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Warfarin Use Is Associated With Progressive Coronary Arterial Calcification

Insights From Serial Intravascular Ultrasound

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ABSTRACT

OBJECTIVES This study compared serial changes in coronary percent atheroma volume (PAV) and calcium index (CaI) in patients with coronary artery disease who were treated with and without warfarin.

BACKGROUND Warfarin blocks the synthesis and activity of matrix Gla protein, a vitamin K–dependent inhibitor of arterial calcification. The longitudinal impact of warfarin on serial coronary artery calcification in vivo in humans is unknown.

METHODS In a post hoc patient-level analysis of 8 prospective randomized trials using serial coronary intravascular ultrasound examinations, this study compared changes in PAV and CaI in matched arterial segments in patients with coronary artery disease who were treated with ($n = 171$) and without ($n = 4,129$) warfarin during an 18- to 24-month period.

RESULTS Patients (mean age 57.9 ± 9.2 years; male 73%; prior and concomitant 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statin) use, 73% and 97%, respectively) demonstrated overall increases in PAV of 0.41 ± 0.07% ($p = 0.001$ compared with baseline) and in CaI (median) of 0.04 (interquartile range [IQR]: 0.00 to 0.11; $p < 0.001$ compared with baseline). Following propensity-weighted adjustment for clinical trial and a range of clinical, ultrasonic, and laboratory parameters, there was no significant difference in the annualized change in PAV in the presence and absence of warfarin treatment (0.33 ± 0.05% vs. 0.25 ± 0.05%; $p = 0.17$). A significantly greater annualized increase in CaI was observed in warfarin-treated compared with non–warfarin-treated patients (median 0.03; IQR: 0.0 to 0.08 vs. median 0.02; IQR: 0.0 to 0.06; $p < 0.001$). In a sensitivity analysis evaluating a 1:1 matched cohort ($n = 164$ per group), significantly greater annualized changes in CaI were also observed in warfarin-treated compared with non–warfarin-treated patients. In a multivariate model, warfarin was independently associated with an increasing CaI (odds ratio: 1.16; 95% confidence interval: 1.05 to 1.28; $p = 0.003$).

CONCLUSIONS Warfarin therapy is associated with progressive coronary atheroma calcification independent of changes in atheroma volume. The impact of these changes on plaque stability and cardiovascular outcomes requires further investigation. (J Am Coll Cardiol Img 2017;■:■–■) © 2017 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

CaI = calcium index

CI = confidence interval

IPTW = inverse probability of treatment weight

IQR = interquartile range

IVUS = intravascular ultrasound

MGP = matrix G1a protein

PAV = percent atheroma volume

Coronary artery calcification correlates with cardiovascular events (1,2). However, emerging evidence increasingly supports the concept that greater calcium density at any level of calcium volume is associated with lower cardiovascular risk (3). Corroborating these findings, serial coronary intravascular ultrasound (IVUS) examinations revealed the procalcific effects of long-term 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statin) therapy despite an association with concomitant atheroma regression, thereby highlighting that plaque delipidation and subsequent calcification could be mechanisms underlying statin-induced plaque stabilization (4).

Underlying coronary artery calcification is complex pathophysiology involving factors either promoting or inhibiting its process. Matrix Gla protein (MGP) is a vitamin K-dependent protein synthesized by vascular smooth muscle and one of the key inhibitors of the metabolism of coronary artery calcium (5). The absence of MGP leads to extensive and lethal medial calcification in mice (6), and it correlates with arterial calcification in humans (7,8). Warfarin blocks the synthesis and activity of MGP by reducing gamma-carboxylation of vascular MGP (5). However, the impact of warfarin on serial coronary artery calcification in humans in vivo is currently unknown.

Serial coronary IVUS has been pivotal in elucidating factors promoting coronary atheroma progression and regression (9), as well as their relationships with clinical events (10,11). Measuring plaque calcification and atheroma volume with the high imaging resolution of coronary IVUS is well described and validated (12). In patients with coronary artery disease, we tested the hypothesis that serial changes in coronary artery calcium are greater in warfarin-treated patients compared with patients not receiving warfarin, independent of changes in coronary plaque volume.

