Increased Vascular Calcification in Patients Receiving Warfarin

Ekamol Tantisattamo, Kum Hyun Han, W. Charles O’Neill

Objective—Matrix gla protein is a vitamin K–dependent inhibitor of medial arterial calcification whose synthesis and activity are blocked by warfarin. Warfarin induces arterial calcification in experimental models, but whether this occurs in humans is unclear. This was addressed by examining breast arterial calcification, which is exclusively medial and easily identified on mammograms.

Approach and Results—Screening mammograms from women with current, past, or future warfarin use were examined for the presence of arterial calcification and compared with mammograms obtained in untreated women matched for age and diabetes mellitus. Women with a serum creatinine ≥2.0 mg/dL or a history of end-stage renal disease were excluded. In 451 women with mammograms performed after ≥1 month of warfarin therapy, the prevalence of arterial calcification was 50% greater than in 451 untreated women (39.0% versus 25.9%; \( P \leq 0.0001 \)). However, in 159 mammograms performed before warfarin therapy, the prevalence of arterial calcification was not increased (26.4% versus 25.8%). The increased prevalence varied with duration of treatment, from 25.0% for <1 year to 74.4% for >5 years. In a multivariable logistic model, only age and duration of warfarin, but not the period of time after stopping warfarin, were significant determinants of arterial calcification in women with current or past warfarin use.

Conclusions—The prevalence of breast arterial calcification is increased in women with current or past warfarin use independent of other risk factors and conditions predating warfarin use. This effect appears to be cumulative and may be irreversible. (Arterioscler Thromb Vasc Biol. 2015;35:237-242. DOI: 10.1161/ATVBBAHA.114.304392.)

Key Words: anticoagulation ▪ matrix proteins ▪ vascular calcification

Arterial calcification frequently accompanies cardiovascular disease and portends poorer outcomes.1–3 Calcification occurs either within atherosclerotic plaques (neointimal calcification) or within the muscular layer unrelated to atheromatous disease (medial calcification). While neointimal calcification may merely be a marker of atherosclerotic burden, medial calcification seems to be directly pathogenic by decreasing vascular compliance.4,5 The medial form is associated with advanced age, diabetes mellitus, advanced chronic kidney disease, and rare genetic disorders.7

The pathophysiology of arterial calcification is complex and involves factors that either promote or inhibit the process. One key inhibitor is matrix gla protein (MGP), a vitamin K–dependent protein synthesized by vascular smooth muscle.8 Absence of MGP leads to extensive and fatal medial arterial calcification in rats both ex vivo and in vivo.8,9,10 Warfarin reduces γ-carboxylation of vascular MGP and induces medial arterial calcification in rats.8,10 Whether warfarin also promotes atherosclerotic calcification is unknown.

The role of MGP has led to concern that warfarin therapy may increase vascular calcification, but studies to date have been small or limited to specific populations and have yielded conflicting results.15–17 Furthermore, the imaging used does not distinguish between medial and atherosclerotic calcification, and the abundance of atherosclerotic calcification at the sites studied could obscure effects on medial calcification. The results were also not fully controlled for effects of underlying cardiovascular disease that is often present in patients receiving warfarin.

Calcification of breast arteries is exclusively medial,18,19 easily detected on mammograms, and correlates with arterial calcification in the extremities.20 Routine screening mammography therefore provides a unique opportunity to examine the effect of warfarin on medial arterial calcification in a large cohort.

Materials and Methods
Materials and Methods are available in the online-only Data Supplement.

Results
The computerized search yielded 16 555 women with screening mammograms, of whom 637 were identified as having received warfarin. Of these, the duration of warfarin was <1
month in 100, 38 had a history of end-stage renal disease, 28 had a serum creatinine ≥2.0, and 20 had no documented warfarin use. The characteristics of the remaining 451 women and the matched controls are shown in Table 1. The use of hydroxymethylglutaryl CoA reductase inhibitors (statins) was twice as prevalent in the warfarin group, probably reflecting the greater prevalence of underlying cardiovascular disease. There were significant, but quantitatively small differences in the serum concentrations of creatinine, calcium, low-density lipoprotein, high-density lipoprotein, and triglycerides that did not fit a particular pattern. In 90% of the patients, the indication for anticoagulation was either atrial fibrillation or deep venous thrombosis with or without pulmonary embolus. Mechanical heart valves (4.7%) and left ventricular thrombus (2.9%) accounted for most of the remainder. Slightly more than half of the patients were receiving warfarin at the time of the mammogram. The remaining 44% had discontinued the warfarin at a mean of 2.7 years before the mammogram with a range of 3 days to 8.5 years. Two separate courses of warfarin were prescribed in 11% of the patients, and the mean interval between treatments was 2.7 years with a maximum of 19 years.

