulation is the most effective form of thromboprophylaxis in patients with AF [2,7]. Until recent years the only therapeutic option for oral anticoagulation relied on vitamin K antagonists (VKA) [1]. However, newer molecules (direct oral anticoagulant - DOACs) directly and selectively inhibiting either thrombin (Dabigatran) or factor Xa (Rivaroxaban,

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Edoxaban and Apixaban) were developed to overcome some of the VKA limitations such as a narrow therapeutic index and need of frequent blood monitoring [8]. On the other hand, the various degree of real clearance of DOACs limits drug prescription in CKD and current guidelines on clinical management of AF [4–6] suggest the use of direct oral anticoagulant (DOACs) unless the residual renal function declines below stage 4 (eGFR ≥ 15 ml/min). On the other hand, vitamin K antagonists (VKA), such as Warfarin, are recommended for end – stage renal disease (ESRD with eGFR < 15 ml/min) and patients on renal replacement therapies (RRTs) [4–6].

A growing body of evidence suggests that DOACs are safe, well tolerated and more effective than VKAs in stroke prevention [4–6,9] possibly even in patients with reduced renal function [8,10]. Although the reasons are still unclear, the higher selectivity of DOACs for factors implicated in the coagulation cascade represents a plausible explanation of the favorable risk-to-benefit ratio of DOACs [1,11]. By altering vitamin K metabolism, VKAs interfere with the posttranscriptional modification of all factors dependent on gamma-glutamyl carboxylation [12]. In these regards, inhibition of matrix Gla protein (MGP) may predispose to cardiovascular calcifications deposition and progression [13] that in turn portend poor prognosis in normal kidney function individuals [14] as well as patients with impaired renal function [15,16]. Other lines of evidence suggest that the inhibition of coagulation factor Xa promoted by Rivaroxaban, Apixaban and Edoxaban, can lead to stabilization and/or regression of cardiovascular calcifications [17]. Indeed, factor Xa has been shown to act as chemoattractant for fibroblasts, mitogen for vascular smooth muscle cells (VSMC) and inducer of inflammatory activation also leading to endothelial cells senescence and apoptosis [17].

We herein sought to investigate in a retrospective, multicenter, longitudinal study involving subjects with CKD stage 3B – 4 and chronic AF on oral anticoagulants the impact of Warfarin or Rivaroxaban on heart valve calcifications.

2. Methods

2.1. Patient selection

A detailed description of the study cohort has been described elsewhere [8]. Briefly, we report on an observational, multicenter, retrospective, longitudinal study. All consecutive CKD stage IIIb – IV (according to NKF – KDOQI guidelines) patients attending at 8 cardiologic outpatient clinics between May 2015 and October 2017 were considered for eligibility for current analysis (Fig. 1). The inclusion criteria enforced were: [1] non–valvular atrial fibrillation (NVAF); [2] stage IIIb – IV chronic kidney disease according to NKF – KDOQI guidelines; [3] age > 18 years; [4] established oral anticoagulant therapy (defined as at least 12 months of treatment). According to available literature, this time interval was deemed sufficient to detect meaningful changes of mitral and aortic valve calcification. In consideration drug prescription limitations of DOACs in patients with [1] end stage renal disease (ESRD); [2] age > 75 years; [3] malignancies and [4] chronic inflammatory diseases, subjects meeting these criteria were excluded from current analyses. Written informed consent to utilized clinical data was obtained from patients at the first outpatient visit. The study complies with the Declaration of Helsinki. The local ethics committee was informed of the study analysis in compliance with the local regulations.

From the initial patient charts interrogation, a total of 377 records were retrieved. However, 20 patients were excluded because of age older than 75 years, 5 patients were excluded due to malignancies and 5 were excluded due to missing follow-up data. Thus, a final cohort of 347 subjects meeting the inclusion and exclusion criteria were identified and were used for current analyses.

Of these, 247 and 100 patients were treated with Rivaroxaban and Warfarin, respectively. Indication to anticoagulation and type of oral anticoagulant were decided by the attending clinician and not mandate by a study protocol. Although various molecules are approved for moderate-to-advance CKD (i.e. Rivaroxaban, Apixaban, Dabigatran), Rivaroxaban was the prescribed DOAC in the cardiologic outpatient clinics enrolled in this study due to the once daily regimen. Rivaroxaban was administered at the dose of 15 mg once daily (OD) in compliance with the Summary of Product Characteristics while Warfarin dose was modulated to reach an International Normalized Ratio (INR) between 2 and 3 according to what suggested by current recommendations of the European Society of Cardiology (ESC) guidelines on anticoagulation in CKD patients with Atrial Fibrillation (AF) [6].

