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Vitamin K antagonists and cardiovascular calcification: A systematic review and meta-analysis

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Background: Many patients treated with Vitamin K antagonists (VKA) for anticoagulation have concomitant vascular or valvular calcification. This meta-analysis aimed to evaluate a hypothesis that vascular and valvular calcification is a side-effect of VKA treatment.

Methods: We conducted a systematic literature search to identify studies that reported vascular or valvular calcification in patients treated with VKA. The associations between VKA use and calcification were analyzed with random-effects inverse variance models and reported as odds ratios (OR) and 95% confidence intervals (95% CI). In addition, univariate meta-regression analyses were utilized to identify any effect moderators.

Results: Thirty-five studies were included (45,757 patients; 6,251 VKA users). The median follow-up was 2.3 years [interquartile range (IQR) of 1.2–4.0]; age 66.2 ± 3.6 years (mean ± SD); the majority of participants were males [77% (IQR: 72–95%)]. VKA use was associated with an increased OR for coronary artery calcification [1.21 (1.08, 1.36), $p = 0.001$], moderated by the duration of treatment [meta-regression coefficient B of 0.08 (0.03, 0.13), $p = 0.0005$]. Extra-coronary calcification affecting the aorta, carotid artery, breast artery, and arteries of lower extremities, was also increased in VKA treated patients [1.86 (1.43, 2.42), $p < 0.00001$] and moderated by the author-reported statistical adjustments of the effect estimates [B: −0.63 (−1.19, −0.08), $p = 0.016$]. The effect of VKA on the aortic valve calcification was significant [3.07 (1.90, 4.96), $p < 0.00001$]; however, these studies suffered from a high risk of publication bias.

Conclusion: Vascular and valvular calcification are potential side effects of VKA. The clinical significance of these side effects on cardiovascular outcomes deserves further investigation.

KEYWORDS

cardiovascular calcifications, atherosclerosis, coronary artery disease, breast arterial calcifications (BAC), peripheral arterial disease (PAD), aortic calcification index, carotid atheroma, calcific aortic valve disease (CAVD)

Introduction

It is well-recognized that vascular calcification is an independent predictor of cardiovascular disease (CVD) and mortality (1). Studies have shown that calcification correlates with clinically-significant coronary artery disease (CAD) (2–4), acute cardiac and cerebrovascular events (5, 6), arterial stiffness and hypertension (7), and aortic valve disease (8). CVD is the leading cause of death, accounting for over 30% of mortality worldwide. Coronary artery calcium scoring has emerged as a non-invasive imaging platform for atherosclerotic CVD risk stratification and guiding lipid-lowering therapies for primary prevention (9, 10).

Warfarin, a vitamin K antagonist (VKA), was introduced into clinical practice as an anticoagulant in the 1950 s (11). Over the years, warfarin and other VKAs have been approved for the prophylaxis of thrombotic events in recurrent venous thrombosis, atrial fibrillation, valvular heart disease, and valve replacement (12). Although the use of VKA has declined in the past few years due to the introduction of safer non-vitamin K oral anticoagulants [NOAC or DOAC (direct oral anticoagulants)], VKAs remain widely prescribed and is the only guideline-recommended therapy for patients with prosthetic valves (13–16). Moreover, older patients and patients with comorbidities are more likely to receive warfarin for anticoagulation (17).

VKA inhibits coagulation factors II, VII, IX, X, and several other proteins by suppressing vitamin K-dependent post-translational gamma-carboxylation required for their function (18). Calcification is suppressed under normal physiologic conditions by several endogenous inhibitors, including matrix Gla protein (MGP), pyrophosphate, and plasma fetuin-A (19). MGP belongs to the same group of gamma-carboxylated proteins as coagulation factors and requires gamma-carboxylation for its inhibitory activity (18). Long-term use of VKA is associated with increased vascular calcification, presumably due to the reduction of vitamin K-dependent gamma-carboxylation of MGP (20, 21).

The role of VKA in vascular calcification is still poorly recognized, and the clinical significance is undefined (22). Here, we present the first meta-analysis of clinical studies on this topic. We aimed to provide objective evidence for the association between VKA use and cardiovascular calcification.

Methods

Search strategy

All clinical studies except case studies and case series were considered, and the inclusion was not limited to a specific indication for VKA use. Primary outcomes were coronary artery calcification, extra-coronary calcification (abdominal or thoracic aorta, carotid arteries, breast arteries, and arteries of the extremities), and valvular calcification. The core systematic literature search was conducted in PubMed with a stepwise keyword search strategy (Supplementary Table 1) up until March 29, 2022. The search results were filtered using pubmed filters to exclude reviews, case reports, guidelines, and study protocols. The reference lists of the relevant articles and "similar articles" suggested by pubmed were also considered. Other databases, including CINAHL, cochrane register of studies, and google scholar, were searched for additional references. Abstracts were screened for the inclusion criteria: (1) VKA treatment and (2) at least one vascular or valvular calcification outcome, e.g., calcium score or index, calcified plaque volume, presence/absence of calcification, calcification severity grade, or an annual rate of progression. Two investigators screened the abstracts, and any disagreements were resolved by finding a consensus.

Data extraction and management

Data were selected based on a full-text assessment. The extracted data included a study identifier, country, study design, sample size, mean or median age, percent of males, VKA treatment or exposure, duration of VKA therapy, calcification outcome(s), methods of assessment of calcification, effect size estimates, and a brief description of statistical models used for the effect estimates. The effect sizes were extracted as incidence, prevalence, odds ratios (OR), mean change from baseline, regression coefficients, ratios of expected counts (REC), and F statistics. The coronary outcomes were coronary artery calcium (CAC) score, measured *via* computed tomography (CT), calcified plaque volume determined by coronary CT angiography (CCTA), and CAC index obtained *via* intravascular ultrasound (IVUS). Extra-coronary outcomes were the presence or absence of calcification, severity grade, calcification score, or an annual rate of progression (detected by CT, X-ray, mammography, or histopathology). Lastly, the aortic valve calcification outcomes included the presence or absence of calcification on transthoracic ultrasound (US), the number of affected aortic valve leaflets, CT calcification score, or positive findings on histopathology.

Risk of bias assessment

The risks of bias were assessed using the Revised Cochrane risk of bias tool (RoB 2) for randomized trials (downloaded February 9, 2022, from https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2) (23) or the Newcastle—Ottawa quality scale (NOS) (24) for observations studies.

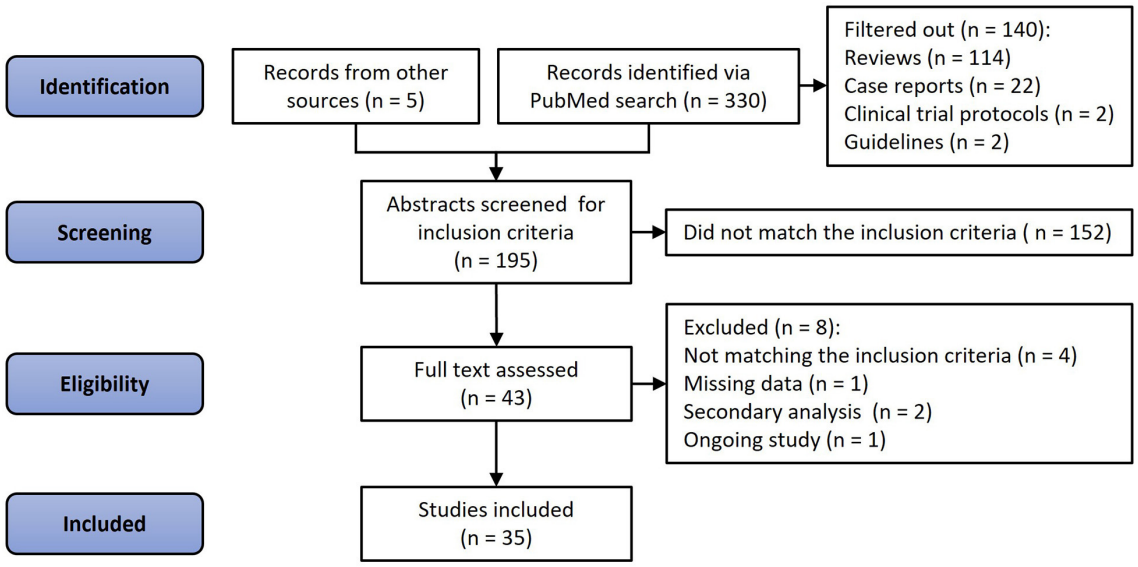

FIGURE 1
Flow diagram of the literature selection.

