Statistical Analysis Plan for the study: ”Effects of menaquinone-7 supplementation in patients with aortic valve calcification”

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Introduction

This document specifies the planned statistical analysis for the study ”Effects of menaquinone-7 supplementation in patients with aortic valve calcification: study protocol for a randomised controlled trial” as carried out following the protocol published in BMJ Open (1).

Sample size considerations

According to the protocol (1): “The study was designed to have 80% power to detect a 20 % difference in progression of aortic valve calcification score between the treatment groups. At least 354 patients were required for the study to be conclusive, and we planned to include 400 patients.”

Randomizations

Subjects were be randomized 1:1 stratified by site (Odense University Hospital - Odense, Svendborg Hospital, Vejle Hospital or Silkeborg Hospital) and AVC score (300–599 or ≥600).

Interim analyses

During conduct of the study, an interim analysis was conducted to determine if the intervention should be prolonged by 6 months. The interim analyses concluded that this was not necessary. As the interim analysis did only influence how the intervention was performed, but neither sample size nor possible discontinuation of the study, we did not take the interim analysis into account in the final analyses.

Statistical principles

All analyses were performed in the intention-to-treat population and as superiority analyses. Two-sided p-values of 0.05 or less were considered to indicate statistical significance and associations and effect estimates will be reported with 95% probability symmetric confidence interval (2.5% to 97.5% quantile).

Practical considerations

Data were collected using REDCap (2, 3) hosted by OPEN (Open Patient data Explorative Network, Odense University Hospital, Odense Denmark) with project number OP_530. Data were analyzed using Stata (4) on OPEN's secure analysis server (OPEN Analyse). A prespecified random seed of 7042021 will be used for bootstrapping and imputation.

Planned analyses for primary publication: Change in AVC score

Exclusion criteria

Participants with AVC < 300 at baseline will be excluded from all analyses in this publication.

Outcomes

Primary outcome: Main analysis

The primary outcome was the change in aortic valve calcification (AVC) score (numerical) from baseline to 24 month for the full cohort of included patients.

Secondary outcomes to be included in the primary publication

Secondary outcomes were

- Change in AVC from baseline to 24 months in two prespecified subgroups (baseline AVC score 300-599 and ≥600).
- Change in AVC from baseline to 12 months and from 12 months to 24 months, both for the full cohort as well as for the two subgroups.
- Changes in aortic valve area from baseline to 24 months (numerical)
- Change in peak aortic-jet velocity from baseline to 24 months (numerical)
- Need of heart valve surgery (dichotomous, occurred / not occurred between baseline and 24 months)

- Change in MGP with different phosphorylation and carboxylation forms (numerical)
- Change in QoL from baseline to 24 months (numerical)

Safety outcomes

Safety outcomes were

- Death (all cause and cardivascular cause) (dichotomous)
- Cardiovascular events (a composite endpoint of myocardial infarction, coronary revascularization, stroke, aortic disease, significant peripheral artery disease (surgery), as well as these endpoints separately) (all dichotomous)
- Progressive aortic valve disease (more than 50% increase in AVC score) (dichotomous)
- Venous thromboembolism (dichotomous)
- Severe bleeding
- Low energy or spontaneous fracture
- Cancer (recurrent cancer or new cancer) (dichotomous)
- The following laboratory measurements: hemoglobin, leucocytes, thrombocytes, albumin, creatinine (eGFR), urea, calcium, phosphate, magnesium, troponine, creatine kinase, alanine aminotransferase, lactate dehydrogenase, bilirubin, alkaline phosphatase, total-cholesterol, low-density lipoprotein, high-density lipoprotein, parathyroid hormone, vitamin D, prothrombin time-international normalised ratio (PT-INR) and activated partial thromboplastin time (all numerical)

Dichotomous safety outcomes were evaluated as occurred / not occurred between baseline and 24 months. Numerical safety outcomes were evaluated as change from baseline to 12 and 24 months.

Planned tables and figures and corresponding analyses

Table 1. Characteristics of patients at baseline

Characteristics (as listed in Table 1 below, numerical characteristics marked with [numerical]) of patients at baseline will be reported separately for the two treatment groups. Characteristics will be reported as

- Mean and standard deviation (SD) for numerical characteristics, which are approximately normally distributed in both treatment groups as investigated by normal quantile-quantile plots. These characteristics will be compared between groups by two-sample t-test with assumed equal variance, or with assumed unequal variance if indicated by Levene's test for equality of variance (5).
- Median and 1st and 3rd quartile (inter quartile range (IQR)) for numerical characteristics, which are not approximately normally distributed in at least one treatment group as indicated by normal quantile-quantile plots. These characteristics will be compared between groups by Wilcoxon rank-sum test.

- Counts and proportions for categorical characteristics (with categories as specified in Table 1 below). These characteristics will be compared between groups by χ^2-test if all counts are at least 5, or with Fisher's exact test otherwise.

No adjustments for multiple testing will be carried out in Table 1 and no effect sizes for differences between groups will be estimated. Missing observations will be excluded from tests in Table 1, and number of missing observations in each group will be specified.

