Increased Peripheral Arterial Calcification in Patients Receiving Warfarin

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Background—Matrix Gla protein is a vitamin K–dependent inhibitor of vascular calcification. Warfarin use is associated with increased breast arterial calcification, but whether this is reflective of other arteries or occurs in men is unclear. In this study, the prevalence of calcification in peripheral arteries was compared in patients with and without warfarin therapy.

Methods and Results—This retrospective matched cohort study assessed 430 patients with radiographs performed during or after warfarin therapy who were identified by a computerized search of medical records. Each patient was matched to a patient without warfarin exposure based on age, sex, and diabetes status. Patients with warfarin exposure <1 month, history of end-stage renal disease, or serum creatinine >2.0 mg/dl were excluded. Radiographs were reviewed visually for arterial calcification. The prevalence of arterial calcification was 44% greater in patients with versus without warfarin use (30.2% versus 20.9%, P=0.0023) but not on radiographs performed before warfarin therapy (26.4% versus 22.4%, n=156) or prior to 5 years of warfarin therapy. The increase was noted only in the ankle and foot, was limited to a medial pattern of calcification, and was similar in men and women.

Conclusions—Warfarin use is associated with lower extremity arterial calcification in both men and women independent of age, sex, diabetes status, and other patient characteristics. This may have implications for the choice of therapies for long-term anticoagulation. (***J Am Heart Assoc***. 2016;5:e002665 doi: 10.1161/JAHA.115.002665)

Key Words: anticoagulants • peripheral vascular disease

Arterial calcification is common in cardiovascular disease and is associated with poorer outcomes.[1–4] In particular, calcification within the medial layer of arteries occurs with advanced age, diabetes, and chronic kidney disease[5] and appears to be directly pathogenic by decreasing vascular compliance.[6,7] Calcification also occurs within the neointima of atherosclerotic plaques, but its clinical impact remains uncertain. Endogenous inhibitors play a critical role in preventing arterial calcification, and a key inhibitor is matrix Gla protein, a vitamin K–dependent protein synthesized by vascular smooth muscle.[8] Its absence leads to medial arterial calcification in humans[9,10] and in mice.[11] The inhibitory action of matrix Gla-protein presumably requires vitamin K–dependent γ-carboxylation because warfarin induces medial calcification in rat arteries both ex vivo and in vivo.[8,12] Whether matrix Gla protein plays a role in atherosclerotic calcification has not been studied.

The role of matrix Gla protein has raised concerns about possible effects of warfarin on vascular calcification in humans. Initial studies to address this issue were small, were limited to specific populations, or did not fully control for underlying cardiovascular disease and yielded conflicting results.[13–17] Because the vessels examined develop both medial and atherosclerotic calcification, which cannot be distinguished radiologically, effects on medial calcification may have been obscured. The results were also not fully controlled for preexisting vascular disease. Using mammography to detect breast arterial calcification, which is exclusively medial,[18,19] we recently demonstrated a 50% increase in prevalence that was independent of other cardiovascular risk factors in a large cohort of women with current or past warfarin use.[20] To demonstrate that the results are generalizable to other arteries and to men, we performed a similar study of arterial calcification in lower extremities detected on standard radiographs.

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Methods

Study Design

This retrospective matched cohort study was designed to test the hypothesis that use of warfarin is associated with increased

arterial calcification in the lower extremities and in men. Inclusion criteria were a radiograph of a lower extremity, a measurement of serum creatinine, and warfarin use for at least 1 month. Exclusion criteria were end-stage renal disease, renal transplantation, or serum creatinine >2.0 mg/dl. Based on our previous study of breast arterial calcification,[20] we anticipated prevalence of arterial calcification of 39% and 26% in warfarin-treated patients and in patients never treated with warfarin, respectively. This yielded a sample size of 216 matched pairs necessary for an 80% power to achieve a significance of P=0.05. This was doubled to obtain enough patients to detect an effect of warfarin in each sex. In addition to sex, prespecified subset analyses were duration of warfarin use (<1, 1–5, and >5 years), age (<60 versus >60 years), and diabetes status, identical to those in the previous study of breast arterial calcification. To control for other patient characteristics besides age, sex, diabetes status, and renal failure that might influence the prevalence of arterial calcification in patients receiving warfarin, we also compared radiographs performed prior to warfarin use with radiographs from matched patients who never received warfarin.