Demographic, clinical and laboratory data were extracted retrospectively from patients' chart. Diabetes mellitus was defined as fasting glucose ≥126 mg/dl or treatment with hypoglycemic drugs (insulin, oral hypoglycemic therapy or dietary advice); hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mm Hg or treatment with anti-hypertensive drugs; ischemic heart disease was defined as history of myocardial infarction or coronary artery revascularization procedure. Smoking was defined as active smoking.

2.2. Laboratory methods

Laboratory data were assayed as part of the standard of care in fasting state and were not centralized. Creatinine levels were measured with immune-enzymatic method and renal function estimated via the CKD – EPI formula. CKD stage was determined according to KDIGO guidelines [18]. LDL cholesterol, triglycerides, PTH, and glycated hemoglobin were assessed and measured by standard methods.

2.3. Cardiac valve calcification assessment

Presence and extension of aortic and mitral valve calcification were investigated at study inception as well as at the end of the study follow-up by trans – thoracic echocardiography (Fig. 1). Ultrasound exams were performed as part of the standard of care according to physician discretion and according to current guidelines [19]. All studies were performed by General Electrical Medical echography, model P7 with a 3.5 MhZ micro convex probe (GE Healthcare, WI, USA) according to the recommendations of the European Society of Cardiology (ESC) and Italian Society of Cardiovascular Echography (SIEC) [19]. All studies were performed as part of the routine care at the physician discretion. Long and short axis parasternal views as well as four- and five-chamber apical views were acquired to provide a comprehensive assessment of heart chambers size and function. Mitral valve study was completed via four-chamber apical view. Presence and extension of mitral valve calcification was scored according to the Wilkins score [20]. Briefly, presence and extension of mitral valve calcification is scored according to the following criteria: no calcifications (0 point); single area of increased echo brightness (1 point); presence of scattered areas of brightness confined to leaflet margins (2 points); presence of scattered areas of brightness extending into the mid-portion of the leaflets (3 points); presence of areas of extensive brightness throughout much of the leaflet tissue (4 points). Of note, "brightness" means hyper-echogenic area due to calcification.

Because no scoring system is currently validated for aortic valve calcification assessment, the presence of aortic valve calcification was evaluated via the five-chamber apical view by means of a semi-quantitative score (0 point = no calcification; 1 point = presence of valvular calcification). Presence of aortic valve calcification was defined as evidence of at least an area of brightness (hyper-echogenic) at any level in the field of view of the aortic valve.

2.4. Statistical analysis

Data are summarized as mean and standard deviation (normally distributed data), median and interquartile range (non-normally distributed data), or as percent frequency, and between groups comparisons were made by independent *T*-Test, Mann Whitney Test, or Chi Square Test, as appropriate.

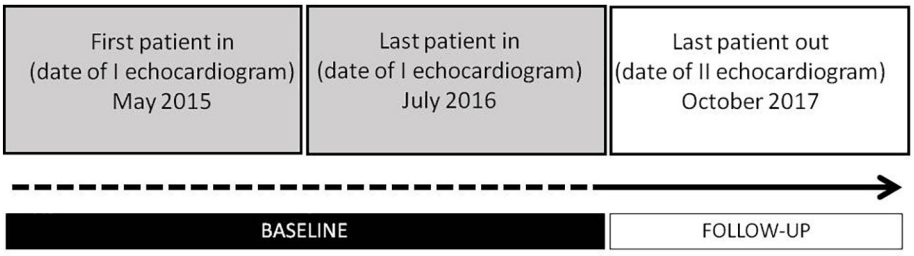

Fig. 1. study flow chart.