Mammograms performed before warfarin therapy were available in 159 women, who were matched to a different set of controls. The characteristics of this group are shown in Table 2. This includes 98 women in the previous cohort who also had mammograms after starting warfarin but with different matched controls because of the differences in age. Serum concentrations of creatinine and phosphorus were slightly higher and high-density lipoprotein slightly lower in the warfarin group. There were no significant differences between the patients with mammograms performed after warfarin therapy (Table 1) and patients with mammograms performed before treatment (Table 2), except for a lower use of statins in the former.

Arterial calcification was identified as thin linear opacities along the edges of vessels, and mammograms were scored as positive only if calcification was present on both sides of ≥1 vessel (Figure 1, top). This calcification is easily distinguished from other forms of calcification (Figure 1, middle and bottom), and in the rare cases of uncertainty, the mammogram was scored as negative. In mammograms performed after initiation of warfarin therapy, the prevalence of arterial calcification was 50% greater than in the control mammograms.
However, the prevalence of arterial calcification in mammograms performed before warfarin therapy was not increased compared with matched controls (26.4% versus 25.8%) and did not differ from the control group for mammograms performed after warfarin therapy. These data are displayed graphically in Figure 2.

The effect of warfarin was dependent on the duration of therapy (Figure 3), with a nonsignificant 25% increase in the prevalence of arterial calcification after <1 year (mean duration, 0.48±0.02), a 67% increase between 1 and 5 years (mean duration, 2.7±0.1), and a 74% increase beyond 5 years (mean duration, 11.4±0.5). In the 20% of patients receiving warfarin for ≤6 months, the prevalence of arterial calcification was not increased (24% versus 27%). In patients who had discontinued warfarin before the mammogram, the prevalence of arterial calcification was still increased compared with the controls (28.8% versus 22.2%), although this was not statistically significant. However, many of these patients had received a short course of warfarin compared with the life-long therapy in most of the subjects receiving warfarin at the time of the mammogram.

To determine whether the greater use of a hydroxymethylglutaryl CoA reductase inhibitors in the warfarin-treated patients affected the results, subjects were analyzed separately based on the use of this medication. In the 125 warfarin-treated subjects using statins, the prevalence of breast arterial calcification (BAC) was 46.4% versus 32.0% in the non-warfarin controls matched for age and diabetes mellitus. In the 326

![Figure 1. Breast calcification. A. Dual linear calcifications indicative of arterial calcification. Insets, Enlargements of heavy (top) and light (bottom) calcifications. B. Linear calcifications representing calcification of ductal fluid. Inset, Enlargement of calcifications. C. Nonlinear, nonvascular calcifications. Inset, Enlargement of a calcification.](http://atvb.ahajournals.org/)

| Table 2. Characteristics of Women With Mammograms Performed Before Initiation of Warfarin Therapy and Untreated Controls |
|-------------------------------------------------|------------------|------------------|-----|
| n                                               | Warfarin         | Untreated        | PValue |
| Age, y                                          | 66.1±1.0 (40–88) | 66.4±1.1 (40–88) | 1.00 |
| Diabetes mellitus, %                            | 32.7             | 32.7             | 1.00 |
| Race, %                                         | White 61.3       | Black 31.3       | 0.85 |
|                                                | Other 2.0        | Unknown 5.3      |      |
| Serum values, mg/dL                             |                  |                  |      |
| Creatinine                                      | 0.92±0.02 (0.33–1.85) | 0.83±0.02 (0.30–1.62) | 0.002 |
| Calcium                                         | 9.27±0.04 (7.1–10.7) | 9.31±0.04 (6.9–10.4) | 0.46 |
| Phosphorus                                      | 3.62±0.10 (1.7–7.4; 84) | 3.33±0.08 (1.9–4.6; 55) | 0.04 |
| LDL cholesterol                                 | 103±3 (27–322; 136) | 108±3 (40–195; 134) | 0.24 |
| HDL cholesterol                                 | 56±1 (15–100; 137) | 59±1 (24–114; 135) | 0.035 |
| Triglycerides                                   | 121±6 (28–463; 139) | 113±5 (33–485; 135) | 0.31 |
| Statin use, %                                    | 10.1             | 9.4              | 1.00 |
| Indication*, %                                   | AF 43.4          | DVT/PE 47.2      |      |
| Years before warfarin                           | 0.80±0.06 (0.01–6.2) |                  |      |