METHODS

STUDY POPULATION. The present analysis included patients participating in 8 clinical trials that used IVUS to assess the impact of medical therapies on serial changes in coronary atheroma burden (Online Table 1). Included in this analysis were trials assessing the following: intensive lipid lowering with statins (REVERSAL [Reversal of Atherosclerosis With Aggressive Lipid Lowering], ASTEROID [A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden], and SATURN [The Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin]) (13–15); antihypertensive therapies (AQUARIUS [Aliskiren Quantitative Atherosclerosis Regression Intravascular Ultrasound Study] and NORMALISE [Norvasc for Regression of Manifest Atherosclerotic Lesions by Intravascular Sonographic Evaluation]) (16,17); the antiatherosclerotic efficacy of acyl-coenzyme A:cholesteryl ester transfer protein inhibition (ACTIVATE [ACAT Intravascular Atherosclerosis Treatment Evaluation]) (18); cholesteryl ester transfer protein inhibition (ILLUSTRATE [Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation]) (19); and peroxisome proliferator-activated receptor-gamma agonism (PERISCOPE [Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation]) (20). From each of these trials, patients receiving warfarin (n = 171) or no-warfarin therapy (n = 4,129) were included in the present analysis.

ACQUISITION AND ANALYSIS OF SERIAL IVUS IMAGES. The acquisition and serial analysis of IVUS images in each of these trials were previously described in detail (13,15–21). Briefly, target vessels for imaging were selected if they contained no luminal stenosis >50% angiographic severity within a segment at least 30 mm long. Imaging was performed within the same coronary artery at baseline and at study completion, which ranged from 18 to 24 months. Imaging in all trials was screened by the Atherosclerosis Imaging Core Laboratory of the Cleveland Clinic Coordinating Center for Clinical Research in Cleveland, Ohio. Patients meeting prespecified requirements for image quality were eligible for randomization. An anatomically matched segment was defined at the 2 time points on the basis of proximal and distal side branches (fiduciary points). Cross-sectional images spaced precisely 1 mm apart were selected for measurement. Leading edges of the lumen and external elastic membrane (EEM) were traced by manual planimetry. Plaque area was defined as the area occupied between these leading edges. The accuracy and reproducibility of this method were reported previously (22). The percent atheroma volume (PAV) was determined by calculating the proportion of the entire vessel wall occupied by atherosclerotic plaque throughout the segment of interest, as follows:

$$PAV = \frac{\sum(EEM_{\text{area}} - Lumen_{\text{area}})}{\sum EEM_{\text{area}}} \times 100$$

Calcium was identified by an echogenic signal brighter than the adventitia with corresponding acoustic shadowing. A calcium grade was assigned for each analyzed image, thus reflecting the degree of acoustic shadowing (0 = no calcium; 1 = calcium with acoustic shadowing <90°; 2 = calcium with shadowing ≥90° but <180°; 3 = calcium with shadowing ≥180° but <270°; 4 = calcium ≥270°) (Figure 1) (4,12,23). For images containing multiple calcium deposits, the grade represented the summation of all angles of acoustic shadowing. For each pullback, a calcium index (CaI) was thus calculated as follows (4,24):

$$CaI = \frac{Total\ no.\ of\ analyzed\ frames\ with\ any\ calcium}{Total\ no.\ of\ analyzed\ frames} \times \frac{Maximal\ arc\ of\ calcium}{4}$$

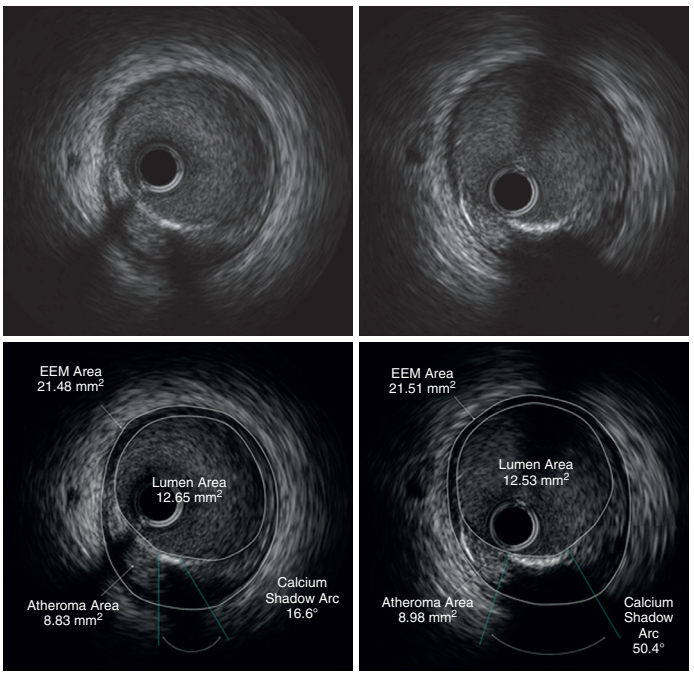
A change in CaI was defined as follow-up CaI minus the baseline CaI (4).