Subjects

Patients and control subjects were identified through a computerized search of inpatient and outpatient records from Emory University Hospital, Emory University Hospital Midtown, and the Emory Clinic for all lower extremity x-rays (knee, tibia/fibula, ankle, and foot) scheduled from January 1, 2010, to August 18, 2014. The following variables were recorded: date of birth; scheduled date of x-ray; the presence of warfarin or Coumadin (Bristol-Myers Squibb Co) on a medication list; sex; diabetes (from diagnoses or problem lists); and serum calcium, phosphorus, and creatinine. A random sample of patients was obtained by choosing all patients with birthdates between March 1 and July 31.

Medical Record Review

The medical record of each patient was reviewed to confirm the use of warfarin and the assignment of diabetes status, to determine the indication for anticoagulation and the start and stop dates of warfarin, and to identify a history of end-stage renal disease.

Controls

Each patient was matched at random to a control subject without a history of warfarin use on the basis of age (in years by integers), sex, and diabetes status at the time of the radiograph. This was performed randomly by a comput-

erized algorithm from a list of all patients without a history of warfarin use sorted by diabetes status, by age, and lastly by medical record number. When an exact match to age could not be made (which occurred in only 4 subjects aged >75 years), controls were matched manually to patients based on closest age, but never >2 integers, using the same list. Diabetes status was matched exactly for all subjects. A random chart review of 82 control subjects revealed that none had a history of warfarin use and that the assignment of diabetes status was accurate for all of them.

Radiographs

Radiographs were selected based on the dates of warfarin use. For patients who were currently receiving warfarin, the most recent x-ray was selected; otherwise, the first x-ray after discontinuation of warfarin was selected. Only radiographs of the knee, tibia/fibula, ankle, or foot were evaluated. The x-rays were scored as arterial calcification present or absent by a single reviewer who was otherwise blinded. In the case of multiple x-rays on the same day, calcification was deemed positive if present on any x-ray. Positive scoring required densities (at least 2 in close proximity) over the location of arteries. Unless the reviewer was certain, calcification was scored as absent. An additional reviewer analyzed 70 radiographs at random, and agreement between the 2 reviewers was 94%. In those few cases of disagreement, the radiographs were reviewed again, and if disagreement remained, calcification was scored as absent. All repeated reviews resulted in agreement between the reviewers. Calcifications were classified as either medial (linear calcifications in a train-track pattern) or atherosclerotic (distinct, nodular calcifications).

This study was approved by the institutional review board of Emory University. Informed consent was waived.

Statistics

Differences between continuous variables was assessed by Student t test. Testing of proportions was by Fisher exact test for 2×2 comparisons and by chi-square test for larger arrays. Logistic regression was performed using StatPages (http://statpages.org/logistic.html) with sequential omission of variables with P>0.20. Interaction terms were used to assess any differences in the warfarin effect between subgroups. Warfarin duration was log-transformed to render its relationship to calcification more linear, and radiograph mismatch was assigned as follows: 1 for ankle or foot in a warfarin-treated patient and knee or tibula/fibula in a control subject, 0 for no mismatch, and −1 for knee or tibula/fibula in a warfarin-treated patient and ankle or foot in a control subject.