Statistical analysis

Data were analyzed using the Review Manager computer program, Version 5.4 (RevMan5, the Cochrane Collaboration, 2020). Effect sizes were expressed as OR and standard errors (SE) using the RevMan5 effect size calculator or an online effect size calculator tool [Practical Meta-Analysis Effect Size Calculator (25)]. Inverse-variance random-effects models were used for data synthesis. Studies were grouped according to the site of calcification, coronary, extra-coronary, and valvular. The combined estimates were calculated as OR and 95% confidence intervals (95% CI) for the presence of vascular or valvular calcification in VKA-treated patients compared with other patients (non-VKA), which included patients treated with non-VKA anticoagulants and those who had no indications for anticoagulation and were not treated with any anticoagulants. The statistical heterogeneity was evaluated using the I^2 test calculated in RevMan5. The risk for publication bias was assessed by an Egger regression and Begg & Mazumdar rank correlation tests, using the Meta-Essentials tool (downloaded on February 9, 2022, from https://www.erim.eur.nl/research-support/meta-essentials) (26). The Meta-Essentials tool was also used for the univariate meta-regression analyses considering the year of publication, geographic region (continent), study design, sample size, patient characteristics, median age, a ratio of participants by sex, duration of VKA treatment, calcium imaging modality, and whether or not the effect estimates were adjusted for confounders. Sub-group analyses were conducted according to each significant modifier detected by meta-regression. Furthermore, the sensitivity analysis was performed by excluding one study at a time from the corresponding meta-analysis. The significance was accepted at $p < 0.05$.

Results

Search results and characteristics of the included studies

A total of 330 articles were identified *via* PubMed search, and five articles were retrieved from other sources. Of these 335 papers, 114 were reviews and editorials, 22 case studies, two study protocols, and two clinical guidelines. Another 152 were deemed irrelevant by consensus between two investigators (NDK and OVS) if articles did not pertain to human subjects, lacked VKA treatment or effect estimates, or had no standardized method of detecting and quantifying calcification. After a full-text review of the remaining 43 articles, an additional seven were excluded due to missing data ($n = 1$), not matching the inclusion criteria ($n = 4$), being a secondary analysis of an already included study ($n = 2$), or an ongoing study ($n = 1$, Figure 1). Thus, 35 studies and 45,757 participants were included in the analysis, and 6,251 were treated with VKA (27–61). Of these, three studies were randomized trials (4 independent analysis cohorts, 333 patients, 169 VKA users) (29, 32, 39), and 32 observational studies (38 cohorts; 45,424 participants; 6,082 VKA users) (27, 28, 30, 31, 33–38, 40–61). Thirteen studies investigated the effects of VKA on coronary artery calcification (15 cohorts, 23,768 participants, 2,625 received VKA) (27, 28, 30, 31, 33–38). Sixteen studies that

reported extra-coronary (any artery but coronary) calcification included 18 cohorts, 4,740 participants, and 1,595 patients treated with VKA (40–54). Finally, nine studies investigated the effects of VKA treatment on aortic valve calcification (9 cohorts; 17,161 participants; 1,987 VKA-treated patients) (31, 49, 55–61).

Characteristics of the included studies are shown in Table 1. Studies were published between 2005 and 2022. Twenty studies were conducted in Europe (29–31, 34–36, 38, 41–43, 46–51, 53, 55, 57–59), thirteen in North America (27, 28, 32, 33, 37, 39, 40, 44, 45, 52, 56, 58, 60), and two in Asia (54, 61). As stated above, we identified three randomized trials (29, 32, 39), one meta-analysis of patient-level data from eight randomized trials (27), twenty one retrospective cohort studies (30, 31, 34–38, 40–42, 45, 48–52, 54, 55, 57, 59, 60), one prospective cohort (61), and nine cross-sectional studies (28, 33, 43, 44, 46, 47, 53, 56, 58). The sample size ranged from 37 to 17,254 participants. The median sample size was 207, interquartile range (IQR) of 108 to 387 patients. The age of participants was 66.2 ± 3.6 years (weighted mean ± SD); 77% (IQR 72–95%) of participants were males. The weighted median duration of VKA treatment in 33 groups of prospectively or retrospectively followed patients was 2.3 years (IQR 1.2–4.0) (27, 29–32, 34–42, 45, 48–52, 54, 55, 57, 59–61). Nine study cohorts were cross-sectional with an unspecified duration of treatment (28, 33, 43, 44, 46, 47, 53, 56, 58). The medical history of the patients included coronary artery disease (CAD) (27, 32, 39), chronic kidney disease CKD (including patients with ESRD) (29, 40–43, 46, 47, 53, 55), calcific aortic valve disease or aortic stenosis (CAVD/AS) (28, 56, 57, 60), atrial fibrillation (AF/NVAF) (28, 29, 32, 34, 38, 39, 49, 54, 55, 58, 61), metallic prosthetic valves (36), lower limb amputation (44), carotid atherectomy (48), non-traumatic cerebral hemorrhage (50) or underwent cardiac CT (33, 35) or mammography (40, 52) tests for diagnostic or screening purposes. Two studies included the general population from health registries (30, 59). For the remaining two studies, patients' medical history was not specified (37, 51).

Quality assessment

Among the three randomized trials, one "per-protocol" trial (29) had a high risk of bias due to missing outcome data, whereas two other "intention-to-treat" studies had concerns regarding missing outcome data (32) and selective reporting (32, 39) (Supplementary Table 2). Observational studies were assessed for the risk of bias on a 9-point Newcastle-Ottawa quality scale. The majority of studies were of at least moderate quality (27, 28, 30, 31, 34–38, 40–43, 45, 46, 48–59, 61) [median score 7 (IQR 5-9)], except five studies in which the risk of bias was considered low to moderate on the Newcastle-Ottawa quality scale (31, 33, 44, 47, 53) (Supplementary Table 3).

Effects of VKA use on the coronary, extra-coronary, and aortic valve calcification

VKA use was associated with increased vascular and valvular calcification. The OR for the coronary artery calcification in VKA-treated patients was 1.21 (95% CI 1.08, 1.36), $p = 0.001$ compared to patients not treated with VKA (Figure 2A). VKA use was also associated with extra-coronary vascular calcification in the aorta, carotid arteries, breast arteries, and arteries of lower extremities [OR 1.86 (1.43, 2.42), $p < 0.00001$, Figure 2B]. Furthermore, we found an association between VKA use and aortic valve calcification [OR 3.07 (1.90, 4.96), $p < 0.00001$, Figure 2C]. Between-study heterogeneity was significant at I^2 of 69, 78, and 90% in the coronary ($n = 15$), extra-coronary vascular ($n = 18$), and aortic valve studies ($n = 9$), respectively.