STATISTICAL ANALYSIS. A total of 171 patients were treated with warfarin, and 4,129 patients did not receive warfarin treatment. Continuous variables were reported as mean ± SD if normally distributed and as median (interquartile range) if non-normally distributed. Categorical variables were recorded as frequency (percentage). Demographics, baseline clinical characteristics, baseline and concomitant medications, laboratory biochemical data, and baseline PAV were compared. Two-sample Student *t* tests were used for normally distributed continuous variables, Wilcoxon rank sum tests were used for non-normally distributed continuous variables, and chi-square tests (or Fisher exact tests) were used for categorical variables. Because the CaI (both baseline and change) had many zero values, rank transformation was performed.

Because of differences in various baseline characteristics between concomitant warfarin use and non-warfarin use, a propensity score weighting method was applied. Two sets of propensity scores and a corresponding inverse probability of treatment weight (IPTW) (the reciprocal of the propensity scores) were estimated by a generalized linear model, using all the confounders detected for warfarin use versus change in CaI ranks as covariates (see Online Figure 1 for the list of confounders for IPTW on CaI). The balance of the pre-treatment covariates was assessed by checking absolute standardized differences in baseline confounders between concomitant warfarin use and non-warfarin use before and after IPTW adjustment, and significant improvement in baseline balance was achieved following weighting, as judged by a 10% threshold (Online Figure 1 for IPTW on CaI). IPTW on PAV shared the same covariate set and therefore had the same magnitude of weighting.

All subsequent analyses of the warfarin effect were weighted by IPTW, except the analysis for baseline CaI. Serial changes in IVUS measurements were analyzed by analysis of covariance, by adjusting for their baseline counterparts, study time duration, and clinical trial to account for heterogeneity across the trials, and were reported as least-squares mean ± SE. A full multivariable linear model was created subsequently. Similar multivariable analysis was conducted for rank-transformed CaI change, to eliminate residual imbalance in baseline characteristics, in which PAV was also adjusted because calcium is a component of plaque. Given that each trial's duration varied between 18 and 24 months, changes in PAV and CaI were also interpolated at 1 year and thus were reported as annualized changes. To assess clinical risk factors of coronary calcification further, a multivariable logistic regression model was developed for CaI increase versus no increase.

FIGURE 1 Procalcific Effects Associated With Warfarin Use Measured With IVUS



(Top left) A single cross-sectional image of a coronary artery acquired with intravascular ultrasound (IVUS) at baseline with a small amount of calcium. **(Top right)** The same cross-sectional image at follow-up with an increase in calcium and no significant increase in atherosclerotic plaque burden. **(Bottom)** The same cross-sections but with measurements superimposed. EEM = external elastic membrane.

TABLE 1 Demographics, Baseline Clinical and Angiographic Characteristics, and Baseline and Concomitant Medications According to Concomitant Warfarin Use

	Overall (n = 4,300)	Warfarin (n = 171)	No Warfarin (n = 4,129)	p Value
Age, yrs	57.9 ± 9.2	62.2 ± 9.2	57.7 ± 9.1	<0.001
Female	27.3	34 (19.9)	1,138 (27.6)	0.027
BMI, kg/m^2	30.1 ± 5.5	31.4 ± 6.1	30.0 ± 5.4	0.001
Diabetes	27.3	52 (30.4)	1,121 (27.1)	0.35
Hypertension	75.9	138 (80.7)	3,127 (75.7)	0.14
History of MI	29.1	43 (25.1)	1,207 (29.2)	0.25
History of PVD	4.5	12 (7.0)	180 (4.4)	0.10
History of CVA	3.1	12 (7.0)	121 (2.9)	0.002
History of AF	3.6	58 (33.9)	98 (2.4)	<0.001
Prior statin use*	73.0	116 (67.8)	3,022 (73.2)	0.12
Baseline aspirin	93.5	153 (89.5)	3,866 (93.6)	0.03
Baseline beta-blockers	75.0	130 (76.0)	3,095 (75.0)	0.75
Baseline ACE inhibitor or ARB	60.7	109 (63.7)	2,503 (60.6)	0.41
Baseline nitrates	18.7	32 (18.7)	772 (18.7)	0.996
Concomitant statin	96.6	168 (98.2)	3,986 (96.5)	0.23
Concomitant aspirin	94.2	153 (89.5)	3,896 (94.4)	0.008
Concomitant beta-blockers	76.2	141 (82.5)	3,135 (75.9)	0.049
Concomitant ACE inhibitor or ARB	66.5	116 (67.8)	2,744 (66.5)	0.71
Concomitant nitrates	18.8	36 (21.1)	773 (18.7)	0.45
Maximum diameter stenosis†	41.0 ± 17.4	43.7 ± 16.8	40.9 ± 17.5	0.10
Subjects with maximum stenosis >50%	25.6	30.3	25.3	0.29