Results

A total of 98 829 x-rays were identified in 77 543 patients, of whom 25 165 had a measurement of serum creatinine. Of these, 1662 had a history of warfarin use, and 685 were selected at random for chart and x-ray review. A total of 590 patients remained after exclusion of patients with end-stage renal disease, renal transplantation, or serum creatinine >2.0 mg/dl; patients without x-rays; and patients in whom the use of warfarin could not be documented. Of these 590 patients with a history of warfarin use, a serum creatinine value <2.0 mg/dl, and no history of end-stage renal disease, 101 had no x-rays performed after warfarin was started, 14 had an indeterminate duration of warfarin use, and 45 had x-rays only during the first month of warfarin use. After these exclusions, 430 patients remained, and information on the warfarin therapy in these patients is presented in Table 1.

Of the 291 patients receiving warfarin at the time of the radiograph, 34 had 2 courses of warfarin separated by a median of 651 days (range: 7–4749 days). In the 139 patients who were not receiving warfarin at the time of the radiograph, warfarin had been discontinued for a median of 596 days (range: 1–9046 days). Thirteen of these patients had received 2 courses of warfarin separated by a median of 983 days (range: 33–9091 days). A precise duration of warfarin could not be determined for 218 patients because of uncertain start or stop dates; for all but 25 of these patients, minimum and maximum durations could be calculated. For Table 1, the midpoints of these ranges were used. In the other 25 patients, warfarin use could be determined only as preceding a specified date, so only a minimum duration could be calculated, and that was used for Table 1. Equal numbers received warfarin for atrial fibrillation or venous thrombosis. In most of the remaining 11%, the indication was cardiac (mechanical valves, atrial or ventricular thrombus).

The characteristics of these 430 warfarin-treated patients and the patients with no history of warfarin use who were matched individually for age, sex, and diabetes status are shown in Table 2. Diabetes was present in 34%, and 40.5% were male. Due to differences between scheduled date and actual x-ray date and the inability to match some older patients to exact age, the difference in age between patients and control subjects was occasionally >1 year. The mean difference in age between control subjects and patients was 0.11±0.03 years, with a range of −1.83 to 2.23 years. Aside from the slightly lower serum calcium level in the warfarin-treated patients, there were no significant differences between the groups. The mean interval between the radiograph and the serum chemistries was slightly >1 year. The warfarin-treated patients had more tibia/fibula and ankle radiographs and fewer foot radiographs; however, the proportion of radiographs of the leg versus the ankle and foot did not differ between the groups. Among the warfarin-treated patients with knee or tibia/fibula radiographs, 41% of the patients without warfarin use also had tibia/fibula radiographs, representing a radiograph mismatch of 59%. Among warfarin-treated patients with ankle or foot radiographs, the match was 56% (44% radiograph mismatch).

Radiographs performed within 3 years prior to starting warfarin were available in 156 patients, including 69 patients in whom radiographs were also available after starting warfarin. These patients were matched to different patients without warfarin use because of a difference in age. The mean time from the radiograph to the start of warfarin was 245±21 days (median: 156 days). The mean interval between the radiograph and the serum chemistries was slightly >1 year. The characteristics of these patients and their matched controls are shown in Table 3. Diabetes was present in 37.2%, and 36.5% were male. As in the previous cohort, serum calcium was lower in the warfarin group, which also had a lower serum creatinine level.

The prevalence of arterial calcification, depicted in Figure 1, was 44% greater in the patients with current or past warfarin use compared with patients without warfarin use (30.2% versus 20.9%, *P*=0.0023). By comparison, there was no significant difference in arterial calcification in radiographs performed prior to warfarin therapy (26.4% versus 22.4%, *P*=0.51). The relationship between total duration of warfarin therapy and arterial calcification is shown in Figure 2. This analysis excluded 42 subjects in whom the range of possible durations did not fall entirely within 1 of the ranges shown. The increased calcification was accounted for almost entirely by patients receiving warfarin for >5 years, in whom the prevalence was increased by 100%. The proportion of ankle or foot radiographs (52% versus 55%) and the indications for warfarin (atrial fibrillation: 45% versus 43%; deep vein thrombosis or pulmonary embolism: 39% versus 55%) did not differ between this group and the remainder of the cohort.