Range and number of subjects if less than total are given in parentheses.
AF indicates atrial fibrillation; DVT, deep venous thrombosis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PE: pulmonary embolus.

*Some patients had >1 indication.
warfarin-treated subjects not using statins, the prevalence of BAC was 36.2% compared with 23.6% in the non-warfarin controls matched for age and diabetes mellitus status. The percent increase in BAC associated with warfarin was similar in each group (45% versus 53%). Subjects using statins were significantly older than those not using statins (73 versus 66).

Mammograms performed before and after starting warfarin were available in 98 women, but the duration of warfarin therapy was only 1.05±0.12 years. The prevalence of BAC increased from 28% to 30% because of the development of new BAC in 2 patients, who were treated with warfarin for 4.2 and 5.4 years. Among the 14 patients receiving warfarin for ≥2 years, this represented an incidence of 14%. Based on the time between mammograms in this group (4.6±0.3 years) and the linear relationship between age and prevalence of BAC after age 50 in all the non-warfarin controls (1.5% per year; data not shown), this is twice the expected incidence. However, the data lack statistical significance because of the small numbers.

Figure 4 shows the prevalence of BAC in different groups based on age or diabetes mellitus. Calcification was much less common in women aged <60 years, but the increase associated with warfarin was much greater (400%) compared with women aged ≥60 years (41%). The increase was also greater in non-diabetics compared to diabetics (60% versus 34%). In a logistic model that included age, diabetes mellitus, warfarin duration, and warfarin-free duration, only age and the duration of warfarin therapy were significant determinants of BAC (Table 3). Race, serum creatinine, serum calcium, and statin use were not determinants in either the warfarin subjects or the untreated controls (not shown). Serum lipids showed no significant effect when added to the model. In the simplest model, age (odds ratio, 1.08; P<0.00001), warfarin duration (odds ratio, 1.05; P=0.0057), and atrial fibrillation (odds ratio, 1.73; P=0.023) were significant determinants of BAC.

Radiographs of a lower extremity (knee or below) performed within 2 years of the mammogram were available in 248 of the women. Among women with BAC, the prevalence of arterial calcification visible on lower extremity radiographs was 40% compared with only 18% in those without BAC (P=0.0006). The quantity of radiographs was insufficient to analyze the effect of warfarin.

**Discussion**

Warfarin use was associated with a 50% greater prevalence of BAC in women undergoing screening mammography. The effect was particularly large in women aged <60 years, who exhibited a 5-fold increase. This association of warfarin with medial arterial calcification is consistent with inhibition of MGP, a vitamin K–dependent inhibitor of vascular calcification that is synthesized by vascular smooth muscle and whose action is blocked by warfarin, resulting in arterial calcification ex vivo and in vivo in animals. The effect of warfarin could not be explained by other known risk factors for medial arterial calcification because the control subjects were matched for age and diabetes mellitus, and subjects with serum creatinine ≥2 or a history of end-stage renal disease were excluded. Although serum creatinine was significantly greater in the warfarin subjects, likely reflecting underlying cardiovascular disease, the difference was small and unlikely to have influenced the results. Serum creatinine was not a significant determinant of arterial calcification in either the warfarin group or the control group, and our previous studies have shown only a
small, nonsignificant increase in BAC in stage 3 chronic kidney disease.21

Because cardiovascular disease is common in patients requiring anticoagulation, it was important to establish that underlying disease was not responsible for the increased prevalence of BAC. In particular, there is the real possibility that vascular calcification or other ectopic calcification could lead to atrial fibrillation and subsequent warfarin therapy. Although there were some significant differences in serum lipid values between the warfarin and non-warfarin patients, they were small and did not fit an atherosclerotic risk profile. It is possible that the greater use of statins in this group could have influenced these values. Importantly, none of the differences in lipid values showed an effect on BAC in regression models. Statin use was substantially greater in the warfarin group, most likely related to an increased prevalence of cardiovascular disease. Although BAC was more prevalent in those using statins, probably explained by the greater age, statin use did not correlate with calcification.