Correlates of the two key dependent variables at baseline (namely: Wilkins score and aortic calcifications) were investigated, respectively, by ordinal and logistic regression analyses according to the nature of the dependent variable. Due to the similar follow-up of all study participants (mean follow-up: 16 ± 0.3 months, IQR: 13–20), time was not included as covariate in the analyses performed to identify predictors of cardiac valve calcification progression. The strength of the relationships between the baseline value of the Wilkins score and its changes over time as well as between baseline aortic calcifications and their changes across the follow-up was investigated by Spearman correlation coefficient (rho) and *P* value. The association between baseline and overtime changes of CRP (both normally distributed variables) was investigated by Pearson correlation coefficient (r) and P value. To account for the potential confounding effect of baseline values of Wilkins score, aortic calcifications and CRP on the link between the study arm (Warfarin/Rivaroxaban) and the changes of these variables overtime, the preliminary crude data analysis was stratified according to the baseline values of these variables [Wilkins score, 1st stratum, score = 0; 2nd stratum, score = 1; 3rd stratum, score = 2 (no patients presented a score of 3 and 4); Aortic calcifications, 1st stratum, no calcification; 2nd stratum, with calcification; CRP, 1st stratum, ≤ median (0.6 mg/l); 2nd stratum, > median]. To adjust the link between study arm and overtime changes in Wilkins score and aortic calcifications (both codified as: −1 = reduced, 0 = stable; 1 = increased) for potential confounders we used multiple ordinal regression analyses. In these analyses, data were expressed as ordered log-odds (logit) regression coefficients, 95% CI and *P* value. Standard interpretation of the ordered logit coefficient is that for a one unit increase in the covariate, the response variable level is expected to change by its respective regression coefficient in the ordered log-odds scale while the other variables in the model are held constant. A multiple linear regression model was used to investigate the independent effect of study drugs on the CRP changes over time. The effect modification by baseline CRP on the effect of Warfarin/Rivaroxaban on changes in Wilkins score and aortic calcifications was investigated by simultaneously introducing into the same ordinal regression model baseline CRP, the study arm (Warfarin = 0; Rivaroxaban = 1) and their multiplicative term. In all regression models, data were expressed as regression coefficient, 95% CI and *P* value. All analyses were performed on the Intention-to-Treat (ITT) bases since no data on drug compliance and adherence were collected. Data analyses were performed by SPSS for Windows, Version 22, IBM, Chicago, Illinois, USA).

3. Results

The study population included 100 patients on treatment with Warfarin and 247 patients on treatment with Rivaroxaban. The two groups did not statistically differ as for age, gender, smoking, diastolic BP, history of hypertension and ischemic cardiomyopathy, LDL cholesterol, triglycerides, and CRP. Patients on Warfarin had more frequently diabetes and nephroangiosclerosis in their CKD etiology as compared to those on Rivaroxaban (Table 1). No patients on Warfarin were affected by Alport syndrome, chronic glomerulonephritis, and polycystic kidney whereas the prevalence of these diseases was 1.2%, 2.8% and 8.9%, respectively, in the Rivaroxaban arm. Systolic BP, glycated hemoglobin and PTH were higher and eGFR was lower in the Rivaroxaban than in the Warfarin arm (see Table 1). The Wilkins score was higher in patients on Rivaroxaban than in those on Warfarin whereas the prevalence of aortic calcifications was similar between the two groups (see Table 2). The follow-up of the study cohort was similar for all study participants and was 16 ± 0.3 months (interquartile range: 13–20).

3.1. Correlates of Wilkins score and aortic calcifications at baseline

On univariate ordinal regression analyses, none of the variables listed in Table 1 resulted to be related to the Wilkins score neither in Warfarin (P ranging from 0.20 to 0.95) and nor in Rivaroxaban (P ranging from 0.07 to 0.98) treated patients. Of interest, although there was a nominally significant difference in aortic valve area between groups at baseline (2.15 ± 0.23 vs 2.23 ± 0.26 cm^2 in warfarin and Rivaroxaban group respectively, $p < 0.001$) and follow-up (2.12 ± 0.23 vs 2.27 ± 0.24 cm^2 in warfarin and Rivaroxaban group respectively, $p < 0.001$), these small differences are of little clinical relevance. By the same token, on univariate logistic regression analyses no association was found between variables listed in Table 1 and the presence of baseline aortic calcifications neither in the Warfarin (P ranging from 0.24 to 0.96) and nor in the Rivaroxaban (P ranging from 0.06 to 0.95) arm.

Table 1
Main demographic, clinical and biochemical characteristics of warfarin and rivaroxaban treated subjects.