Table 1. Parameters of Warfarin Use

	%	Days on Warfarin Median (Range)	Days off Warfarin Median (Range)
All patients	100	940 (31–13 826)	0 (0–4749)
Currently receiving warfarin	68	1389 (32–13 826)	0 (0–4749)
Past warfarin use	32	297 (31–7729)	655 (1–9046)
Atrial fibrillation	45	1128 (32–13 826)	0 (0–3144)
DVT/PE	44	477 (33–13 401)	3 (0–9046)

DVT indicates deep vein thrombosis; PE, pulmonary embolism.

Table 2. Characteristics of Patients With X-Rays Performed During and After Warfarin and Control Patients Without Warfarin

	Warfarin	Control	P Value
Patients, n	430	430	
Age, y	66.9.0±0.8 (21–98)	67.1±0.8 (21–98)	0.86
Serum creatinine, mg/dL	1.01±0.02 (0.31–1.98)	1.04±0.02 (0.30–1.99)	0.29
Serum calcium, mg/dL	9.04±0.03 (5.3–11.7)	9.16±0.03 (7.3–11.4)	0.005
Serum phosphorus, mg/dL*	3.39±0.05 (0.8–6.0)	3.43±0.06 (1.4–6.5)	0.62
X-ray type (%)			0.052
Knee	37.4	38.4	
Tibia/fibula	7.9	4.7	
Ankle	24.9	20.9	
Foot	29.8	36.0	

*Available in 247 warfarin-treated patients and 150 control patients.

The effect of warfarin in other subgroups is shown in Figure 3. The increased prevalence of arterial calcification was similar and significant in both men and women. Although increases were seen in those both with and without diabetes, it was greater in those without diabetes (57% versus 30%) and was significant only in that group. As expected, the prevalence of arterial calcification was much lower in patients aged <60 years, but the increase with warfarin was much greater in this group compared with those aged ≥60 years (138% versus 35%). The *P* values for the interaction of warfarin with age, sex, or diabetes status were 0.25, 1.0, and 0.83, respectively, indicating that there was no significant difference in the warfarin effect between any subgroups. The effect of warfarin was observed in both the atrial fibrillation (38.3% versus 29.9%) and deep vein thrombosis and pulmonary embolism (25.0% versus 13.3%) groups. The greater prevalence of arterial calcification in the patients without warfarin use in the atrial fibrillation group can be explained by older age (74.4 versus 68.0 years) and prevalence of diabetes (39.7% versus 28.2%).

The type of calcification was classified as medial or atherosclerotic based on the pattern noted on the radiograph. Medial and atherosclerotic calcifications were judged to be present in 95% and 19% of calcified arteries, respectively, with 12% having both types. The prevalence of medial arterial calcification was 49% greater in patients with current or past warfarin use than in those without warfarin use but did not differ in radiographs obtained prior to warfarin therapy (Figure 4). Warfarin use was not associated with an increased prevalence of atherosclerotic calcification (5.6% versus 5.3%), but the numbers were small.

The effect of artery type is shown in Figure 5. Although the prevalence of arterial calcification did not vary much among sites in the patients without warfarin use, the effect of warfarin was apparent only in the distal arteries, in which the prevalence of arterial calcification was almost doubled. The

Table 3. Characteristics of Patients With X-Rays Performed Before Warfarin and Control Patients Without Warfarin

	Warfarin	Control	P Value
Patients, n	156	156	
Age, y	66.6±1.1 (18–94)	66.7±1.1 (18–95)	0.95
Serum creatinine, mg/dL	1.01±0.03 (0.37–1.97)	1.13±0.03 (0.50–1.99)	0.005
Serum calcium, mg/dL	9.05±0.05 (7.3–10.7)	9.23±0.04 (7.4–10.8)	0.005
Serum phosphorus, mg/dL*	3.42±0.08 (1.0–6.8)	3.40±0.10 (1.9–6.1)	0.88
X-ray type (%)			0.99
Knee	49.4	48.7	
Tibia/fibula	6.4	5.8	
Ankle	17.9	19.2	
Foot	26.3	26.3	