Values are mean ± SD or n (%). *Prior statin use was defined as statin use on any occasion before study enrollment. †Represents the maximum percent diameter stenosis across all available lesions.

ACE = angiotensin-converting enzyme; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; MI = myocardial infarction; CVA = cerebrovascular accident; PVD = peripheral vascular disease.

Multicollinearity was examined in the full models by using variance inflation factor and/or a collinearity analysis through the condition index. None of the variance inflation factor values were larger than 4, and the condition indices were less than 10 after adjusting for the intercept, thus indicating no collinearity issues. Given that propensity scores may generate extreme scores or degrees of overlap, a sensitivity analysis with trimmed outliers (≈5% of total population) was performed, removing those with scores >0.1 to improve the data distribution (Online Tables 2 and 3, Online Figure 2).

Finally, considering the large difference in sample size between the warfarin group and the no-warfarin group, a sensitivity analysis was performed on the CaI data, by using propensity score greedy 5→1digit matching. A 2-sided probability value of 0.05 was considered statistically significant. Analyses were performed using SAS software version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

CLINICAL CHARACTERISTICS OF THE STUDY POPULATION. Table 1 describes baseline demographics, clinical characteristics, medication use, and baseline angiographic characteristics according to concomitant warfarin use. Overall, patients had a mean age of 57.9 ± 9.2 years, and 73% were men; 73% of the study population received prior statins, and 97% received concomitant statins. Significant between-group differences were noted across certain baseline variables. Patients in the warfarin-treated group were older, more likely male, had a higher body mass index, and had a higher incidence of prior cerebrovascular accident, atrial fibrillation (AF), and aspirin use compared with the no-warfarin group. Angiographic characteristics between groups did not differ.

BASELINE AND CHANGES IN LABORATORY MEASURES. Table 2 describes baseline values, follow-up values, and changes from baseline of laboratory biochemical measures within both treatment groups. There were no significant lipoprotein measurement differences between groups at baseline, but serum creatinine levels were significantly higher in the warfarin-treated group. At follow-up, warfarin-treated compared with non-warfarin-treated patients had lower high-density lipoprotein cholesterol levels (47.3 ± 14.7 mg/dl vs. 49.2 ± 15.0 mg/dl) and higher creatinine levels (1.03 ± 0.21 mg/dl vs. 0.94 ± 0.1 mg/dl; $p < 0.001$). Both treatment groups demonstrated significant decreases in low-density lipoprotein cholesterol levels (−20.0% and −19.4% in warfarin-treated and non-warfarin-treated patients, respectively) from baseline following medical therapies.

BASELINE AND CHANGES IN CORONARY ATHEROMA VOLUME ACCORDING TO WARFARIN THERAPY. Table 3 describes baseline values and changes in PAV in each treatment group, as well as comparisons of changes in PAV following propensity weighting. There were no differences in PAV between the groups at baseline. At follow-up and following propensity weighting and covariate adjustment, there were no significant differences of annualized changes in PAV in the presence and absence of warfarin therapy, respectively (0.33 ± 0.05% vs. 0.25 ± 0.04%; $p = 0.17$).