	On Warfarin ($n = 100$)	On Rivaroxaban ($n = 247$)	P
Demographic characteristics			
Age (years)	66.5 ± 4.6	66.0 ± 4.4	0.39
Male gender n. (%)	58(58.0%)	134(54.3%)	0.52
Smokers n. (%)	69(69.0%)	163(66.0%)	0.59
Systolic pressure (mm Hg)	146 ± 12	150 ± 16	0.003
Diastolic pressure (mm Hg)	81 ± 7	83 ± 9	0.07
Clinical characteristics			
CKD etiology			
Nephrongiosclerosis	100 (100%)	140 (56.6%)	<0.001
Diabetes	47 (47%)	75 (30.4%)	0.004
Alport syndrome	–	3 (1.2%)	0.64*
Chronic glomerulonephritis	–	7 (2.8%)	0.2*
Polycystic kidney disease	–	22 (8.9%)	0.004*
Hypertension n. (%)	100 (100%)	240 (97%)	0.20
Ischemic heart disease n. (%)	6 (6%)	22 (8.9%)	0.51
Previous stroke n. (%)	0 (0%)	0 (0%)	…
Previous hemorrhage n. (%)	0 (0%)	0 (0%)	…
Laboratory characteristics			
CRP (mg/l)	0.59 ± 0.23	0.62 ± 0.24	0.40
LDL cholesterol (mg/dl)	129 ± 36	134 ± 32	0.29
Triglycerides (mg/dl)	146 ± 42	144 ± 51	0.67
eGFR (ml/min/1.73 m^2)	38.4 ± 2.4	37.6 ± 2.3	0.005
Glycated hemoglobin (mMol/mol)	27.4 ± 5.9	36.2 ± 11.1	<0.001
PTH (pg/ml)	26.5 (22.2–34.0)	56.0 (32.0–132.0)	<0.001
Calcium (mg/dl)	9.24 ± 0.23	9.43 ± 0.60	0.03
Phosphate (mg/dl)	4.74 ± 0.29	4.73 ± 0.28	0.002
Alkaline phosphatase (IU/l)	79.1 ± 20.2	74.6 ± 18.9	0.92

Table legend: Data are given as mean ± SD if normally distributed, median and interquartile range if not normally distributed, or as percent frequency. Parametric and non-parametric tests are used accordingly. *Yates correction. Abbreviations: CKD: chronic kidney disease; HDL: high density lipoprotein; LDL low density lipoprotein; eGFR: estimated glomerular filtration rate; PTH: parathyroid hormone.

3.2. Effect of Warfarin and Rivaroxaban on Wilkins score, aortic calcifications and CRP over time

In both study arms, the baseline value of Wilkins score was significantly and inversely related to changes of the same variable over time (Warfarin arm, rho = −0.25, $p = 0.01$; Rivaroxaban arm, rho = −0.72, $p < 0.001$) and this was also true when investigating the relationship of baseline aortic valve calcifications with changes of this biomarker during follow-up (Warfarin arm, rho = −0.27, $p = 0.007$; Rivaroxaban arm, rho = −0.61, $p < 0.001$). Similarly, baseline serum CRP was significantly and inversely related to changes of this biomarker over time in both Warfarin ($r = -0.39$, $p < 0.001$) and Rivaroxaban ($r = -0.78$, $p < 0.001$) arm. Overall, these associations indicate that the baseline values of these three variables may confound the assessment of the relationship between Warfarin/Rivaroxaban and changes in the Wilkins score, aortic calcifications and CRP over time. For these reasons, the between-arms comparisons of changes in Wilkins score, aortic calcifications and CRP were performed according to relevant patients' strata (*see Methods-Statistical Analysis*). In patients with a baseline Wilkins

Table 2
Prevalence of mitral (according to the Wilkins score) and aortic valve calcifications in Warfarin and Rivaroxaban treated patients at study inception.

	On Warfarin (n = 100)	On Rivaroxaban (n = 247)	P value
Wilkins score n. (%)			
0	59 (59%)	97 (39.3%)	
1	33 (33%)	117 (47.4%)	0.004
≥2	8 (8%)	33 (13.3%)	
Aortic calcifications n. (%)	36 (36%)	98 (39.7%)	0.52