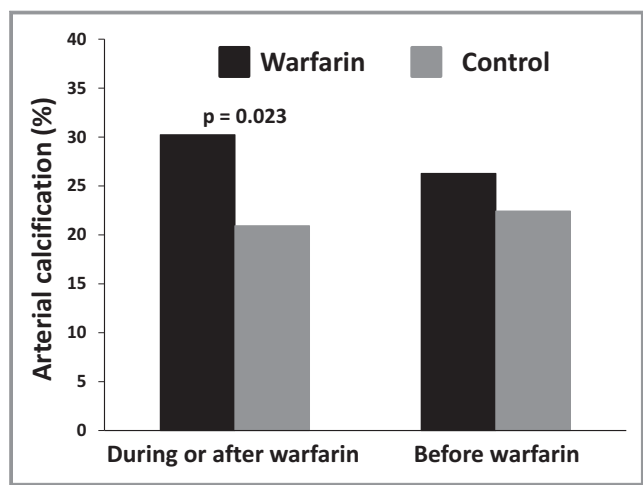*Available in 104 warfarin-treated patients and 54 control patients.

Figure 1. Prevalence of arterial calcification in low-extremity radiographs performed during or after warfarin therapy (n=430) and before warfarin therapy (n=156) compared with control patients (no warfarin therapy) matched for age, sex, and diabetes status.

Figure 3. Prevalence of arterial calcification in warfarin-treated patients and control subjects in different groups: age <60 vs ≥60 years, male (M) vs female (F), diabetes vs no diabetes. The numbers of patients are given in parentheses.

prevalence of diabetes (36.9% versus 30.4%) and the duration of warfarin use (4.9±0.4 versus 4.7±0.4 years) were slightly but not significantly greater in patients with ankle or foot radiographs, but age was significantly lower (66.0±1.09 versus 69.2±1.05 years, P=0.029). Serum creatinine was significantly higher in patients with ankle and foot radiographs (1.06±0.02 versus 0.96±0.02, P=0.003); however, there was no difference in serum creatinine between patients and control subjects with either type of radiograph. Because of mismatch between patient and control radiograph types, the data were reanalyzed in those pairs with matching radiograph types (Figure 6). The prevalence of arterial calcification remained greater in patients with versus without warfarin
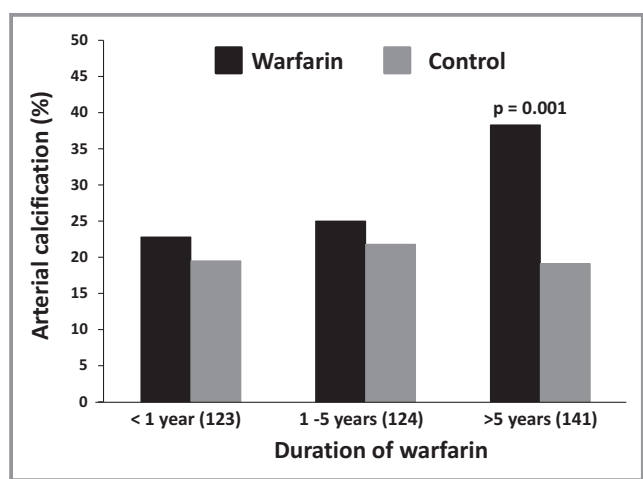
use who had ankle or foot radiographs (30.5% versus 19.8%, n=131, P=0.06) and did not differ for those with knee or tibia/fibula radiographs (23.8% versus 22.5%, n=80).

Logistic regression for arterial calcification in the patients with warfarin use is shown in Table 4 and included the following variables: age, diabetes status, sex, indication for warfarin, serum creatinine, radiograph type, radiograph mismatch, warfarin duration, and interval without warfarin. As expected, age and diabetes were significant predictors of arterial calcification, but duration of warfarin therapy remained a significant independent determinant despite inclusion of these other variables.