BASELINE AND CHANGES IN CaI ACCORDING TO WARFARIN THERAPY. Table 4 describes baseline and changes in CaI of each treatment group and comparisons of changes in CaI following propensity weighting and further adjustment for clinical trial, baseline measures of plaque burden and calcium, and other covariates. Compared with non-warfarin-treated patients, warfarin-treated patients had a significantly higher CaI at baseline (0.30 [IQR: 0.14 to 0.59] vs. 0.28 [IQR: 0.09 to 0.54]; $p = 0.024$). Both treatment

TABLE 2 Laboratory Findings*

	Overall (n = 4,300)	Warfarin (n = 171)	No Warfarin (n = 4,129)	p Value
Baseline				
LDL-C, mg/dl	107.7 ± 35.6	106.6 ± 36.9	107.8 ± 35.6	0.73
HDL-C, mg/dl	44.2 ± 11.8	43.0 ± 10.8	44.2 ± 11.8	0.22
Non–HDL-C, mg/dl	138.1 ± 41.2	138.1 ± 45.0	138.1 ± 41.0	0.78
Triglycerides, mg/dl	136 (133.7 to 138.3)	135 (122.3 to 147.7)	136 (133.7 to 138.3)	0.92
CRP, mg/l	2.1 (2.0 to 2.2)	2.3 (1.8 to 2.8)	2.1 (2.0 to 2.2)	0.14
Creatinine, mg/dl	0.96 ± 0.21	1.01 ± 0.21	0.96 ± 0.21	0.002
Follow-up				
LDL-C, mg/dl	81.9 ± 27.4	81.2 ± 30.0	81.9 ± 27.3	0.62
HDL-C, mg/dl	49.1 ± 15.0	47.3 ± 14.7	49.2 ± 15.0	0.05
Non–HDL-C, mg/dl	109.5 ± 32.7	108.6 ± 35.7	109.5 ± 32.6	0.52
Triglycerides, mg/dl	126.5 (124.7 to 128.3)	125.9 (117.4 to 134.4)	126.6 (124.7 to 128.5)	0.59
CRP, mg/l†	1.5 (1.4 to 1.6)	1.8 (1.4 to 2.2)	1.5 (1.4 to 1.6)	0.09
Creatinine, mg/dl	0.94 ± 0.21	1.03 ± 0.21	0.94 ± 0.21	<0.001
Change from baseline				
LDL-C				
Median of % change	−19.4 (−20.5 to −18.3)	−20.0 (−25.3 to −14.7)	−19.4 (−20.5 to −18.3)	0.86
p value‡	–	<0.001	<0.001	–
HDL-C				
Median of % change	7.4 (6.8 to 8.0)	3.8 (1.1 to 6.5)	7.5 (6.9 to 8.1)	0.07
p value‡	–	<0.001	<0.001	–
Non-HDL-C				
Median of % change	−17.0 (−17.9 to −16.1)	−19.1 (−24.0 to −14.2)	−17.0 (−18.0 to −16.0)	0.88
p value‡	–	<0.001	<0.001	–
Triglycerides				
Median of % change	−7.2 (−8.2 to −6.2)	−9.7 (−14.5 to −4.9)	−7.1 (−8.1 to −6.1)	0.35
p value‡	–	0.006	<0.001	–
CRP				
Median of % change	−22.4 (−24.9 to −19.9)	−14.8 (−27.3 to −2.3)	−22.6 (−25.1 to −20.1)	0.80
p value‡	–	0.28	<0.001	–
Creatinine				
Median of % change	0.0 (−0.5 to 0.5)	0.0 (−2.7 to 2.7)	0.0 (−0.5 to 0.5)	0.006
p value‡	<0.001	0.12	<0.001	–

Values are mean ± SD or median (95% CI). The 95% CIs of the medians are calculated using the formula 95% CI_{median} = median ± 1.57 • IQR/√N, where N is the size of the patient cohort. *Unless otherwise noted, laboratory values obtained during treatment are the time-weighted averages of all post-baseline values. †Last observation. ‡p value for Wilcoxon signed rank test.

CI = confidence interval; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol.

groups demonstrated significant progression of coronary calcium from baseline ($p < 0.001$ from baseline); however, the change in CaI was significantly greater in those patients treated with warfarin (warfarin-treated patients, +0.03 [IQR: 0.00 to 0.08]; non-warfarin-treated patients, +0.02 [IQR: 0.00 to 0.06]; $p = 0.003$ for between group difference).

FACTORS ASSOCIATED WITH PROGRESSIVE CORONARY CALCIFICATION. Table 5 describes a multivariable logistic regression model describing factors independently associated with an increase in CaI following propensity weighting. Concomitant warfarin use was independently associated with progressive coronary artery calcification (odds ratio: 1.16; 95% confidence interval [CI]: 1.05 to 1.28; $p = 0.003$). Other factors associated with a rising CaI included female sex, higher body mass index, history of diabetes mellitus, prior myocardial infarction, baseline and concomitant statin use, baseline aspirin use, baseline beta-blocker use, greater baseline CaI, greater baseline PAV, and increase in PAV. Follow-up renal function was not associated with an increase in CaI.