Publication bias

We constructed funnel plots of the effect sizes against their standard errors [log (OR), SE] and examined them using the Egger funnel plot asymmetry test and Begg & Mazumdar rank correlation test to evaluate the risks of publication bias. No significant risks of publication bias were found among the coronary artery calcification studies (Egger $p = 0.097$, Begg & Mazumdar $p = 0.441$) or the extra-coronary calcification studies (Egger $p = 0.307$, Begg & Mazumdar $p = 0.172$, Figures 3A,B). However, the risk of publication bias was significant in the studies of aortic valve calcification (Egger $p = 0.0037$, Begg & Mazumdar $p = 0.0030$, Figure 3C).

Meta-regression and subgroup analysis

We performed meta-regression analyses to identify potential effect modifiers. We calculated univariate random-effects regressions to assess the effects of the year of publication, geographic region (continent), study design, sample size, patient characteristics, median age, ratio of participants by sex, duration of VKA treatment, calcium imaging modality, and whether or not the effect estimates were reported adjusted for the confounders (Table 1). The estimates of coronary artery calcification were influenced by three explanatory variables, the year of publication [B regression coefficient of −0.04 (95% CI: −0.08, 0.00), $p = 0.035$]; the gender ratio expressed as a percent of male participants [$B = -0.01$ (−0.03, 0.00), $p = 0.039$]; and the duration of VKA treatment [$B = 0.08$ (0.03, 0.13), $p = 0.0005$, Table 2; Figure 4A]. The effects on the extra-coronary vascular calcification were modified by whether or not

TABLE 1 Characteristics of the included studies.

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Coronary artery calcification												
Andrews et al. (27)	United States	Patient-level meta-analysis	171/4129	CAD	62/58	80/72	Warfarin/no exposure	18–24 mo	CAC index	IVUS	OR for an increase of calcium index	Multivariable regression adjusted for age, BMI, rank-transformed baseline calcium index, baseline percent atheroma volume (PAV), change in PAV, and last observation of creatinine, clinical trial, treatment, study time duration
Chaikriangkrai et al. (28)	United States	Cross-sectional	154/706	AF, no CAD	63 (all)	65 (all)	Warfarin/no exposure	n/a	CAC score >0	CT	OR for calcification	Univariable regression; unadjusted
De Vriese et al. (29)	Belgium	Prospective randomized	44/46	ESRD, NVAF	80/80	57/76	VKA/rivaroxaban	18 mo	CAC	CT	Change from baseline	Kruskal–Wallis test
Hasific et al. (30)	Denmark	Retrospective cohort	1748/15506	no CAD	67 (all)	75 (all)	Warfarin time-updated exposure	14 mo	CAC score	CT	OR for higher CAC category per year	Multivariable regression adjusted for age, gender, smoking, BMI, diabetes, hypertension, hypercholesterolemia, family history of CVD, eGFR, NOAC treatment duration
Koos et al. (31)	Germany	Retrospective cohort	23/63	CAVD	71 (all)	62 (all)	VKA/no exposure	7.3 yrs	CAC score	CT	CAC score mean, SD	Student's *t*-test
Lee et al. (32)	United States	Prospective randomized	51/46	NVAF, CAC >10	60/63	77/65	Warfarin/rivaroxaban	12 mo	Calcified plaque volume	CCTA	Regression coefficient	Multivariable regression adjusted for age, gender, BMI, hypertension, diabetes, dyslipidemia, baseline LDL cholesterol, current smoking, family history, statin use, and baseline normalized plaque volume
Palaniswamy et al. (33)	United States	Cross-sectional	28/205	cardiac CT testing	67/63	71/56	Warfarin/no exposure	n/a	CAC score	CT	Incidence of CAC score >100	Prevalence

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TABLE 1 (Continued)

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Plank et al. (34)	Austria	Retrospective cohort	101/101	NVAF, no CAD	60/60	73/70	VKA/no exposure	20 mo	CAC score	CT	CAC score mean, SD	ANOVA. Cohorts were matched according to the propensity score for age, male sex, hypertension, hyperlipidemia, diabetes, family history of premature cardiac death, smoking, BMI
Schurgers et al. (35)	Netherlands	Retrospective cohort	44/44	diagnostic cardiac CT	58/58	66/66	VKA/no exposure	2.5 mo	CAC score	CT	CAC score mean, SD	ANOVA. Patients were matched according to the Framingham risk score (FRS)
Schurgers et al. (35)	Netherlands	Retrospective cohort	44/44	diagnostic cardiac CT	60/60	61/61	VKA/no exposure	19 mo	CAC score	CT	CAC score mean, SD	ANOVA. Patients were matched according to FRS
Schurgers et al. (35)	Netherlands	Retrospective cohort	45/45	diagnostic cardiac CT	64/59	78/78	VKA/no exposure	7.2 yrs	CAC score	CT	CAC score mean, SD	ANOVA. Patients were matched according to FRS
Unlu et al. (36)	Turkey	Retrospective cohort	43/65	metallic prosthetic valve	57/54	35/39	VKA/no exposure	15 yrs	CAC score	CT	CAC score mean, SD	Mann–Whitney *U*-test. Patients were matched according to atherosclerotic risk factors
Villines et al. (37)	United States	Retrospective cohort	28/31	no CAD	73/64	68/68	5.9 yrs/1 mo warfarin	5.9 yrs	CAC score	CT	CAC score median, IQR, Min, Max	ANOVA
Weijs et al. (38)	Netherlands	Retrospective cohort	71/86	AF, no CAD	58/56	79/62	VKA time-updated exposure	3.8 yrs	CAC score	CT	OR for an increase of CAC category/ year	Multivariable regression adjusted for age, left atrium diameter, use of statins and ACE inhibitors
Win et al. (39)	United States	Prospective randomized	30/26	NVAF	55/60	80/58	Warfarin/apixaban	12 mo	Calcified plaque volume	CCTA	Regression coefficient	Multivariable regression adjusted for age, gender, BMI, hypertension, diabetes, dyslipidemia, smoking, family history, prior percutaneous coronary intervention, coronary bypass surgery, aspirin use, statin use, and baseline plaque volume

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TABLE 1 (Continued)

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Extra-coronary arterial calcification												
Alappan et al. (40)	United States	Retrospective cohort	35/57	BAC, no CKD	76/74	0/0	Warfarin/no exposure	8.3 yrs	BAC rate (mm/yr)	Mammogram	Log-modulus BAC rate per year	Kruskal-Wallis test
Alappan et al. (40)	United States	Retrospective cohort	29/95	BAC, CKD	79/76	0/0	Warfarin/no exposure	4.1 yrs	BAC rate (mm/yr)	Mammogram	Log-modulus BAC rate per year	Kruskal-Wallis test
Alappan et al. (40)	United States	Retrospective cohort	14/36	BAC, ESRD	61/60	0/0	Warfarin/no exposure	3.9 yrs	BAC rate (mm/yr)	Mammogram	Log-modulus BAC rate per year	Kruskal-Wallis test
De Vriese et al. (29)	Belgium	Prospective randomized	44/46	ESRD, NVAF	80/80	57/76	VKA/rivaroxaban	18 mo	TA calcification score	CT	Change from baseline	Kruskal–Wallis test
Eren-Sadioglu et al. (41)	Turkey	Retrospective cohort	32/44	ESRD	68/65	56/50	Warfarin/no exposure	5.5 yrs	AA Kauppila score (62) >6	X-ray	OR of Kauppila score of >6	Multivariable regression adjusted for age, PTH, serum calcium, serum phosphorus; dialysis vintage; patients were matched according to age, sex, comorbidities, dialysis vintage, and dialysis center.
Fusaro et al. (42)	Italy	Retrospective cohort	46/341	ESRD	70/63	59/63	Warfarin/no exposure	4.2 yrs	AA calcification grade	X-ray	OR of calcification	Multivariable regression adjusted for age, angina, AF, PPI use, total BGP
Fusaro et al. (43)	Italy	Cross-sectional	101/213	ESRD	72 (all)	63 (all)	VKA/no exposure	n/a	AA calcification score (63)	X-ray	OR of severe calcification	Multivariable regression adjusted for age, sex, dialysis vintage, HF, PAD, stroke, plasma vitamin D, vertebral fractures
Han et al. (44)	United States	Cross-sectional	29/79	Lower limb amputation	64 (all)	51 (all)	Warfarin/no exposure	n/a	Lower extremity calcification	Histopathology	Incidence of calcification	Fisher's exact test