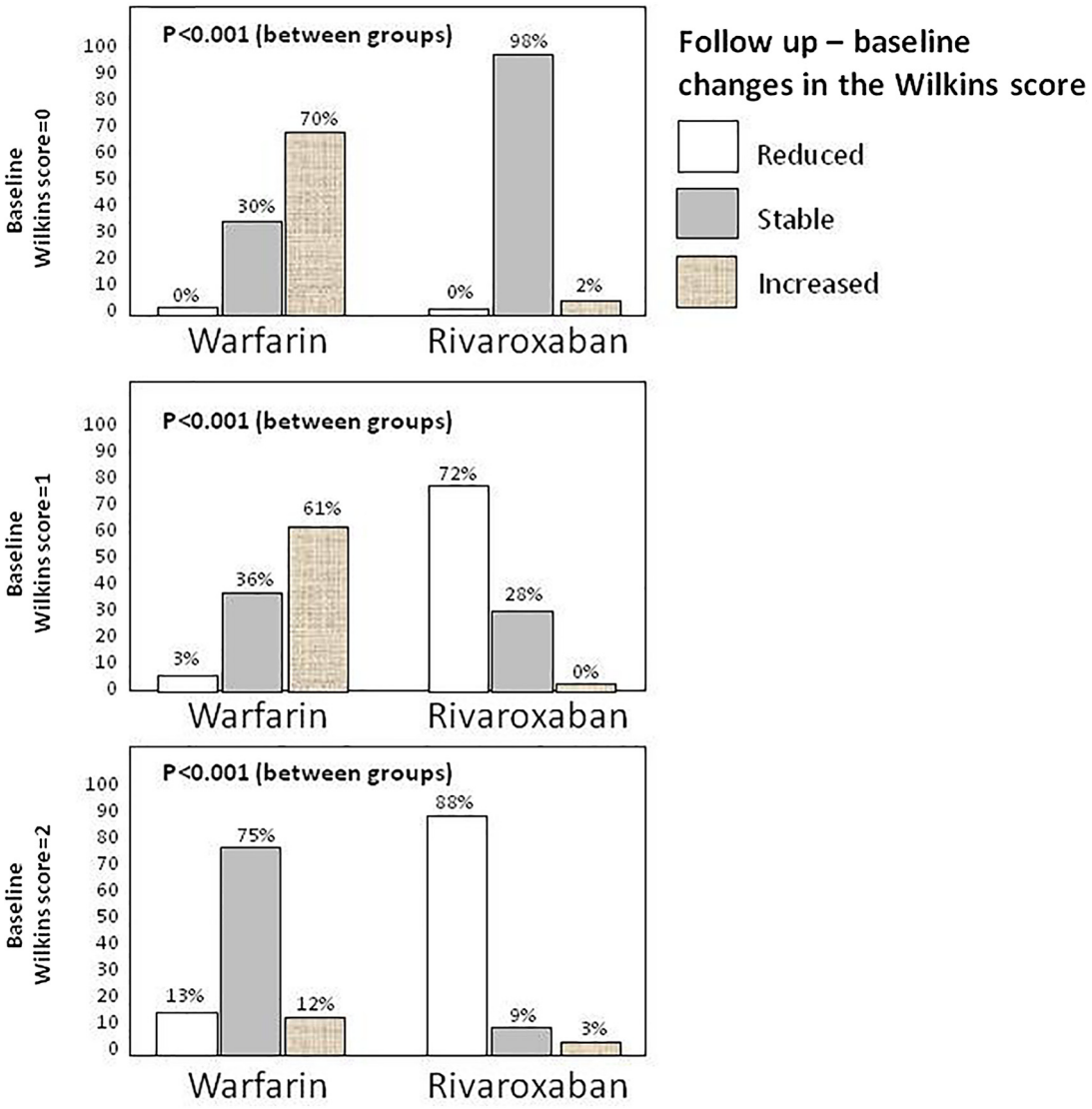

Fig. 2. mitral valve calcification changes during follow-up according to severity of valvular calcification (Wilkins' score) at study inception and type of oral anticoagulant.