Characteristic	**Vitamin K +D group (N=XX)**	**Placebo group (N=XX)**	**P-value**
Age – years [numerical]			
Baseline AVC – AU [numerical]			
Echocardiography • Bicuspid aortic valve – no (%) • V_{max} - cm/sec [numerical] • Aortic valve area - cm^2 [numerical] • Left ventricular ejection fraction -% [numerical]			
Estimated GFR – ml/min/1.73 m^2 [numerical]			
dp-ucMGP – pmol/L [numerical]			
Body-mass index [numerical]			
Systolic Blood pressure – mmHg [numerical]			
Smoking • Active smokers - no (%) • Former smokers – no (%) • Non-smokers – no (%)			
Coexisting condition – no (%) • Hypertension • Diabetes • Ischemic heart disease • Atrial fibrillation • Renal failure (eGFR<60)			
Medications – no (%) • ACE inhibitor or ARB • Beta-blocker • Mineralocorticoid-receptor antagonist • Antiplatelet therapy • NOAC • Statin therapy			

ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, AVC aortic valve calcification, dp-ucMGP dephosphorylated-uncarboxylated Matrix Gla-Protein, GFR glomerular filtration rate, NOAC new-onset anticoagulant
† The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Primary outcome and numerical secondary outcomes

Primary outcome and numerical secondary outcomes will be analyzed by mixed effects linear regression. The mixed effects linear regression models will include a fixed effect for treatment, a fixed effect for time point (baseline, 12 and 24 months) and a fixed effects interaction between treatment and time point. As baseline measurements are obtained before randomization, treatment at baseline will be modelled as a

separate common treatment category, constraining baseline measurements to no systematic treatment effect between the two treatment arms, as suggested in literature (6). All mixed effects models will include a random intercept for each included patient. Normality assumptions on residuals and random effects will be evaluated by normal quantile-quantile plots. In case deviations from normality assumptions are detected analyses will be repeated with nonparametric bootstrapping with 1,000 bootstrapping samples (handling individual patients as bootstrapping clusters, and treatment groups as bootstrapping strata).

Missing data will be excluded from analyses in Table 2 (but see sensitivity analyses below).

		Vitamin K +D group	Placebo group		
		Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Primary outcome: Main analysis					
AVC score (all patients)	0 – 24 months				
Numerical secondary outcomes					
AVC score (all patients)	0 – 12 months				
	12 – 24 months				
AVC score (baseline AVC score 300-599)	0 – 24 months				
	0 – 12 months				
	12 – 24 months				
AVC score (baseline AVC score ≥600)	0 – 24 months				
	0 – 12 months				
	12 – 24 months				
Aortic valve area	0 – 24 months				
Peak aortic-jet velocity	0 – 24 months				
dp-ucMGP	0 – 24 months				
QoL	0 – 24 months				

Table 3. Dichotomous secondary outcomes and adverse events

Dichotomous secondary outcomes (valvular surgery between baseline and 24 months) and adverse events are reported as counts and proportions separately for each treatment group and compared between groups by χ^2-test if all counts are at least 5, or with Fisher's exact test otherwise. If a patient reported multiple events of the same outcomes, this will be handled dichotomously as experiencing the outcome in the analyses, but the count of such patients will be reported in a parenthesis or footnote to the table.

Missing observations will be excluded from tests in Table 2, and number of missing observations in each group will be specified.

Event	**Vitamin K +D group**	**Placebo group**	**p-value**

	(N=XX)	(N=XX)	
Outcome			
Valvular Surgery – no (%)			
Adverse Events			
Any event – no (%)			
Death – no (%)			
Cardiovascular events (combined) – no (%) Myocardial infarction Coronary revascularization Stroke Aortic disease Peripheral artery surgery			
Progressive aortic valve disease (more than 50% increase in AVC score)			
Severe bleeding – no (%)			
Venous thromboembolism – no (%)			
Low energy fracture– no (%)			
Incident cancer – no (%)			

Table 4. Numerical safety outcomes

Numerical safety outcomes will be analyzed by linear mixed models corresponding to the analyses specified for the primary outcome in Table 2. No subgroup analyses will be performed.

		Vitamin K +D group	Placebo group		
		Mean change (95 % CI)	Mean change (95 % CI)	Treatment effect (95 % CI)	P-value
hemoglobin	0 - 24 months				
creatinine (eGFR)	0 - 24 months				
calcium	0 - 24 months				
phosphate	0 - 24 months				
parathyroid hormone	0 - 24 months				
vitamin D	0 - 24 months				
activated partial thromboplastin time	0 - 24 months				
prothrombin time-international normalised ratio (PT-INR)	0 - 24 months				

Table 5. Stratified analyses of the primary outcome

Analyses of the primary outcome (AVC score) will be repeated stratifying for age (<70 or ≥70 years at baseline), diabetes (yes / no at baseline), hypertension (yes / no at baseline), atrial fibrillation (yes / no at baseline), ischemic heart disease (yes / no at baseline), renal failure (yes / no at baseline), statin therapy (yes / no at baseline), and MGP (below / above median). In these analyses, patients will not be stratified by baseline AVC score 300-599 and ≥600. The same models as for Table 2 will be applied for these analyses.

	Vitamin K +D group	Placebo group		
	Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Age < 70 years				
Age ≥70 years				
No diabetes				
Diabetes				
No hypertension				
Hypertension				
No atrial fibrillation				
Atrial fibrillation				
No ischemic heart disease				
Ischemic heart disease				
No renal failure				
Renal failure				
No statin therapy				
Statin therapy				
Baseline MGP < median				
Baseline MGP >= median				

Supplementary Table S1. Sensitivity analysis: Primary outcome and numerical secondary outcomes with imputation

If more than 5% of observations are missing at 24 months for the main analysis of the primary outcome reported in Table 2 the analysis will be repeated using multiple imputation (assuming missing at random) by linear regression with all baseline characteristics included in Table 1 as covariates generating 100 imputation. Results will be reported in a table structured as Table 2.