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TABLE 1 (Continued)

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Han and O'Neill. (45)	United States	Retrospective cohort	430/430	no ESRD	67/67	41/41	Warfarin time-updated exposure	9.8 mo	Lower extremity calcification	X-ray	OR of calcification per log days of warfarin	Multivariable regression adjusted for age, diabetes status, sex, duration of warfarin use, serum creatinine, radiograph type
Jean et al. (46)	France	Cross-sectional	32/129	ESRD	67 (all)	55 (all)	Warfarin/no exposure	n/a	AA, IA, FA calcification score	X-ray	OR of calcification score 2 or 3	Multivariable regression adjusted for age, sex, FGF-23, diabetes, smoking, peripheral vascular disease, CAD, albumin, OPG, CRP
Jean et al. (47)	France	Cross-sectional	44/163	ESRD	70 (all)	57 (all)	Warfarin/no exposure	n/a	AA Kauppila score (86) >7	X-ray	OR of Kauppila score >7	Prevalence
Nuotio et al. (48)	Finland	Retrospective cohort	82/418	carotid atherectomy	75/69	73/67	Warfarin/no exposure	19 mo	CCA calcification, Y/N	CT	OR of calcification	Multivariable regression adjusted for age, sex, and smoking
Peeters et al. (49)	Netherlands	Retrospective cohort	71/86	AF, no prior CAD	58/56	80/62	VKA/no exposure	2.3 yrs	AscA calcification score	CT	OR of calcification	Multivariate regression adjusted for the propensity score for age, sex, BMI, systolic BP, family history of MI, hyperlipidemia, blood glucose, LA dimension
Peeters et al. (50)	Netherlands	Retrospective cohort	77/299	Non-traumatic cerebral hemorrhage	78/70	54/53	VKA/no exposure	2.9 yrs	ICA calcification score	CT	OR of high calcification score	Multivariable regression adjusted for age, sex, hypertension, hypercholesterolemia, and diabetes
Rennenberg et al. (51)	Netherlands	Retrospective cohort	19/18	Risk of thrombosis, no prior CAD	48/56	79/50	Coumarin/no exposure	13 yrs	FA calcification, Y/N	X-ray	Regression coefficient	Multivariable regression adjusted for age, smoking, BMI, and triglycerides
Tantisattamo et al. (52)	United States	Retrospective cohort	451/451	Mammography	68/68	0 (all)	VKA time-updated exposure	4.6 yrs	BAC, Y/N	Mammogram	OR of calcification per year	Multivariable regression adjusted for age, sex, diabetes, indications for warfarin, warfarin-free duration, serum creatinine, serum calcium, and statin use
Van Berkel et al. (53)	Belgium	Cross-sectional	24/286	CKD, ESRD, renal Tx	59 (all)	0 (all)	VKA/no exposure	n.a	BAC Y/N	Mammogram	BAC, Y/N	Prevalence

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TABLE 1 (Continued)

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Wei et al. (54)	China	Retrospective cohort	79	NVAF	64 (all)	51 (all)	Warfarin time-updated exposure	5 mo	AA calcification score	CT	OR of score change by 1 SD per year	Multivariable regression adjusted for age, BMI, smoking, ALP, LDL cholesterol, CRP, warfarin dose, and INR
Aortic valve calcification												
Di Lullo et al. (55)	Italy	Retrospective cohort	100/247	NVAF, CKD	67/66	58/54	Warfarin/rivaroxaban	16 mo	AVC, change from baseline	US	Regression coefficient	Multivariable regression adjusted for baseline aortic calcification, systolic BP, eGFR, diabetes, glycated hemoglobin, PTH
Ing et al. (56)	United States	Cross-sectional	11/184	AS	71 (all)	78 (all)	VKA/no exposure	n/a	AV ossification Y/N	Histopathology	OR of presence of ossification	Multivariable regression adjusted for sex, sex, diabetes
Koos et al. (31)	Germany	Retrospective cohort	23/63	CAVD	71 (all)	62 (all)	VKA/no exposure	7.3 yrs	Agatston score	CT	Mean, SD	Student's *t*-test
Koos et al. (57)	Germany	Retrospective cohort	27/164	CAVD	71 (all)	71 (all)	VKA/no exposure	> 4 yrs	AVC score	CT	F statistics	ANCOVA adjusted for sex, age, sex, BMI, diabetes, smoking, hypertension, hypercholesterolemia, eGFR, use of the beta-blockers, ACE inhibitors, diuretics, cholesterol-lowering medications, thyroid hormones, and antidepressants
Lerner et al. (58)	United States	Cross- sectional	725/430	NVAF	74/74	61/61	Warfarin/no exposure	n/a	AV calcification, Y/N	US	OR of calcification	Multivariable regression adjusted for age, sex, race, eGFR, serum ALP, calcium, phosphate, and calcium-phosphate product
Peeters et al. (49)	Netherlands	Retrospective cohort	71/86	AF, no CAD	58/56	80/62	VKA/no exposure	2.3 yrs	AVC score	CT	OR of calcification	Multivariable regression adjusted for the propensity score for age, sex, BMI, systolic BP, family history of MI, hyperlipidemia, blood glucose, and LA dimension

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TABLE 1 (Continued)

References	Country	Study design	Sample size VKA - Y/N	Patients characteristics	Age VKA - Y/N	% Males VKA - Y/N	Treatment or exposure	Duration	Outcome	Assessment	Effect size estimate	Parameter estimate - methods and adjustments
Sonderskov et al. (59)	Denmark	Retrospective cohort	873/13,731	general population	67 (all)	95 (all)	VKA/no exposure	2.5 yrs	AVC score (arbitrary)	CT	REC per year	Multivariable negative binomial regression adjusted for age, sex, hypertension, diabetes mellitus, creatinine clearance, statins, and sq root AVC score at baseline
Tastet et al. (60)	Canada	Retrospective cohort	35/166	AS (mild)	79/65	71 (all)	Warfarin/no exposure	24 mo	AVC Agatston score rate (100 pe year)	CT	Regression coefficient	Multivariable regression adjusted for gender, age, BMI, diabetes mellitus, hypertension, dyslipidemia, smoking status, known CVD, family history of CVD, and eGFR
Yamamoto et al. (61)	Japan	Prospective cohort	122/101	NVAF	70/69	79/68	Warfarin/no exposure	4 yrs	AV number of calcified leaflets	US	Incidence of progression	Incidence