score of zero, the Wilkins score remained unchanged in almost all Rivaroxaban treated patients (98%) whereas it increased in a substantial proportion of patients on Warfarin (70%) (Fig. 2). The same analysis carried out in patients with a Wilkins score of 1 and 2 showed that the Wilkins score reduced in the large majority of patients in the Rivaroxaban arm (72% and 88%, respectively) whereas only a minority of patients in the Warfarin arm displayed a reduction of this score (3% and 13%, respectively). The same analysis carried out according to aortic calcifications showed that all Rivaroxaban patients without aortic calcifications at baseline remained calcification free across the follow-up against a 70% observed in those on Warfarin (Fig. 2). Of note, among those with aortic calcifications at baseline, the aortic calcifications reduced in 49% of Rivaroxaban patients against a 14% observed in patients on Warfarin (Fig. 3). Serum CRP reduced in Rivaroxaban treated patients and increased in those on Warfarin and such effects were independent of baseline CRP (Fig. 4). Of note, the advantage of Rivaroxaban versus Warfarin for reducing Wilkins score, aortic calcifications and CRP remained significant also in multiple regression analyses adjusting for a series of potential confounders (Table 3), i.e. for all variables which significantly differed between the two study arms (Table 1). Moreover, no effect modification by baseline CRP was found on the effect of allocation arm on aortic ($p = 0.15$) and Wilkins score ($p = 0.81$) changes over time. Finally, no association of mitral or aortic valve calcification and markers of mineral metabolism (i.e. calcium, phosphate, PTH, alkaline phosphatase) was detected.

4. Discussion

The main findings of this multicenter, retrospective, observational study suggest a potential difference of Rivaroxaban and Warfarin on cardiac valve calcifications and inflammation. Although hypothesis generating, these results lend credibility to the hypothesis that avoiding vitamin K inhibition [13] in patients with moderate-to-advanced CKD and AF may prevent cardiovascular calcification deposition and progression.

Development of vascular calcifications is a major concern in CKD and end stage renal disease (ESRD) patients [15,16]. Epidemiological data suggest a steep increase in prevalence and extension of vascular calcification as renal function declines [15] with about 60% of patients starting dialysis presenting some degree of Coronary Artery Calcification (CAC) [16]. Several mechanisms such as accelerated atherosclerosis or aging have been postulated to explain this phenomenon [21]. Other lines of evidence support the notion that mineral metabolism derangements, chronic inflammation and oxidative stress lead to progressive

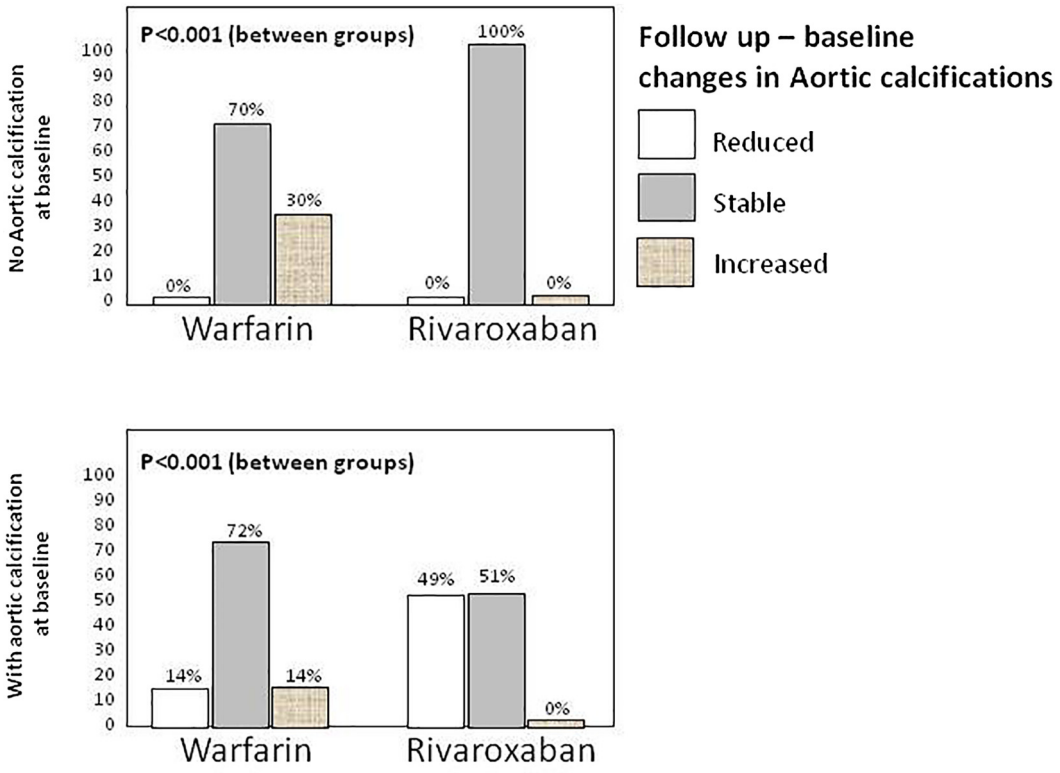

Fig. 3. aortic valve calcification changes during follow-up according to presence of valvular calcification at study inception and type of oral anticoagulant.