Supplementary Table S2. Sensitivity analysis: Primary outcome on subgroup with high compliance

The analysis of primary outcome (all patients 0-24 months) will be repeated (with the same methodology as specified for Table 3) on those patients, who reported a compliance of at least 90% during the study period. Furthermore, number and proportion of patients included in this analysis will be reported in Table S4, together with odds ratio (with 95% confidence interval) and p-value determined by univariate logistic regression for difference in compliance rate between treatment arms will be reported.

		Vitamin K +D group	Placebo group	Group difference	
		Number (proportion)	Number (proportion)	Odds ratio (95 % CI)	P-value
Compliance of at least 90%					
		Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Primary outcome (only including with compliance of at least 90%)					
AVC score (all patients)	0 – 24 months				

Supplementary Table S3. Safety outcomes of clinical biochemistry

Numerical safety outcomes will be analyzed by linear mixed models corresponding to the analyses specified for the primary outcome in Table 2. No subgroup analyses will be performed.

		Vitamin K +D group	Placebo group		
		Mean change (95 % CI)	Mean change (95 % CI)	Treatment effect (95 % CI)	P-value
hemoglobin	0 - 12 months				
	0 - 24 months				
leucocytes	0 - 12 months				
	0 - 24 months				
thrombocytes	0 - 12 months				
	0 - 24 months				
albumin	0 - 12 months				
	0 - 24 months				
creatinine (eGFR)	0 - 12 months				
	0 - 24 months				
urea	0 - 12 months				
	0 - 24 months				
calcium	0 - 12 months				
	0 - 24 months				
magnesium	0 - 12 months				
	0 - 24 months				
phosphate	0 - 12 months				
	0 - 24 months				
troponine	0 - 12 months				
	0 - 24 months				
creatine kinase	0 - 12 months				
	0 - 24 months				
alanine aminotransferase	0 - 12 months				
	0 - 24 months				
lactate dehydrogenase	0 - 12 months				
	0 - 24 months				
bilirubin	0 - 12 months				

	0 - 24 months				
alkaline phosphatase	0 - 12 months				
	0 - 24 months				
total-cholesterol	0 - 12 months				
	0 - 24 months				
low-density lipoprotein	0 - 12 months				
	0 - 24 months				
high-density lipoprotein	0 - 12 months				
	0 - 24 months				
triglyceride	0 - 12 months				
	0 - 24 months				
parathyroid hormone	0 - 12 months				
	0 - 24 months				
vitamin D	0 - 12 months				
	0 - 24 months				
prothrombin time-international normalised ratio (PT-INR)	0 - 12 months				
	0 - 24 months				
activated partial thromboplastin time	0 - 12 months				
	0 - 24 months				

Figure 1. Enrollment and Randomization of Patients.