AA, abdominal aorta; ACE, angiotensin-converting enzyme; AF, atrial fibrillation; ALP, alkaline phosphatase; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AS, aortic stenosis; AscA, ascending aorta; AV, aortic valve; AVC, aortic valve calcification; BAC, breast artery calcification; BGP, bone Gla protein; BMI, body mass index; BP, blood pressure; CAC, coronary artery calcium; CAD, coronary artery disease; CAVD, calcific aortic valve disease; CCA, common carotid artery; CCTA, cardiac computed tomography angiography; CKD, chronic kidney disease; CRP, C-reactive protein; CT, computed tomography, CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FA, femoral artery; FGF-23, fibroblast growth factor 23; FRS, Framingham risk score; HF, heart failure; IA, iliac artery; ICA, internal carotid artery; INR, international normalized ratio; IQR, interquartile range; IVUS, intravascular ultrasound; LA, left atrium; LDL, low-density lipoprotein; MI, myocardial infarction; NOAC, non-VKA oral anticoagulants (DOAC, direct oral anticoagulants); NVAF, non-valvular atrial fibrillation; OPG, osteoprotegerin; OR, odds ratio; PAD, peripheral arterial disease; PAV, percent atheroma volume; PPI, proton pump inhibitor; PTH, parathyroid hormone; REC, ratio of expected counts; SD, standard deviation; TA, thoracic aorta; US, ultrasound; VKA, vitamin K antagonist.

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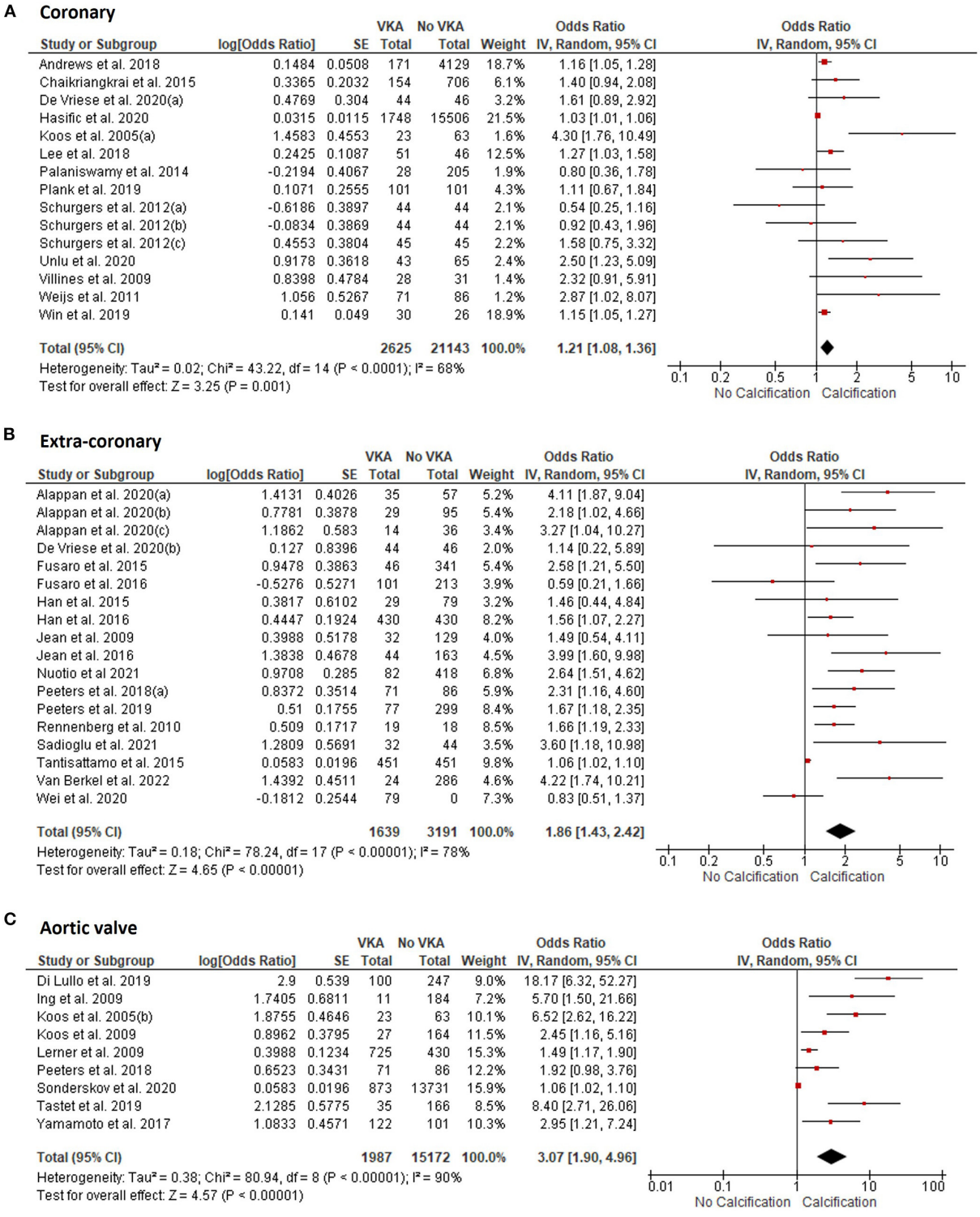


FIGURE 2
Meta-analysis of vascular and valvular calcification studies in VKA-treated patients. **(A)** coronary artery calcification; **(B)** extra-coronary calcification; **(C)** aortic valve calcification studies.