transformation of vascular smooth muscle cells (VSMC) and valvular interstitial cells (VICs) into osteogenic cells promoting calcification development in the context of the arterial wall or cardiac valves [22,23]. In this study cohort, however, we could not detect any association of cardiac valve calcifications and markers of mineral metabolism (i.e. parathyroid hormone – PTH, calcium, phosphate and alkaline phosphatase) or inflammation (C-reactive protein – CRP). Whether this is attributable to the small sample size or the substantially normal levels of serum PTH and CRP in the majority of study participants is a matter of debate. Nevertheless, we detected a profound effect of oral anticoagulant regimens on both cardiac valve calcifications as well as inflammation. At least in this study cohort, inflammation was not the driver of the association of anticoagulant treatments and cardiac valve calcification progression. Indeed, anticoagulant regimens and CRP were both independently associated with mitral and aortic valve calcification change and no effect modulation of these factors on calcification changes was detected.

Although this study does not allow assessing the biological mechanism(s) that explain current findings, several hypotheses can be postulated. VKAs interfere with the posttranscriptional modification of all factors dependent on gamma-glutamyl carboxylation [12]. In these regards, inhibition of matrix Gla protein (MGP) may predispose to cardiovascular calcification deposition and progression [13]. Matrix - Gla – Protein (MGP) is a potent inhibitor of cardiovascular calcification both preventing calcification formation and inhibiting Bone Morphogenic Protein – 2 (BMP – 2), that it is involved in the osteogenic transformation of VSMCs and VICs [23,24]. Hence, VKA may promote cardiovascular calcification development and some clinical data lend credibility to the notion that Warfarin is a risk factor for calcific uremic arteriolopathy (CUA) (also named calciphylaxis), a serious medical

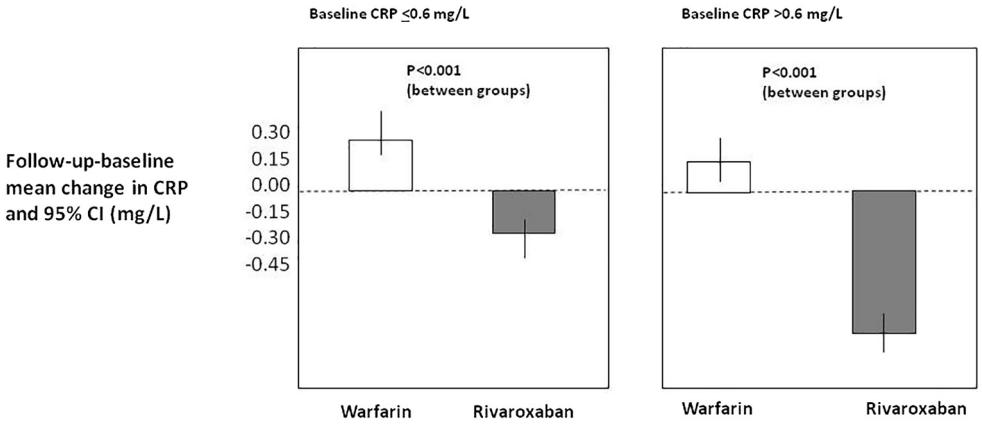

Fig. 4. C-reactive protein (CRP) changes during follow-up according to inflammatory status (equal/below or above the median CRP of the study cohort) at study inception and type of oral anticoagulant.

Table 3
Multiple ordinal regression analyses of changes in Wilkins score and aortic calcifications (a-b) and multiple linear regression analysis of changes in CRP (c).