Further evidence that the increased calcification associated with warfarin was not due to underlying cardiovascular disease came from examining mammograms performed before the start of warfarin therapy. Because the vast majority of these were performed within a year before warfarin therapy, they should reflect the underlying state of vascular calcification. The prevalence of arterial calcification in these mammograms was almost identical to that in mammograms from matched controls and to the prevalence in the controls for the mammograms performed after initiation of warfarin. This demonstrates that the increased prevalence associated with warfarin use is not because of patient characteristics that preceded warfarin use and is directly related to the anticoagulation. Data on incidence of BAC was available in only a small number of women, most of whom received warfarin for only brief periods. Although not statistically significant, the results show a 2-fold increase in incidence after 2 years of warfarin, consistent with the increase in prevalence found for the corresponding duration of warfarin therapy.

The increased prevalence of BAC varied directly with the duration of warfarin therapy, providing further evidence that it is due to the warfarin. Although not significant, the small increase in prevalence seen with durations under 1 year suggests that warfarin might influence calcification within that time frame. Beyond 5 years, the further increase in prevalence was small, despite durations up to 39 years, suggesting that some patients are resistant to the effect of warfarin. Because only prevalence was determined, it is possible that the extent of calcification continued to increase. There was no increase in the prevalence of arterial calcification in patients receiving warfarin for ≤6 months. This suggests that standard therapy for uncomplicated deep venous thrombosis does not promote vascular calcification.

The increased prevalence of BAC varied directly with the warfarin-free duration, providing further evidence that the increased arterial calcification associated with warfarin persists after therapy and raising the possibility that it is irreversible.

Other studies have suggested an effect of warfarin on calcification of coronary13,15 and femoral16 arteries, but no significant effect on coronary artery calcification was found in another study.14 Because these sites exhibit both atherosclerotic and medial calcification, an effect limited to medial calcification may be difficult to discern. An increase in cardiac valve calcification has also been noted in patients receiving warfarin.15,22 The cohorts in most of these prior studies were small, and although traditional risk factors for underlying cardiovascular disease may have been controlled, additional factors may apply specifically to vascular calcification. Furthermore, because only patients currently receiving warfarin were studied, the effect of past warfarin treatment and potential reversibility were not addressed.

Previous data suggest that BAC is a marker of calcification in other arterial beds. It predicts cardiovascular disease in the general population2,23 and correlates with calcification in peripheral arteries in patients with end-stage renal disease.20 The correlation with calcification of peripheral arteries in the current data indicates that this is also true in the general population. We have also shown that BAC is strongly associated with critical limb ischemia in end-stage renal disease patients,24 which is consistent with the finding that calcification of peripheral arteries predicts critical limb ischemia in patients with peripheral arterial disease.1 A significant increase in medial calcification may therefore explain the failure of warfarin to improve outcomes in peripheral arterial disease.15 Furthermore, warfarin use is associated with calciphylaxis,17 a particularly serious complication of vascular calcification. Although this suggests that the increased BAC with warfarin likely reflects generalized medial calcification and could have clinical consequences, further studies will be required to confirm this.

The mechanism by which warfarin increases medial arterial calcification is presumably related to impaired gamma carboxylation of MGP, an important inhibitor of vascular calcification that is synthesized by vascular smooth muscle.2 Absence of this protein in mice results in severe medial arterial calcification,9 and calcification is also seen when it is absent in humans.10,11 The carboxylation of MGP, which is probably required for activity,26,27 is blocked by warfarin,4 resulting in arterial calcification in vivo12 and ex vivo.5 MGP is a highly insoluble protein that adheres to the vascular matrix. Thus, persistence after synthesis may explain why short courses of warfarin (<6 months) were not associated with increased BAC.