Flow chart

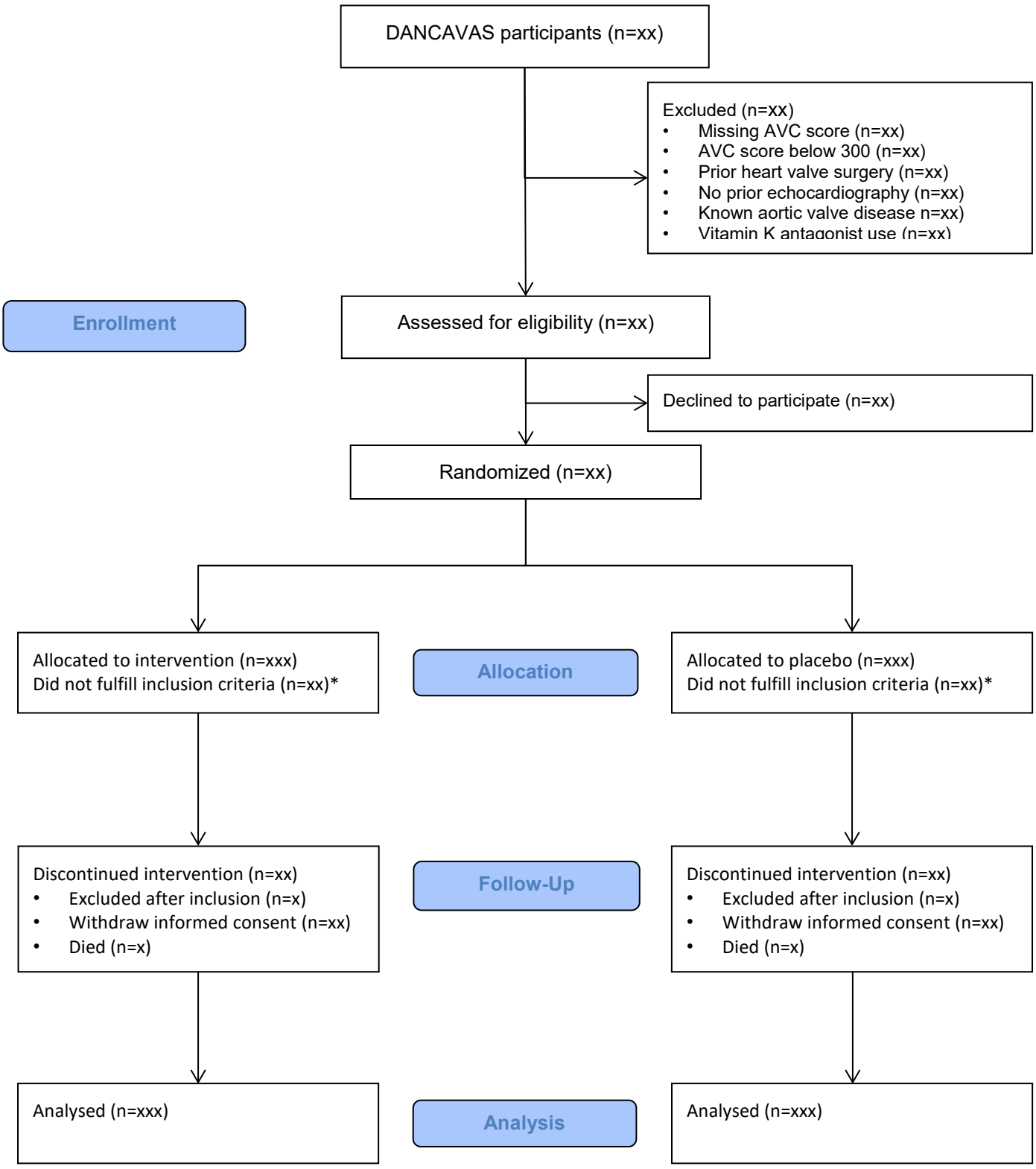


Figure 2. AVC progression according to treatment allocation.

AVC score at baseline, 12, and, 24 months will be presented at line graph for the two treatment groups presenting mean with 95% confidence interval (as obtained from the linear mixed models applied for Table 3). The figure will be presented for all patients (Figure 2A) as well as stratified by the baseline AVC score subgroups 300-599 (Figure 2B) and ≥600 (Figure 2C). (See mockup figure 2A below, 2B and 2C are similar)

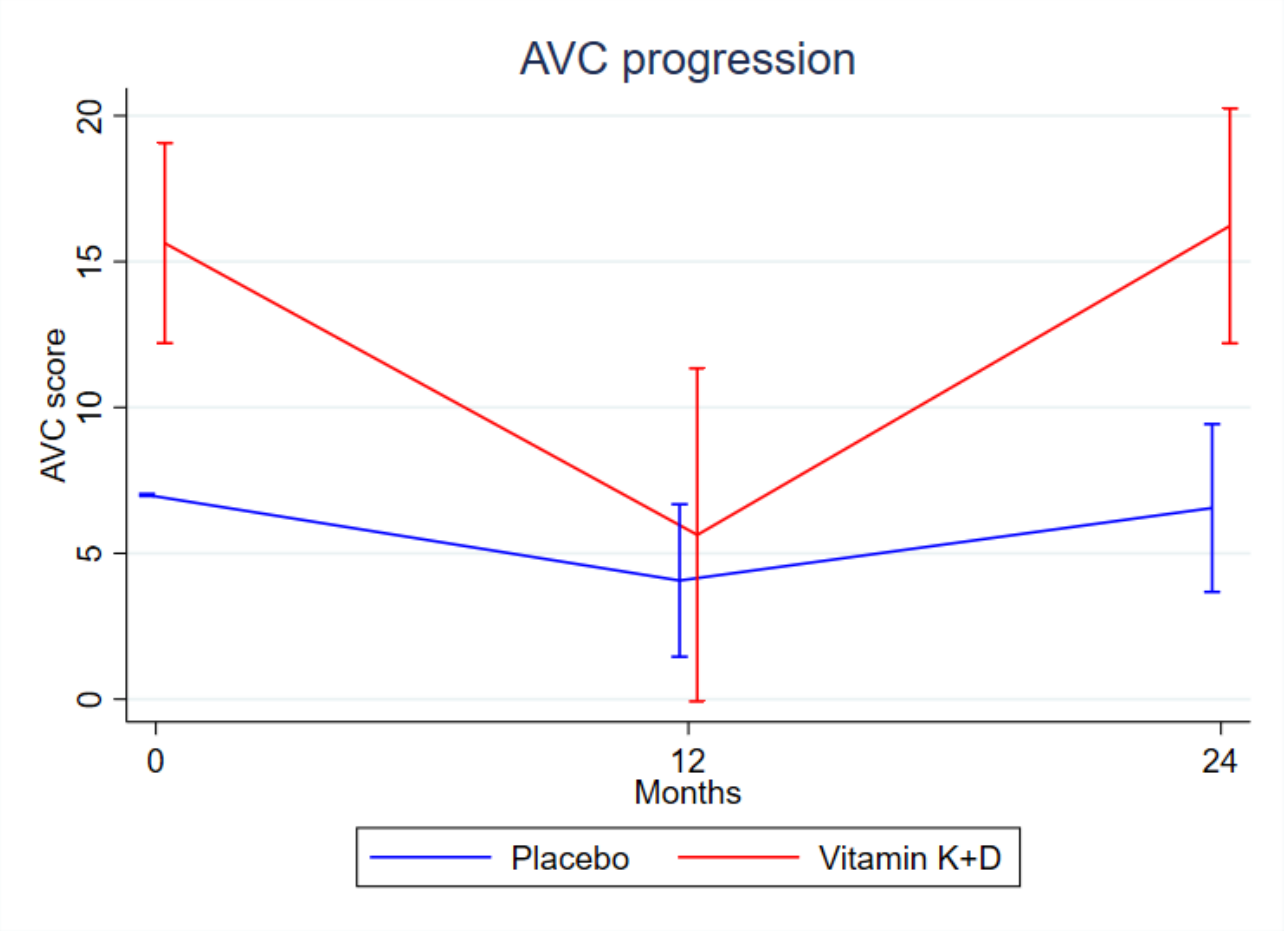


Figure 3. AVA (A) and Vmax (B) changes according to treatment allocation

AVA (Figure 3A) and Vmax (Figure 3B) at baseline, 12, and, 24 months will be presented at line graph for the two treatment groups presenting mean with 95% confidence interval (as obtained from the linear mixed models applied for Table 3). (See mockup figures below)