	Regression coefficient	95% CI of the regression coefficient	P
a) Dependent variable: changes in Wilkins score (follow up – baseline)			
Study arm (0 = Warfarin; 1 = Rivaroxaban)	−4.86	−5.95 to − 3.76	<0.001
Baseline Wilkins score	−2.40	−2.90 to − 1.90	<0.001
Systolic BP (mm Hg)	−0.006	−0.023 to 0.012	0.53
Diabetes (0 = no;1 = yes)	−0.40	−0.98 to 0.18	0.17
eGFR (ml/min/1.73 m^2)	0.031	−0.08 to 0.14	0.58
Glycated hemoglobin (mMol/ml)	0.003	−0.024 to 0.031	0.81
PTH (lg10 of pg/ml)	−0.782	−1.70 to 0.14	0.10
b) Dependent variable: changes in aortic calcifications (follow up – baseline)			
Study arm (0 = Warfarin; 1 = Rivaroxaban)	−2.90	−3.96 to − 1.84	<0.001
Baseline aortic calcifications (0 = no;1 = yes)	−3.54	−4.51 to 2.57	<0.001
Systolic BP (mmHg)	−0.01	−0.03 to 0.01	0.35
eGFR (ml/min/1.73 m^2)	0.099	−0.03 to 0.23	0.13
Diabetes (0 = no = 1 = yes)	0.11	−0.51 to 0.72	0.74
Glycated hemoglobin (mMol/ml)	−0.12	−0.04 to 0.02	0.43
PTH (lg10 of pg/ml)	0.338	−0.68 to 1.36	0.52
c) Dependent variable: changes in CRP (follow up – baseline)			
Study arm (0 = Warfarin; 1 = Rivaroxaban)	−0.36	−0.39 to − 0.32	<0.001
Baseline CRP (mg/l)	−0.46	−0.51 to − 0.41	<0.001
Systolic BP (mmHg)	−0.00003	−0.0009 to 0.0008	0.95
Diabetes (0 = no;1 = yes)	−0.002	−0.02 to 0.03	0.90
eGFR (ml/min/1.73 m^2)	0.00014	−0.006 to 0.006	0.96
Glycated hemoglobin (mMol/ml)	−0.00022	−0.002 to 0.002	0.73
PTH (lg10 of pg/ml)	0.03	−0.017 to 0.069	0.24

condition characterized by extensive and diffuse arterial calcification [25]. In these regards, Yamamoto and coworkers reported a significantly greater incidence of aortic valve calcification associated with warfarin (18.0% vs 6.9% in warfarin vs non warfarin treated subjects; $p = 0.014$) in a large series of 430 subjects free from CKD prescribed anticoagulants for various medical conditions and followed for 4 years [26].

On the other hand, inhibition of factor Xa operated by Rivaroxaban could play a role in the calcification process. In vitro *or animal evidence* suggest that factor Xa is a chemoattractant for fibroblasts, trigger for endothelial cell apoptosis, mitogen for vascular smooth muscle cells (VSMC), and inducer of inflammatory activation also leading to endothelial cells senescence and apoptosis [17,27]. Although we failed to demonstrate an association with a marker of systemic inflammation such as CRP, it is possible that inhibition of factor Xa ultimately reduces local inflammation and risk of calcification progression [28].

Our data confirms and expands available evidence suggesting that, when compared to Warfarin, the use of Rivaroxaban in an unselected cohort of patients with moderate-to-advanced CKD is associated with a reduction of mitral and aortic valve calcification deposition and progression as well as reduced inflammation. However, this study is not without limitations. The retrospective, observational study design precludes any causal inference since residual confounding may apply irrespective of our best effort to control for it. The short follow-up and the limited sample size also limit the generalizability of our findings. The absence of a control group and the open-label design prevents any speculation on whether the observed effects may be due to a protective effect of Rivaroxaban or vice versa to an unfavorable effect of Warfarin. Similarly, current data cannot prove that Rivaroxaban was associated with cardiac valve calcification regression. Our analyses are, also, limited to Rivaroxaban (a direct inhibitor of factor Xa) since this was the only DOACs molecule prescribed to patients with moderate-to-advanced CKD in the outpatient Cardiology Units at the time the study was conducted. Hence, whether these observations also apply to thrombin inhibitors such as dabigatran warrant future confirmation. The lack of information on patient drug adherence is another limitation of the study. Nonetheless, analyses were conducted on the Intention-To-Treat (ITT) bases and poor drug adherence or study arm cross-over during follow-up would have likely resulted in attenuation rather than amplification of the signal.

In summary, current data suggest that Rivaroxaban compared to Warfarin is associated with a lower levels of serum markers of inflammation as well as risk of cardiac valve calcification deposition and progression. Of interest, although inflammation is tightly connected with cardiovascular calcification, in this study cohort the effect associated with the use of anticoagulants seems only partially explained by chronic inflammation. In light of the study limitations, future efforts are needed to confirm our observations and elucidate the underlying biological mechanisms.

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