Table 3. Multivariable Logistic Regression Model for Breast Arterial Calcification in Patients Receiving Warfarin

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.08</td>
<td>1.05–1.10</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.05</td>
<td>0.64–1.71</td>
<td>0.87</td>
</tr>
<tr>
<td>Race (white vs nonwhite)</td>
<td>1.40</td>
<td>0.85–2.32</td>
<td>0.19</td>
</tr>
<tr>
<td>Indication (AF vs non-AF)</td>
<td>1.63</td>
<td>0.99–2.66</td>
<td>0.053</td>
</tr>
<tr>
<td>Warfarin duration, y</td>
<td>1.06</td>
<td>1.02–1.10</td>
<td>0.0047</td>
</tr>
<tr>
<td>Warfarin-free duration, y</td>
<td>1.02</td>
<td>0.92–1.14</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>1.89</td>
<td>0.77–4.64</td>
<td>0.16</td>
</tr>
<tr>
<td>Serum calcium, mg/dL</td>
<td>1.06</td>
<td>0.71–1.60</td>
<td>0.78</td>
</tr>
<tr>
<td>Statin use</td>
<td>1.02</td>
<td>0.63–1.67</td>
<td>0.93</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation.
Although the results provide strong evidence that warfarin promotes vascular calcification, prospective studies will be required to confirm this. The results of this study are necessarily restricted to women, and although it is likely that the findings can be extrapolated to other arterial beds, this will also need to be confirmed. The inability to quantify BAC limits the sensitivity of the analyses, but it does not diminish the findings. Most likely, the effect of warfarin is underestimated because worsening of existing calcifications would not be recognized in this nonquantitative analysis. An effect of anticoagulation on vascular calcification independent of the mechanism of warfarin action cannot be ruled out by the findings of this study, although there is no precedent for this. Unfortunately, the paucity of warfarin-naïve patients treated with other anticoagulants prevented such an analysis, and future studies will be required to address this issue.

Disclosures

None.

References


Significance

The association of warfarin with breast arterial calcification strongly suggests that it promotes medial arterial calcification. This is critically important because of the widespread use of this anticoagulant and the fact that many of the patients treated with warfarin either already have underlying cardiovascular disease or are at risk for it.
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Materials and Methods

Patients. A computerized search was performed of all subjects undergoing screening mammography in 2011 through 2013 at Emory Healthcare. Additional information obtained included date of birth, gender, diabetes status, the presence or absence of warfarin listed as a medication, and serum levels of creatinine, calcium, and phosphorus. Men and subjects with advanced chronic kidney disease (serum creatinine $\geq 2.0$ mg/dl) were excluded. The electronic medical records of all remaining subjects with warfarin identified as a medication were reviewed to determine the indication for warfarin, dates of initiation and cessation, and to identify mammograms performed before and after initiation of warfarin. Women who received warfarin for less than one month or had a history of end-stage renal disease were excluded. Each subject was randomly matched to a subject without a history of warfarin use based on exact age (rounded to the nearest integer) at the time of mammography and the presence or absence of diabetes. This was accomplished by sorting non-warfarin mammography patients by age and diabetes status and selection by last name in alphabetic order. The protocol was approved by the Institutional Review Board of Emory University.

Analysis. Duration of warfarin therapy was determined by subtracting the start date from the stop date. In patients with two treatment periods, these were added. When precise dates could not be determined, a minimum and maximum duration was established based on the available information. The warfarin-free duration was calculated by subtracting the duration of warfarin therapy from the interval between the start date and the mammogram. When there were two periods of warfarin therapy and the first was less than one year and the second was more than one year, the interval from the second stop date to the mammogram was used instead. Digital mammograms were reviewed visually by one of two investigators who were unaware of the clinical information, including the use of warfarin. Arterial calcification was identified as thin linear opacities along the edges of vessels, and mammograms were scored as positive only if calcification was present on both sides of at least one vessel. In the rare cases of uncertainty, the mammogram was scored as negative. In 60 mammograms reviewed by both investigators, the agreement was over 93%. The few discrepancies were resolved by joint review of the mammograms.

Statistical analysis. Quantitative variables were analyzed by two-tailed t-testing and categorical variables by chi-squared testing or Fisher’s exact test for $2 \times 2$ analyses. Multivariable logistic regression was performed using Statpages version 05.07.20 (http://statpages.org/logistic.html). Backward regression was used to obtain the simplest model.