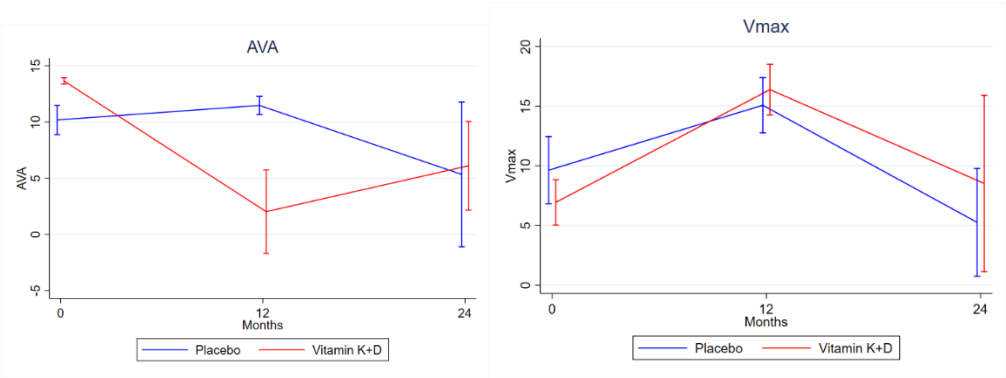
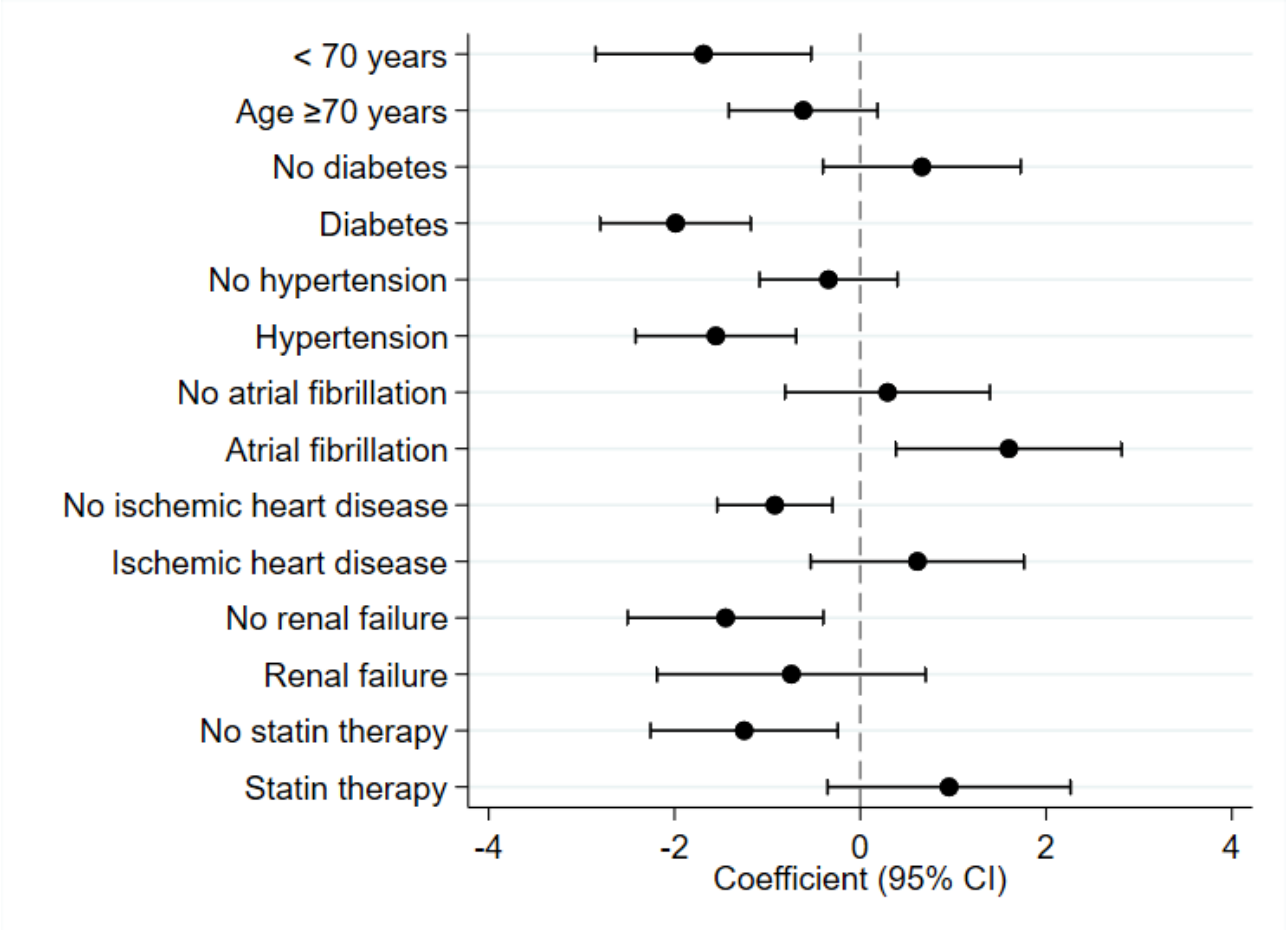


Figure 4. Forest plot of stratified analyses of the primary outcome

Coefficients with 95% confidence intervals from stratified analyses (as reported in Table 4) for differences in primary outcome (change of AVA score from baseline to 24 months) between treatment groups will be presented as a forest plot. (See mockup figure below.)