Discussion

The results demonstrated a greater prevalence of peripheral arterial calcification in patients receiving warfarin. This association cannot be explained by other risk factors for vascular calcification because patients were matched by age, sex, and diabetes status and because serum creatinine, phosphorus, and calcium were not greater in the patients with warfarin use. Furthermore, warfarin remained an independent determinant in a logistic regression that included age, sex, diabetes status, radiograph type, and serum creatinine. Although an effect of other, unmeasured variables cannot be excluded, the fact that increased calcification was not observed in radiographs performed prior to warfarin use eliminates many preexisting patient characteristics.

In addition to confirming our previous data on breast arterial calcification, these results demonstrated that the association of warfarin use with vascular calcification is

Figure 2. Prevalence of arterial calcification in warfarin-treated patients and control subjects at different durations of warfarin: 1 to 12 months, 1 to 5 years, >5 years. The numbers of patients are given in parentheses.
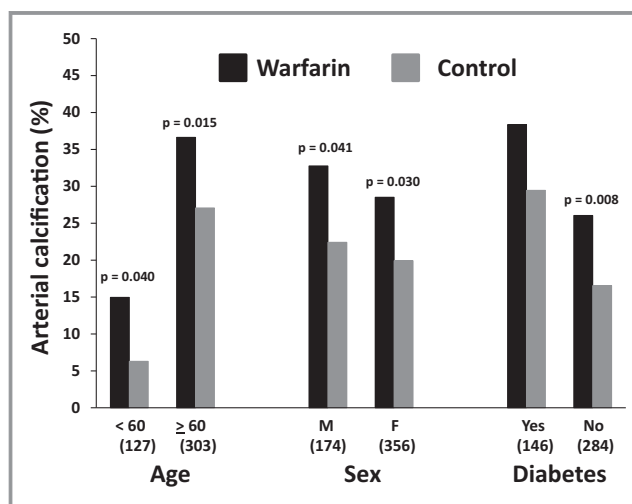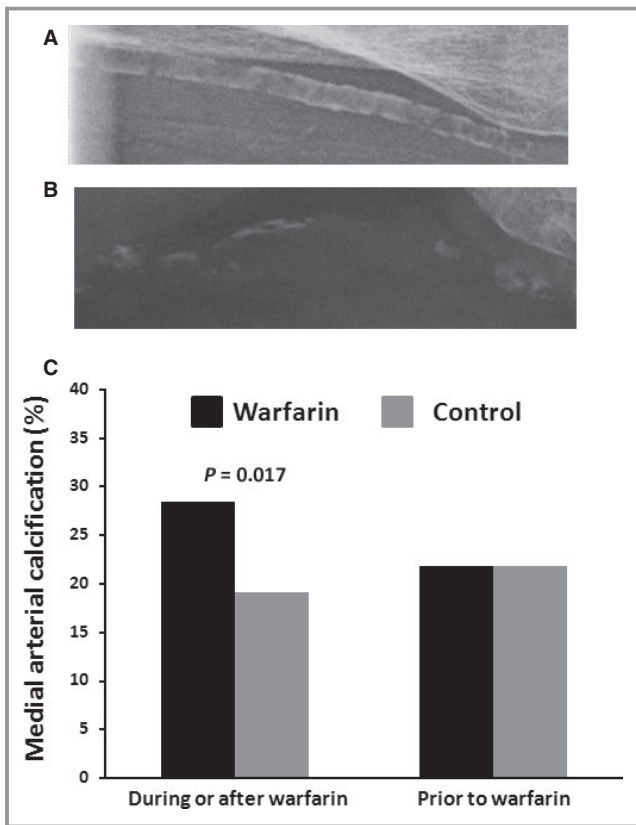
Figure 4. Medial vs atherosclerotic calcification. A, Radiograph of posterior tibial artery showing a medial pattern of calcification. B, Radiograph of femoral artery showing an atherosclerotic pattern of calcification. C, Prevalence of medial arterial calcification in low-extremity radiographs performed during or after warfarin therapy (n=430) and before warfarin therapy (n=156) compared with control patients matched for age, sex, and diabetes status.