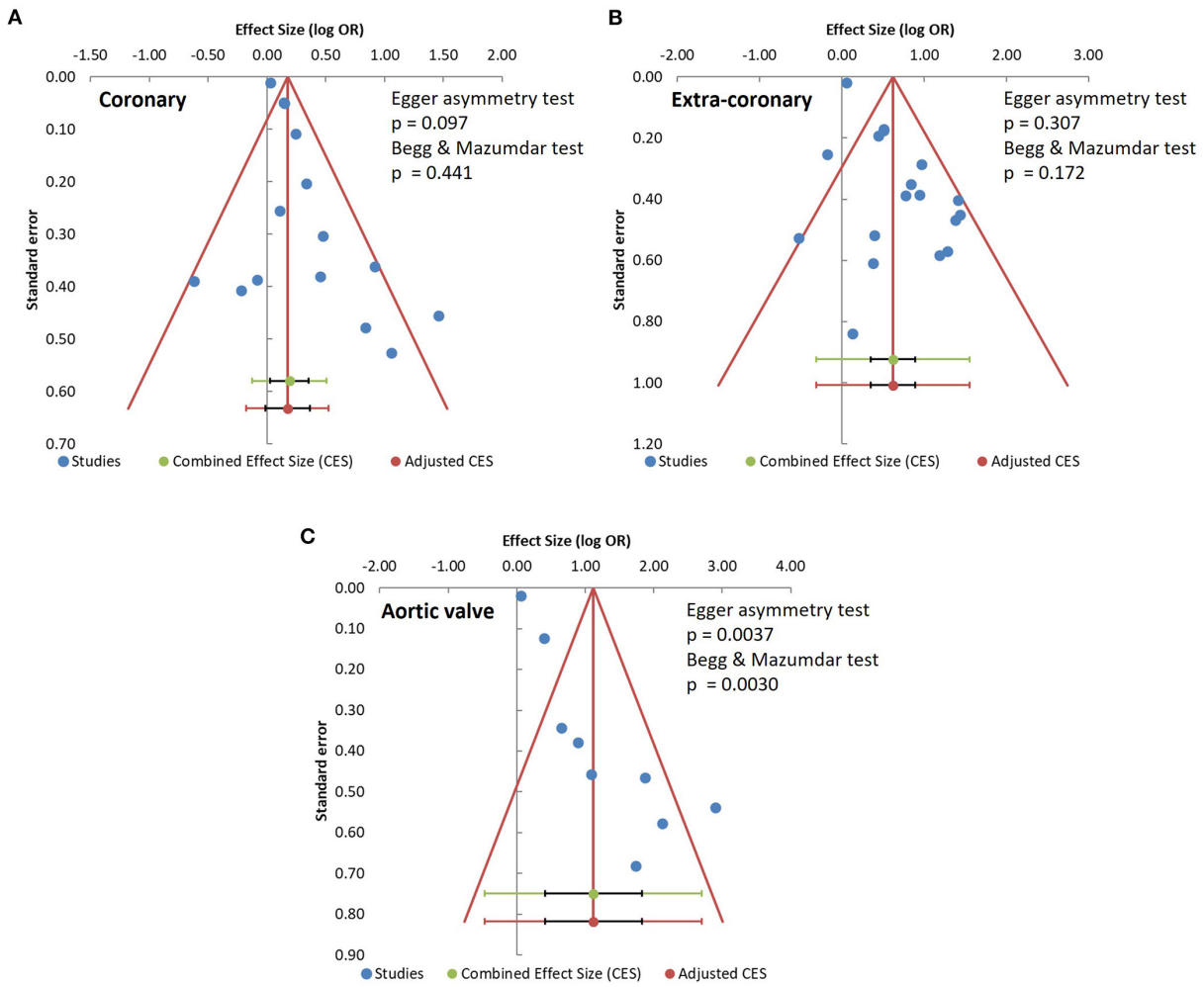


FIGURE 3
Analysis of publication bias. **(A)** coronary artery calcification; **(B)** extra-coronary calcification; **(C)** aortic valve calcification studies.

the reported estimates were adjusted or not adjusted for the confounders [$B = -0.63$ (-1.19, -0.08), $p = 0.016$, Table 2; Figure 5A]. Although the number of the aortic valve studies was low ($n = 9$) and suffered from a significant risk of publication bias, we performed a meta-regression analysis and found that the effect estimates were potentially modified by the sample size [$B = -0.32$ (-2.35, -0.04), $p = 0.009$, Table 2].

We consequently performed subgroup analyses comparing the top and bottom half of the studies with respect to each of the identified modifiers (publication year, sex ratio, duration of VKA treatment, and statistical adjustment). We found that the effect of VKA duration on the coronary artery calcification was borderline significant when comparing studies of a longer duration (>1.7 years) and studies of ≤1.7 years duration (groups difference test $I^2 = 72\%$, $p = 0.06$; Table 3; Figure 4B). We also found that, as predicted by meta-regression, the estimates of VKA effects of the extra-coronary calcification were modified by whether or not the reported models were adjusted for plausible confounders ($I^2 = 79\%$, $p = 0.005$, Table 3; Figure 5B).

Sensitivity analysis

To explore the influence of any single study on the overall effect sizes, we excluded studies from the corresponding analyses, one at a time. No significant effects of any individual study on the effect sizes of VKA were observed (Supplementary Table 4).

Discussion

We present the first meta-analysis of the effects of vitamin K antagonists on vascular and valvular calcification.

TABLE 2 Meta-regression analysis of potential effect moderators.

Effect moderator	Coronary		Extra-coronary		Aortic valve	
	B coefficient (95% CI)	*P*-value	B coefficient (95% CI)	*P*-value	B coefficient (95% CI)	*P*-value
Publication year	−0.04 (−0.08, 0.00)	0.035	0.05 (−0.03, 0.12)	0.183	0.00 (−0.14, 0.14)	0.953
Geographic region	−0.03 (−0.33, 0.26)	0.803	−0.18 (−0.70, 0.34)	0.458	0.11 (−1.51, 1.73)	0.873
Study design	−0.01 (−0.24, 0.22)	0.908	0.11 (−0.53, 0.75)	0.711	−0.35 (−2.35, 1.65)	0.688
Sample size, log (*N*)	−0.04 (−0.10, 0.02)	0.164	−0.08 (−0.35, 0.19)	0.520	−0.32 (−0.60, −0.04)	0.009
Patient characteristics	−0.22 (−0.54, 0.11)	0.153	0.33 (−0.24, 0.91)	0.216	0.82 (−0.18, 1.82)	0.059
Age, years	0.01 (−0.01, 0.03)	0.316	0.00 (−0.03, 0.03)	0.953	0.04 (−0.09, 0.17)	0.459
Sex ratio (% males)[a]	−0.01 (−0.03, 0.00)	0.039	0.01 (−0.01, 0.03)	0.279	−0.04 (−0.11, 0.02)	0.111
Duration of treatment, years[b]	0.08 (0.03, 0.13)	0.0005	0.02 (−0.06, 0.09)	0.657	0.01 (−0.40, 0.42)	0.947
Imaging modality[c]	−0.31 (−1.93, 1.31)	0.653	−0.22 (−0.86, 0.43)	0.476	−0.31 (−1.93, 1.31)	0.653
Adjustment for confounders	−0.18 (−0.46, 0.09)	0.154	−0.63 (−1.19, −0.08)	0.016	−0.48 (−1.80, 0.84)	0.401

[a]excluding BAC studies; [b]excluding cross-sectional studies; [c]excluding histopathology.

Our results confirmed a strong association between VKA use and vascular calcification in the absence of significant risks of publication bias. We also found evidence of a positive association between VKA use and aortic valve calcification; however, due to a smaller number of studies and evidence of publication bias, the confidence in this finding is low.

A large number of good-quality observational studies of the effects of VKA on vascular calcification have been published. These studies assessed calcification in thousands of VKA-treated patients. Improved imaging modalities and the use of calcium imaging in diagnostics studies allowed for the analysis of larger cohorts of patients with greater precision. Several authors took a propensity matching approach to eliminate the potential confounders; others used other comprehensive statistical methods to minimize the effects of confounding variables on calcification estimates. The quality of publications on VKA use and vascular calcification is also supported by a lack of significant risk of publication bias and the fact that we could detect that duration of treatment as a modifier of the effect estimates. Lastly, a recent introduction of a new class of non-vitamin K oral anticoagulants, NOAC, allowed for the analysis of calcification in the first head-to-head randomized trials with VKA, although each of the three randomized trials assessed <100 patients so far.

Of 45,000 participants included in this meta-analysis, 99.5% were from observational studies. Therefore, it is important to recognize limitations inherent to observational study designs, such as the difficulty of establishing a cause-and-effect relationship. We also observed that the magnitude of effect estimates was modified by several experimental parameters, including sample size, gender ratio, and adjustments for confounding variables. In addition, we found that studies of VKA in valvular calcification suffered from a significant risk of publication bias, limiting our confidence in that association.

For decades, warfarin, a commonly used VKA, has been a standard and effective treatment option for patients requiring anticoagulation. Early studies in the 1980 s found an association between dystrophic calcification and warfarin in an animal model examining bioprosthetic aortic valves (64). Since the early 1990 s, it has been known that warfarin is associated with soft tissue calcification, such as skin calcinosis and tracheobronchial calcification (65, 66). In 2005, Koos et al. documented for the first time the effects of warfarin on the coronary artery and aortic valve calcification (31).

Cardiovascular calcification presents several morphologically distinct forms, including the intimal, medial, and heart valve calcification. Coronary arteries are primarily affected by atherosclerotic intimal calcification, whereas the peripheral arteries and aorta show different degrees of medial and intimal involvement. Despite the anatomical difference, many significant correlations exist between calcification burdens at different vascular sites, suggesting a common mechanism and the influence of systemic factors. Thus, calcification in the abdominal aorta, breast and the arteries of the extremities artery correlates with coronary artery calcification (67–69). An independent association between aortic valve calcification and the severity of coronary artery calcification has also been reported (70).