Planned analyses for secondary publication: Change in aortic calcification and diameter

Exclusion criteria

Participants with prior aortic surgery are excluded from this study.

Outcomes

Primary outcome

- Co-primary outcome 1: change in aortic calcification score (numerical) from baseline to 24 month.
- Co-primary outcome 2: change in aortic dilatation (numerical) from baseline to 24 month.

Secondary outcomes

- Change in aortic calcification score from baseline to 12 months and from 12 months to 24 months.
- Change in aortic diameter from baseline to 12 months and from 12 months to 24 months.
- Need of aortic surgery (dichotomous, occurred / not occurred between baseline and 24 months).

Planned tables and figures and corresponding analyses

Table 1. Characteristics of patients at baseline

Statistical methods applied for table 1 are similar to the statistics used for table 1 in the primary publication.

Characteristic	**Vitamin K +D group (N=XX)**	**Placebo group (N=XX)**	**P-value**
Age – years [numerical]			
Baseline aortic calcification score – AU [numerical] • Ascending • Arcus • Descending • Suprarenal • Infrarenal • Renal artery • Iliaca com artery • Iliaca ext artery			
Baseline aortic diameter – mm [numerical] • Ascending • Arcus • Descending • Infrarenal			
Estimated GFR – ml/min/1.73 m^2 [numerical]			
Body-mass index [numerical]			

dp-ucMGP – pmol/L			
Systolic Blood pressure – mmHg [numerical]			
Smoking Active smokers - no (%) Former smokers – no (%) Non-smokers – no (%)			
Coexisting condition – no (%) Hypertension Diabetes Ischemic heart disease Atrial fibrillation Renal failure (eGFR<60)			
Medications – no (%) ACE inhibitor or ARB Beta-blocker Mineralocorticoid-receptor antagonist Antiplatelet therapy NOAC Statin therapy			

ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, AVC aortic valve calcification, dp-ucMGP dephosphorylated-uncarboxylated Matrix Gla-Protein, GFR glomerular filtration rate, NOAC new-onset anticoagulant
† The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Primary outcome and numerical secondary outcomes

Primary outcome and numerical secondary outcomes will be analyzed by mixed effects linear regression. The mixed effects linear regression models will include a fixed effect for treatment, a fixed effect for time point (baseline, 12 and 24 months) and a fixed effects interaction between treatment and time point. As baseline measurements are obtained before randomization, treatment at baseline will be modelled as a separate common treatment category, constraining baseline measurements to no systematic treatment effect between the two treatment arms, as suggested in literature (6). All mixed effects models will include a random intercept for each included patient. Normality assumptions on residuals and random effects will be evaluated by normal quantile-quantile plots. In case deviations from normality assumptions are detected analyses will be repeated with nonparametric bootstrapping with 1,000 bootstrapping samples (handling individual patients as bootstrapping clusters, and treatment groups as bootstrapping strata).

Due to the 12 primary outcome measures, we will apply the Bonferroni-Holm-method to adjust for multiple testing of these 12 p-values. The unadjusted p-values will be reported in the table, but it will be indicated by a * if treatment effects are still significant at the 5% level after Bonferroni-Holm-adjustment. No adjustment for multiple testing will be performed for secondary outcomes.

Missing data will be excluded from analyses in Table 2 (but see sensitivity analyses below).

		Vitamin K +D group	Placebo group		
		Mean change (95 % CI)	Mean change (95 % CI)	Treatment effect (95 % CI)	P-value
Primary outcome					

Aortic calcification score • Ascending • Arcus • Descending • Suprarenal • Infrarenal • Renal artery • Iliaca com artery • Iliaca ext artery	0 – 24 months				
Aortic diameter • Ascending • Arcus • Descending • Infrarenal	0 – 24 months				
Numerical secondary outcomes					
Aortic calcification score • Ascending • Arcus • Descending • Suprarenal • Infrarenal • Renal artery • Iliaca com artery • Iliaca ext artery	0 – 12 months and 12 – 24 months				
Aortic diameter • Ascending • Arcus • Descending • Infrarenal	0 – 12 months and 12 – 24 months				

Table 3. Dichotomous secondary outcomes

Dichotomous secondary outcome (aortic surgery between baseline and 24 months) is reported as counts and proportions separately for each treatment group and compared between groups by χ^2-test if all counts are at least 5, or with Fisher's exact test otherwise. If a patient reported multiple events of the same outcomes, this will be handled dichotomously as experiencing the outcome in the analyses, but the count of such patients will be reported in a parenthesis or footnote to the table.

Missing observations will be excluded from tests in Table 3, and number of missing observations in each group will be specified.

Event	**Vitamin K +D group (N=XX)**	**Placebo group (N=XX)**	**p-value**
Outcome			
Aortic surgery – no (%)			

Table 4. Stratified analyses of the primary outcome

Analyses of the primary outcome (change in aortic calcification score and change in aortic dilatation) will be repeated stratifying for and MGP (below / above median). The same models as for Table 2 will be applied for these analyses.

Supplementary Table S1. Sensitivity analysis: Primary outcome with imputation

If more than 5% of observations are missing at 24 months for the main analysis of the primary outcome reported in Table 2 the analysis will be repeated using multiple imputation (assuming missing at random) by linear regression with all baseline characteristics included in Table 1 as covariates generating 100 imputation sets. Results will be reported in a table structured as Table 2.