generalizable to other arterial beds and to men as well as women. The effect of warfarin was similar to the 50% increase observed previously in breast arterial calcification and was
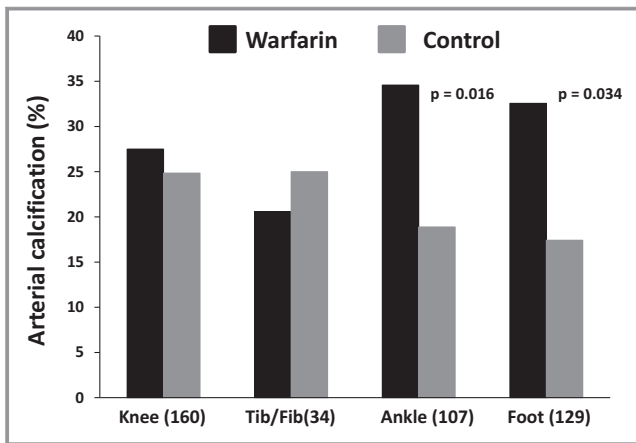

Figure 5. Prevalence of arterial calcification in warfarin-treated patients and control subjects based on anatomic location in warfarin-treated patients. The numbers of patients are given in parentheses. Tib/Fib indicates tibia/fibula.
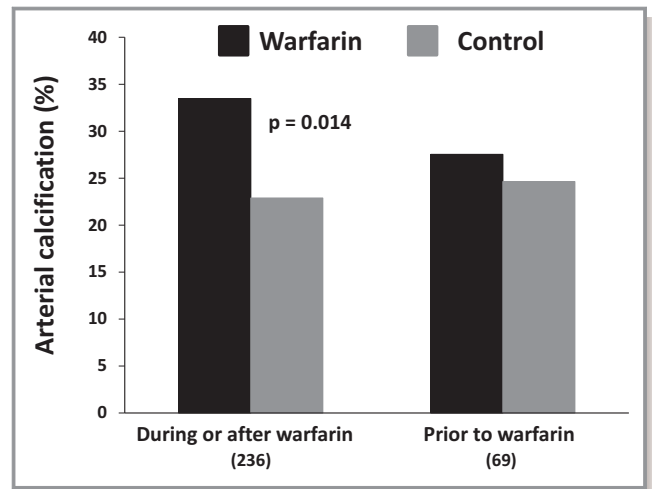

Figure 6. Prevalence of arterial calcification in ankle and foot radiographs performed during or after warfarin therapy (n=236) and before warfarin therapy (n=69) compared with control patients with similar radiographs matched for age, sex, and diabetes status.

dependent on duration, with only durations of >5 years showing an increased prevalence of peripheral arterial calcification. The failure to detect an increase with durations between 1 and 5 years, as seen with breast arterial calcification, may relate to the greater sensitivity of mammography to detect the calcifications. Although the prevalence of arterial calcification was much lower in patients aged <60 years, the increase associated with warfarin was much greater —a phenomenon also observed with breast arterial calcification. Some of this increase may be due to the greater proportion of ankle or foot radiographs in patients aged <60 years than in those aged >60 years (69.3% versus 52.1%).

Most of the arterial calcification had a medial pattern, consistent with a previous study showing much greater prevalence of medial rather than atherosclerotic calcification in amputation specimens.[21] Interestingly, the effect of warfarin was limited to medial calcification, which probably explains why the effect of warfarin was limited to arteries visible on ankle and foot radiographs: Medial calcification is more prevalent and severe in distal arteries.[21] The anatomic

Table 4. Multivariable Logistic Regression for Lower Extremity Arterial Calcification