Calcification is initiated by osteogenic transdifferentiation of vascular cells (71). Transdifferentiated cells secrete mineralizing matrix vesicles that serve as nucleating sites for extracellular

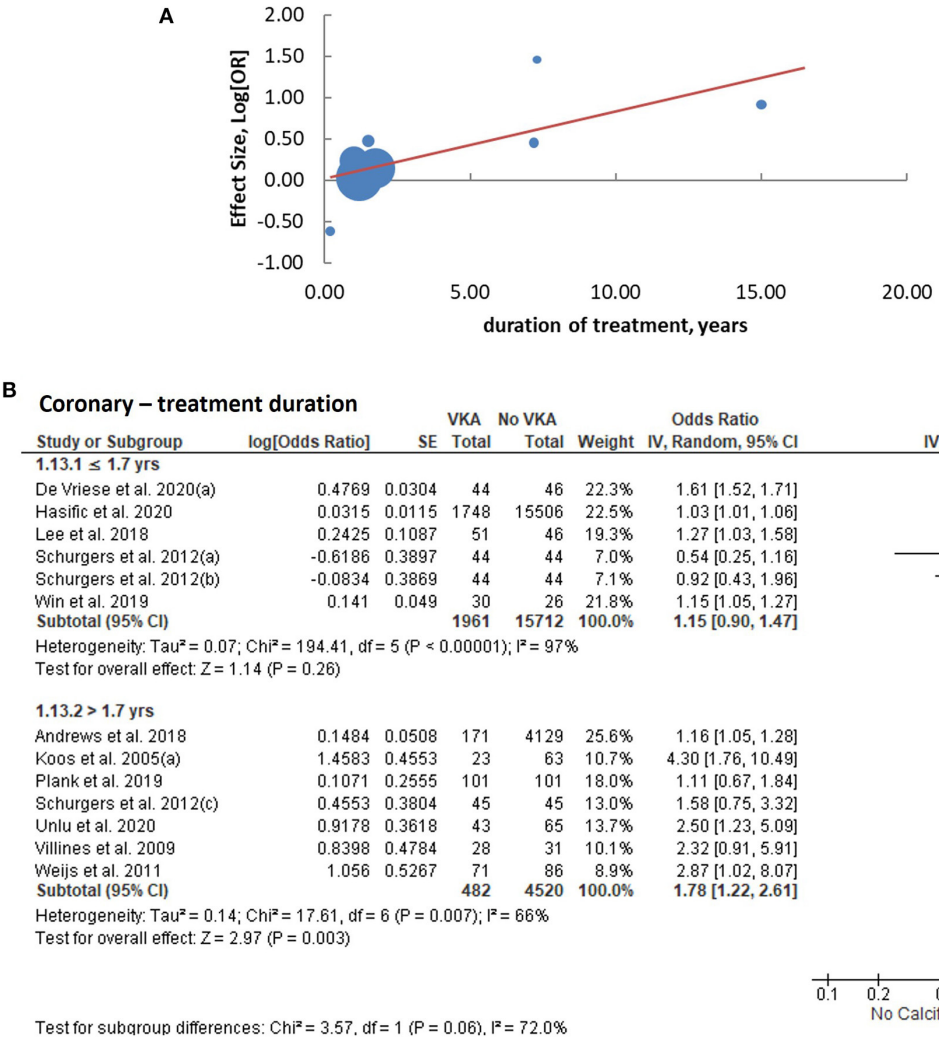
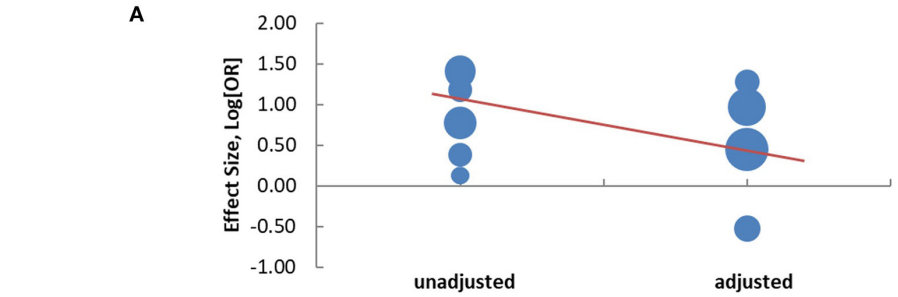


FIGURE 4
Analysis of coronary arterial calcification according to the duration of VKA treatment. **(A)** meta-regression of the effect sizes—less or equal vs. more than the median duration; **(B)** subgroup analysis based on the duration of treatment.

calcium deposition (72–74). Osteogenic transdifferentiation is preceded by inflammation, as has been shown in a longitudinal study of patients' coronary artery calcification employing positron emission tomography (PET) combined with CT (75). Active inflammation and microcalcification detected by specific PET were also shown to co-exist in patients with peripheral arterial or calcific aortic valve disease (76, 77). MGP interferes with both the osteogenic cell transformation and physicochemical process of biomineralization (78). VKA reduces gamma-carboxylation leaving MGP in the inactive state.

Calcification can negatively affect the clinical course of cardiovascular disease in several ways, by increasing arterial stiffness, stability of atherosclerosis plaques, and, in the context of calcific aortic valve stenosis, reducing the opening of the valves. Arterial stiffness promotes microcirculatory damage by increasing the transmission of pressure pulsatility (79). Numerous studies have shown that arterial stiffness predicts cardiovascular outcomes after adjustments for conventional risk factors (80–82). Calcification also changes the composition of atherosclerotic plaque. An earlier study of culprit plaques' characteristics documented that surface erosion over a calcified nodule has likely precipitated an acute ischemic coronary event and death (83). The later studies using ^{18}F-sodium fluoride positron emission tomography (^{18}F-NaF PET), capable of detecting micro-calcification invisible to other imaging technologies, demonstrated that micro-calcification is associated with high-risk plaque features