Supplementary Table S2. Sensitivity analysis: Primary outcome on subgroup with high compliance

The analysis of primary outcome will be repeated (with the same methodology as specified for Table 2) on those patients, who reported a compliance of at least 90% during the study period. Furthermore, number and proportion of patients included in this analysis will be reported in Table S5, together with odds ratio (with 95% confidence interval) and p-value determined by univariate logistic regression for difference in compliance rate between treatment arms will be reported.

		Vitamin K +D group	Placebo group	Group difference	
		Number (proportion)	Number (proportion)	Odds ratio (95 % CI)	P-value
Compliance of at least 90%					
		Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Primary outcome (only including with compliance of at least 90%)					
Aortic calcification score • Ascending • Arcus • Descending • Suprarenal • Infrarenal • Renal artery • Iliaca com artery • Iliaca ext artery	0 – 24 months				
Aortic diameter • Ascending • Arcus • Descending • Infrarenal	0 – 24 months				

Figure 1. Enrollment and Randomization of Patients.

Flow chart

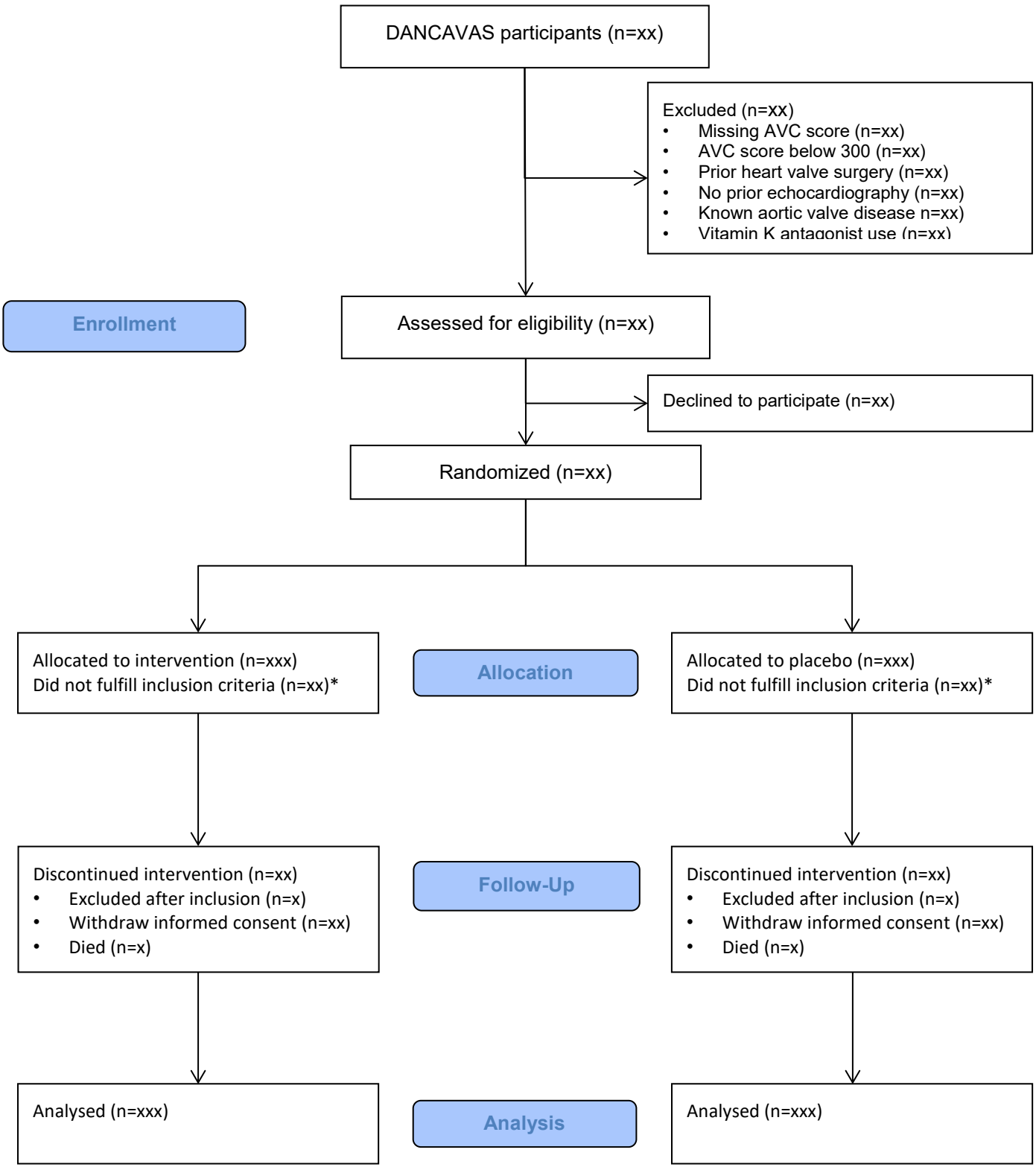


Figure 2A-H. Aortic calcification according to treatment allocation.