SENSITIVITY ANALYSIS. We performed a sensitivity analysis for a cohort of 328 patients (n = 164 per treatment group) obtained by propensity score greedy matching. Baseline CaIs were similar in warfarin-treated and non-warfarin-treated patients (0.29

TABLE 3 Baseline and Change in Ultrasonic Vascular Parameters According to Concomitant Warfarin Use

Percent Atheroma Volume	Overall (n = 4,300)	Warfarin (n = 171)	No Warfarin (n = 4,129)	Predicted Mean Difference (95% CI)	p Value for Predicted Mean Difference
Baseline	38.1 ± 9.1	38.1 ± 9.8	38.1 ± 9.1	0.03 (−1.4 to 1.4)	0.96
Change from baseline	0.25 ± 0.23	0.13 ± 0.33	0.25 ± 0.23	−0.12 (−0.60 to 0.36)	0.63
p value for test of change = 0*	0.29	0.68	0.28		
Annualized changes	0.17 ± 0.13	0.12 ± 0.18	0.17 ± 0.13	−0.05 (−0.30 to 0.20)	0.71
Change from baseline†	0.41 ± 0.07	0.51 ± 0.09	0.34 ± 0.08	0.17 (−0.04 to 0.38)	0.10
p value for test of change = 0*	0.001	<0.001	<0.001		
Annualized changes†	0.28 ± 0.04	0.33 ± 0.05	0.24 ± 0.05	0.09 (−0.03 to 0.20)	0.16
Change from baseline‡	0.42 ± 0.07	0.51 ± 0.09	0.35 ± 0.08	0.17 (−0.04 to 0.37)	0.12
Annualized changes‡	0.29 ± 0.04	0.33 ± 0.05	0.25 ± 0.05	0.08 (−0.04 to 0.20)	0.17

Baseline values are reported as mean ± SD from general linear model. Change values are reported as least-squares mean ± SE from linear mixed models controlling for baseline PAV and clinical trial and treatment. *From Student *t* test. †Values are from the IPTW-weighted linear mixed models controlling for baseline PAV, clinical trial, and study time duration (ignored for annualized changes). ‡Values are from the IPTW-weighted linear mixed model for change in PAV, controlling for baseline PAV, clinical trial, treatment, study time duration (ignored for annualized changes), baseline statin use, concomitant statin use, age, sex, BMI, history of MI, history of diabetes, and use of an ACE inhibitor or ARB at baseline.

IPTW = inverse probability of treatment weight; PAV = percent atheroma volume; other abbreviations as in Tables 1 and 2.

[IQR: 0.14 to 0.55] vs. 0.34 [IQR: 0.14 to 0.65]; p = 0.65 for ranks). However, in accordance with the results of the main analysis, annualized changes in CaI were significantly greater in the warfarin-treated group compared with those patients not treated with warfarin (0.03 [IQR: 0.00 to 0.073] vs. 0.02 [IQR: 0.00 to 0.066]; p = 0.029 for ranks).

A further sensitivity (trimmed) analysis that removed patients with a propensity score >0.1 or ≈5% of the total study population did not yield data that significantly differed from those of the principal analysis (Online Tables 2 and 3).

DISCUSSION

In this post hoc propensity-weighted analysis of patients with coronary artery disease who underwent serial coronary IVUS examinations, we demonstrated the association between warfarin use and serial coronary calcification that is independent of statin use, age, and renal function. There was no difference in the progression of atheroma volume between the 2 groups despite the significantly greater increase in CaI in warfarin-treated patients. This study concomitantly demonstrated the serial effects of warfarin and changes of both atheroma volume and coronary calcification in humans in vivo.