	Odds Ratio	95% CI	*P* Value
Age, y	1.06	1.04 to 1.08	<0.0001
Diabetes	1.87	1.16 to 3.01	0.01
Warfarin duration (log days)	1.56	1.07 to 2.28	0.022
Serum creatinine, mg/dL	1.95	1.00 to 3.83	0.051
Sex	1.60	0.97 to 2.64	0.068
Site (lower leg vs upper leg)	1.51	0.95 to 2.42	0.083

specificity is unlikely to be explained by differences in patient characteristics because the patients were matched for age, sex, and diabetes status, and there were no differences in serum creatinine. The possibility that this effect could be related to other uncollected variables such as race or smoking cannot be excluded. It is also unlikely to be caused by the combined effect of site-specific prevalence of arterial calcification and radiograph mismatch because the prevalence of arterial calcification in the nonwarfarin group was actually lower in ankle and foot radiographs, the degree of mismatch in control radiographs did not differ substantially between patients with knee and tibia/fibula versus ankle and foot radiographs, and similar results were obtained when patients with mismatched radiographs were omitted. This finding differs from a previous study in which warfarin was associated with increased calcification of femoral arteries.[16]

Because patients requiring anticoagulation frequently have cardiovascular disease, the increase in peripheral arterial calcification in warfarin-treated patients could have preceded warfarin use or developed independently of warfarin use. The prevalence of arterial calcification, however, was not increased in x-rays performed prior to warfarin use, and because the majority were performed <6 months before warfarin therapy, they should reflect the underlying state of vascular calcification. The fact that increased calcification was apparent only after 5 years of warfarin is further evidence that it was not caused by coexisting vascular disease in patients receiving warfarin. Consequently, it is unlikely that the increase in peripheral arterial calcification is related to factors other than anticoagulation. Unfortunately, the number of patients with lower extremity x-rays performed both before and after initiation of warfarin was too small to determine the effect of warfarin on the incidence of arterial calcification.

The results are consistent with animal studies in which warfarin produced medial arterial calcification, probably by inhibition of the γ-glutamylation of matrix Gla protein, an inhibitor of vascular calcification produced by vascular smooth muscle.[8] Absence of this protein results in medial arterial calcification in both mice[11] and humans,[9,10] and warfarin produces arterial calcification in rat arteries both ex vivo and in vivo.[8,12] Although this is the likely explanation for the increase in peripheral arterial calcification associated with warfarin, an effect of anticoagulation in general cannot be ruled out. Unfortunately, the number of patients using other anticoagulants was insufficient to address this possibility.

Whether warfarin also promotes atherosclerotic calcification has not been addressed in animal studies. Increased calcification of coronary arteries, which is predominantly atherosclerotic, has been observed in some[13,15] but not all[14] clinical studies; however, medial calcification also occurs in these arteries and could explain the findings. Warfarin use was not associated with an atherosclerotic pattern of calcification in peripheral arteries, but an effect on atherosclerotic calcification cannot be ruled out because of the low prevalence.

The effect of warfarin on arterial calcification is likely to have clinical consequences because medial calcification is associated with poorer outcomes.[6,7] Furthermore, calcification of peripheral arteries predicts critical limb ischemia,[4] which may explain the failure of warfarin to improve outcomes in patients with peripheral arterial disease.[22] Warfarin use is also associated with calciphylaxis,[17] a serious complication of medial calcification in small superficial arteries. Moreover, an increase in cardiac valve calcification has also been noted in patients receiving warfarin.[15,23]

A limitation of this study is the inability to quantify the calcification, which reduces the sensitivity by not detecting increases in patients with preexisting calcification; therefore, it is likely that warfarin had a greater effect than indicated merely by prevalence data. Whether warfarin exacerbates existing calcification or magnifies the effect of other risk factors for medial arterial calcification is an important question that could have implications for the choice of anticoagulants. The prevalence data suggested some additivity of warfarin and age or diabetes, but, quantitatively, this could be much greater. Although not studied, advanced renal failure is another important risk factor for medial arterial calcification that could be exacerbated by warfarin. Future quantitative studies are needed to address the interaction of warfarin with other risk factors for medial arterial calcification.

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Disclosures

None.

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