B **Extra-coronary – statistical adjustment of the effect estimates**

Study or Subgroup	log[Odds Ratio]	SE	VKA Total	No VKA Total	Weight	Odds Ratio IV, Random, 95% CI
1.17.1 unadjusted						
Alappan et al. 2020(a)	1.4131	0.4026	35	57	21.0%	4.11 [1.87, 9.04]
Alappan et al. 2020(b)	0.7781	0.3878	29	95	22.7%	2.18 [1.02, 4.66]
Alappan et al. 2020(c)	1.1862	0.583	14	36	10.0%	3.27 [1.04, 10.27]
De Vriese et al. 2020(b)	0.127	0.8396	44	46	4.8%	1.14 [0.22, 5.89]
Han et al. 2015	0.3817	0.6102	29	79	9.2%	1.46 [0.44, 4.84]
Jean et al. 2016	1.3838	0.4678	44	163	15.6%	3.99 [1.60, 9.98]
Van Berkel et al. 2022	1.4392	0.4511	24	286	16.7%	4.22 [1.74, 10.21]
Subtotal (95% CI)			**219**	**762**	**100.0%**	**2.97 [2.07, 4.27]**
Heterogeneity: Tau² = 0.00; Chi² = 4.97, df = 6 (P = 0.55); I² = 0%						
Test for overall effect: Z = 5.90 (P < 0.00001)						
1.17.2 adjusted						
Eden-Sadioglu et al. 2021	1.2809	0.5691	32	44	4.2%	3.60 [1.18, 10.98]
Fusaro et al. 2015	0.9478	0.3863	46	341	6.9%	2.58 [1.21, 5.50]
Fusaro et al. 2016	-0.5276	0.5271	101	213	4.7%	0.59 [0.21, 1.66]
Han et al. 2016	0.4447	0.1924	430	430	11.9%	1.56 [1.07, 2.27]
Jean et al. 2009	0.3988	0.5178	32	129	4.8%	1.49 [0.54, 4.11]
Nuotio et al 2021	0.9708	0.285	82	418	9.3%	2.64 [1.51, 4.62]
Peeters et al. 2018(a)	0.8372	0.3514	71	86	7.7%	2.31 [1.16, 4.60]
Peeters et al. 2019	0.51	0.1755	77	299	12.4%	1.67 [1.18, 2.35]
Rennenberg et al. 2010	0.509	0.1717	19	18	12.5%	1.66 [1.19, 2.33]
Tantisattamo et al. 2015	0.0583	0.0196	451	451	15.6%	1.06 [1.02, 1.10]
Wei et al. 2020	-0.1812	0.2544	79	0	10.1%	0.83 [0.51, 1.37]
Subtotal (95% CI)			**1420**	**2429**	**100.0%**	**1.55 [1.19, 2.02]**
Heterogeneity: Tau² = 0.12; Chi² = 43.73, df = 10 (P < 0.00001); I² = 77%						
Test for overall effect: Z = 3.22 (P = 0.001)						
Test for subgroup differences: Chi² = 8.06, df = 1 (P = 0.005), I² = 87.6%						

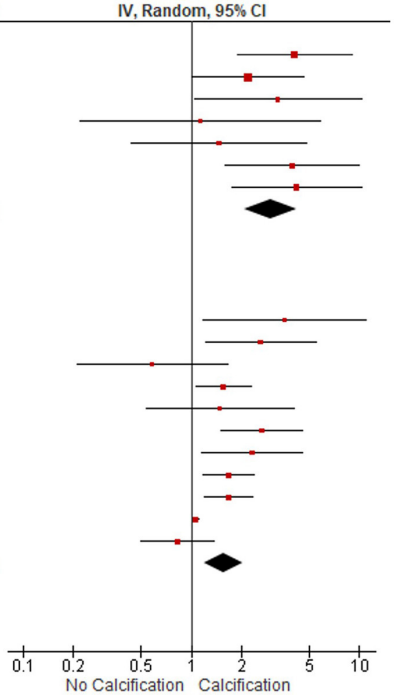


FIGURE 5
Analysis of extra-coronary arterial calcification according to statistical adjustment for confounding variables. **(A)** Meta-regression of the effect sizes—adjusted vs. unadjusted. **(B)** Subgroup analysis based on whether or not statistical adjustments were applied to calculate the effect estimates.

(84). Furthermore, aortic valve calcification and the rate of progression of calcification are strong predictors of aortic valve stenosis outcomes (85, 86). Thus, the association between VKA use and cardiovascular calcification is concerning because it might worsen the course of vascular or valvular disease.

It was suggested that warfarin-induced calcification could result in adverse clinical outcomes (87). One study included in this meta-analysis demonstrated that warfarin had a significant hazard ratio of 1.97 for the overall mortality in hemodialysis patients independent of the confounder variables, age, atrial fibrillation, and diabetes. Furthermore, adjustment for vascular calcification reduced the strength of this association, suggesting that warfarin-induced calcification might have contributed to mortality (42).

In conclusion, our meta-analysis demonstrates that VKA use is associated with vascular calcification. Thus, vascular calcification can be considered a side effect of VKA. However, the clinical significance of VKA-induced calcification and the risk benefits of VKA therapy requires further evaluation.

TABLE 3 Subgroup analysis by study design.

Moderator	Subgroup	Number of studies	Patients—VKA (Y/N)	OR (95% CI)	*P*-value	I^2	Test for subgroup differences *p*-value
Coronary							
Publication year	Before 2016	8	437/1,224	1.43 (0.93, 2.18)	0.10	61%	0.63
	2017–2022	7	2,188/19,919	1.27 (1.04, 1.55)	0.02	97%	
Sample size	≤97	8	309/345	1.31 (1.03, 1.66)	0.03	58%	0.42
	>97	7	2,316/20,798	1.16 (1.01, 1.35)	0.04	65%	
Sex ratio (%males)	≤71%	8	481/1,274	1.33 (0.92, 1.92)	0.13	62%	0.48
	>71%	7	2,144/19,869	1.16 (1.04, 1.28)	0.006	70%	
Duration[a]	≤1.7 yrs	7	1,961/15,712	1.15 (0.90, 1.47)	0.26	97%	0.06
	>1.7 yrs	6	482/4,520	1.78 (1.22, 2.61)	0.003	66%	
Adjustment for confounders	Unadjusted	10	554/1,350	1.40 (1.02, 1.93)	0.04	55%	0.21
	Adjusted	5	2,071/19,793	1.14 (1.03, 1.26)	0.01	76%	
Extra-coronary							
Publication year	Before 2019	9	1,223/1,910	1.59 (1.16, 2.19)	0.04	74%	0.21
	2019–2022	9	416/1,281	2.21 (1.48, 3.29)	<0.0001	63%	
Sample size	≤159	9	352/461	1.93 (1.33, 2.80)	0.0005	53%	0.80
	>159	9	1,287/2,730	1.80 (1.26, 2.58)	0.001	82%	
Sex ratio (%males)[b]	≤56	7	703/1,267	1.65 (1.16, 2.36)	0.006	55%	0.46
	>56	7	407/1,285	2.00 (1.40, 2.86)	0.0001	44%	
Duration[a]	≤4.1 yrs	7	797/1,315	1.68 (1.22, 2.30)	0.001	52%	0.50
	>4.1 yrs	6	612/1006	2.04 (1.28, 3.25)	0.003	84%	
Adjustment for confounders	Unadjusted	7	219/762	2.97 (2.07, 4.27)	<0.00001	0%	0.005
	Adjusted	11	1,420/2,429	1.55 (1.19, 2.02)	0.001	77%	
Aortic valve							
Publication year	Before 2018	5	908/942	2.93 (1.57, 5.48)	0.0008	73%	0.70
	2018–2022	4	1,079/14,230	3.82 (1.14, 12.87)	0.03	93%	
Sample size	≤201	5	167/663	3.83 (2.11, 6.95)	<0.00001	53%	0.20
	>201	4	1,820/14,509	2.24 (1.26, 3.99)	0.006	92%	
Sex ratio (%males)	≤71%	5	910/1,070	4.76 (1.82, 12.43)	0.001	89%	0.16
	>71%	4	1,077/14,102	2.03 (1.01, 4.08)	0.03	79%	
Duration[a]	≤2.5 yrs	4	1079/14,230	3.82 (1.14, 12.87)	0.03	93%	0.89
	>2.5 yrs	3	172/328	3.47 (1.95, 6.19)	<0.0001	29%	
Adjustment for confounders	Unadjusted	2	145/164	4.37 (2.01, 9.50)	0.0002	32%	0.31
	Adjusted	7	1,842/15,008	2.72 (1.64, 4.50)	0.0001	90%	

[a]excluding cross-sectional studies; [b]excluding BAC studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

NDK and OVS conceived, designed the review, analyzed, interpreted the results, and edited the manuscript. NDK and MS performed the literature search and screened the data. NDK and DK extracted the data. OVS verified the extracted data. NDK wrote the first draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.938567/full#supplementary-material

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