Traditionally, warfarin-induced calcification has been thought to involve the arterial media exclusively, a condition commonly known as Mönckeberg sclerosis. However, an atherosclerotic mouse model study demonstrated that short-term administration of warfarin induced intimal calcification and outward arterial remodeling without significantly affecting plaque burden, a finding suggesting that warfarin use may have procalcific effects not limited to the vascular media (25). These observations could have implications for plaque stability in patients

TABLE 4 Baseline and Change in Calcium Index According to Warfarin Treatment

Calcium Index	Overall (n = 4,300)	Warfarin (n = 171)	No Warfarin (n = 4,129)	Difference *t* Statistic	p Value for Difference
Baseline	0.28 (0.09, 0.54)	0.30 (0.14, 0.59)	0.28 (0.09, 0.54)	2.3	p = 0.024*
Change from baseline	0.04 (0.00, 0.11)	0.05 (0.00, 0.15)	0.04 (0.00, 0.11)	3.9	p < 0.001†
p value for test of change = 0‡	<0.001	<0.001	<0.001		
Annualized changes	0.02 (0.00, 0.06)	0.03 (0.00, 0.08)	0.02 (0.00, 0.06)	4.2	p < 0.001§

Calcium index values are reported as median (interquartile range). Calcium index increase is reported as n (%). *Values are from the general linear model for baseline calcium index ranks, controlling for baseline plaque burden (PAV) and clinical trial; the *t* value has 4,290 degrees of freedom. †Values are from the IPTW-weighted linear mixed model for change in calcium index ranks, controlling for clinical trial, study time duration, sex, Caucasian race, BMI, history of diabetes, history of hypertension, baseline statin use, concomitant statin use, baseline beta-blocker use, baseline calcium index (ranks), baseline PAV, change in PAV, baseline HDL-C, baseline creatinine level, and last observation of creatinine. The *t* value has 4,199 degrees of freedom. ‡From Wilcoxon signed rank test. §Values are from the IPTW-weighted linear mixed model for annualized change in calcium index ranks, controlling for clinical trial, treatment, sex, Caucasian race, BMI, history of diabetes, history of hypertension, baseline statin use, concomitant statin use, baseline beta-blocker use, baseline calcium index ranks, baseline PAV, change in PAV, baseline HDL-C, baseline creatinine level, and last observation of creatinine; the *t* value has 4,200 degrees of freedom.

Abbreviations as in Tables 1 to 3.

prescribed long-term warfarin, especially patients with concomitant atherosclerotic risk factors. However, the true impact of warfarin on atheroma phenotype and subsequent clinical events in vivo in humans remains unknown, and further investigation is required. Plaque calcification may also represent a more stable atheroma phenotype with greater resistance to progression and regression (23), and it can occur after statin therapy as a result of plaque delipidation followed by fibrosis and calcification (4). Calcified coronary plaques have been associated with atheroma regression in the setting of aggressive statin therapy (4,26,27), a finding likely underscoring the vasculoprotective effects of statin-induced calcium. It therefore remains unknown whether warfarin-induced plaque calcification represents a more stable plaque phenotype.

Our data are thus consistent with, and extend the findings of, prior imaging studies undertaken to evaluate the effects of warfarin on the human arterial vasculature (28–30). However, these prior analyses were limited by their cross-sectional design and by the issues that concomitant plaque volume measurements were not undertaken and relatively low numbers of patients were evaluated at a single time point. Nevertheless, the association between warfarin use and its procalcific properties has been demonstrated in multiple arterial territories in humans. In 1 cohort study, the presence of lower extremity arterial calcification was greater in patients exposed to warfarin compared with those not exposed to the drug (29). Other human imaging studies have also demonstrated the association between warfarin exposure and calcification involving the femoral (29) and breast (30) arteries. A prospective study undertaken in patients with AF identified that those patients using vitamin K antagonists had higher coronary calcium scores, as measured with coronary computed tomography angiography (28).

Until recently, elevated calcium scores on multidetector computed tomography study were thought simply to correlate with increasing cardiovascular risk. It is now apparent that the density of calcium may be more important than simply measuring its total burden (3). Even so, medial calcification is often seen first within peripheral arteries (31); it has been associated with increasing duration of warfarin use (29), and it is known to correlate with cardiovascular risk and event rates (1). Our data suggest that warfarin use is associated with progressive arterial calcium irrespective of baseline calcium and plaque burden, concomitant statin use, age, renal function, and sex. This finding indicates that in patients prescribed long-term warfarin treatment, progressive vascular calcification likely ensues, and it could affect cardiovascular risk, depending on the presence of other risk factors. Although this hypothesis requires further investigation, the potential clinical implications of these findings are that in such circumstances, clinicians prescribing long-term warfarin treatment may need to consider the impact of induced plaque calcification and subsequent longer-term risk factor modification. Furthermore, newer oral anticoagulant agents that do not inhibit vitamin K are now available, and they could be used as alternative agents in patients considered to be at higher atherosclerotic risk and requiring long-term anticoagulant therapy. Considering the growing burden of AF in society, which often coexists with coronary and cerebrovascular disease in the presence other cardiovascular risk factors, and given that many patients with AF require long-term systemic anticoagulant therapy, the findings of the present analysis could have much broader clinical implications.

TABLE 5 Full Multivariable Model for Calcium Index Increase*

	OR and 95% CI	p Value
Female	2.00 (1.77–2.26)	<0.001
Caucasian	2.24 (1.86–2.70)	<0.001
BMI	1.12 (1.07–1.18)	<0.001
Diabetes	1.51 (1.33–1.71)	<0.001
Prior hypertension	1.67 (1.49–1.89)	<0.001
Prior MI	1.16 (1.04–1.29)	0.010
Baseline statin use	1.28 (1.15–1.44)	<0.001
Baseline aspirin use	1.29 (1.06–1.58)	0.013
Baseline beta-blocker use	1.17 (1.04–1.31)	0.010
Concomitant statin use	1.87 (1.36–2.56)	<0.001
Concomitant warfarin use	1.16 (1.05–1.28)	0.003
Baseline calcium index (ranks)	1.52 (1.43–1.61)	<0.001
Baseline PAV	1.53 (1.44–1.62)	<0.001
Change in PAV	1.65 (1.56–1.74)	<0.001
Creatinine	0.97 (0.92–1.02)	0.26

ORs for age, BMI, rank-transformed baseline calcium index, baseline PAV, change in PAV, and last observation of creatinine are reported per SD; creatinine value reported as last observation. *The model was also adjusted for clinical trial, treatment, study time duration, and was IPTW-weighted.

OR = odds ratio; other abbreviations as in Tables 1 and 3.

STUDY LIMITATIONS. Several caveats of the present analysis warrant further consideration. We could demonstrate significant differences in plaque calcification despite a follow-up period of 18 to 24 months, a relatively modest time period compared with the duration of warfarin therapy usually prescribed for patients with appropriate clinical indications. Given the significant procalcific effects in warfarin-treated patients that were demonstrated within 18 to 24 months, one could therefore speculate on the extent of coronary atheroma calcification following a much

more extended period of warfarin therapy. The current analysis was not a placebo-controlled trial. However, it is highly unlikely that there will ever be such a trial because it is unethical to administer (or withhold) warfarin simply to evaluate its effects on vascular calcification in humans. The present analysis provides evidence of the serial, procalcific effects of warfarin in human coronary atheroma in vivo. There is the possibility of unmeasured confounders influencing our analysis. However, a range of clinical, ultrasonic, and metabolic parameters, as well as clinical trials, was extensively controlled for in the present analysis, thus minimizing the effects of potential biases. Moreover, the inclusion and exclusion criteria for all the included serial IVUS trials were relatively uniform, and the analysis was performed within a single core laboratory using standardized analytical techniques. Furthermore, concordant results in 2 differing propensity models highlighted the consistency and biological plausibility of these findings. Depth analysis of calcium is not a standard component of our core laboratory's IVUS imaging protocol. Consequently, the relative location of the calcium (intimal vs. adventitial) remains unknown. Other than the presence of concomitant AF, indications and serial international normalized ratios were not systematically collected across IVUS trials. Finally, markers of bone mineralization were not measured, and such measurement may have provided added mechanistic insight into the cellular mediators of atherosclerosis that are common to osteogenesis and bone mineralization (32).

CONCLUSIONS

In conclusion, warfarin use is independently associated with serial procalcific effects within the human coronary vasculature in vivo, irrespective of concomitant changes in atheroma volume, concomitant statin therapy, and renal function. These findings are consistent with earlier preclinical and cross-sectional human vascular imaging data. The longer-term clinical implications of these data and their extrapolation to newer oral anticoagulant agents remain unknown, however, thus warranting further investigation.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In patients with angiographically apparent coronary artery disease, warfarin use is associated with progressive coronary artery calcification in vivo, independent of its baseline extent, the degree of baseline plaque burden, baseline or concomitant statin use, or renal function.

TRANSLATIONAL OUTLOOK: Additional mechanistic studies are warranted to determine whether these observations relate to intensity and duration of warfarin exposure and promote an underlying vulnerable (or stable) plaque phenotype. Similar studies may also need to be undertaken to evaluate the effects of some of the newer oral anticoagulant agents on the arterial wall.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.