Aortic calcification score at baseline, 12, and, 24 months will be presented at line graph for the two treatment groups presenting mean with 95% confidence interval (as obtained from the linear mixed models applied for Table 2) for each of the 8 aortic calcification outcomes as Figures 2A to 2I. (See mockup figure 2A below, 2B-I similar)

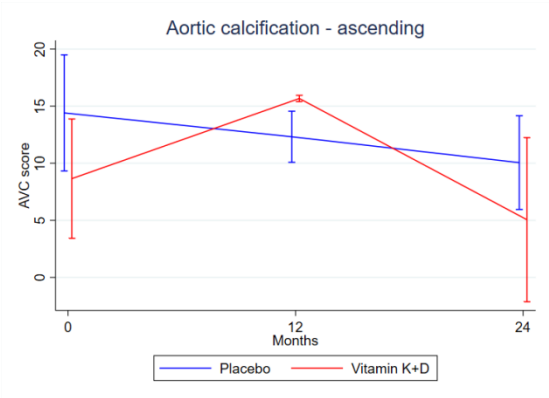
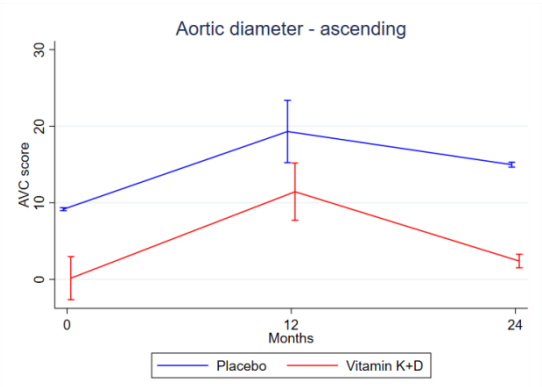


Figure 3A-D. Aortic diameter according to treatment allocation.

Aortic diameter at baseline, 12, and, 24 months will be presented at line graph for the two treatment groups presenting mean with 95% confidence interval (as obtained from the linear mixed models applied for Table 2) for each of the 4 aortic diameter outcomes as Figures 3A to 3D. (See mockup figure 3A below, 3B-D similar)



Planned analyses for secondary publication: Change in coronary artery calcification and plaque composition

Exclusion criteria

Participants with known cardiovascular disease (CVD) defined as previous acute myocardial infarction (AMI), percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) surgery will be excluded.

Outcomes

Primary outcome

The primary outcome in this substudy is the change in coronary artery calcification (CAC) score (Agatston Units) assessed by non-contrast cardiac CT scans from baseline to 24 months follow-up and will be analyzed as continuous variable in the entire population.

Secondary outcomes

The secondary outcomes are as follows:

- Change in CAC score from baseline to 24 months in two prespecified subgroups (baseline CAC score <400 and ≥ 400) (numerical)
- Change in CAC score from baseline to 12 months and from 12 to 24 months, both for the full cohort as well as for the two subgroups (numerical)
- Change in plaque volume (mm^3), composition and plaque burden parameter (%) on per-patient level assessed by contrast cardiac CT scans of total plaque, noncalcified plaque, low-attenuation noncalcified plaque and calcified plaque from baseline to 24 months (numerical)
- Change in presence of normal, non-obstructive and obstructive (>50% in left main stem or >70% in left anterior descending, left circumflex and right coronary arteries in segments >2 mm) coronary artery stenosis by visual assessment in contrast CT scans from baseline to 24 months (numerical)
- Number of patients with myocardial infarction, coronary revascularization and all-cause death in the two groups during the follow-up period (dichotomous)

Planned tables and figures and corresponding analyses

Table 1. Characteristics of patients at baseline

Statistical methods applied for table 1 are similar to the statistics used for table 1 in the primary publication.

Characteristics	Vitamin K +D group	Placebo group	p-value

	(N=XX)	(N=XX)	
Age – years [numerical]			
Body-mass index [numerical]			
Coexisting condition – no (%) Diabetes Hyperlipidemia Hypertension Atrial fibrillation Renal failure, eGFR < 60 mL/min1.73 m^2			
Family history of premature CVD			
Smoking status Active smokers - no (%) Former smokers – no (%) Non-smokers – no (%)			
HDL – mmol/L			
LDL – mmol/L			
Total cholesterol – mmol/L			
Estimated GFR – ml/min/1.73 m^2 [numerical]			
dp-ucMGP – pmol/L			
Systolic blood pressure – mmHg [numerical]			
Diastolic blood pressure – mmHg [numerical]			
Medications – no (%) ACE inhibitor or ARB Beta-blocker Mineralocorticoid-receptor antagonist Antiplatelet therapy NOAC Statin therapy			
Baseline CAC score – AU [numerical] Total population < 400 AU ≥ 400 AU			

ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, AVC aortic valve calcification, CAC coronary artery calcification, dp-ucMGP dephosphorylated-uncarboxylated Matrix Gla-Protein, GFR glomerular filtration rate, HDL high-density lipoprotein, LDL low-density lipoprotein, NOAC new-onset anticoagulant

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Primary outcome – main and secondary analyses

Statistical methods applied for table 2 are similar to the statistics used for table 2 in the primary publication. The main analysis is on mean change and treatment effect from baseline to 24 months follow-up. Secondary analyses as shown in the table are derived from the same statistical model.

	Mean change from 0 to 12 months (95%CI)		**Mean change from 12 to 24 months (95%CI)**		**Mean change from 0 to 24 months (95%CI)**		**Treatment effect from 0 to 12 months (95% CI)**	**Treatment effect from 12 to 24 months (95% CI)**	**Treatment effect from 0 to 24 months (95% CI)**	**p-value[a]**	**p-value[b]**	**p-value[c]**	**p-value[d]**
	Vitamin K+D group	**Placebo group**	**Vitamin K+D group**	**Placebo group**	**Vitamin K+D group**	**Placebo group**							
CAC score (Continuous AU)													
CAC score (baseline CAC score < 400 AU)													
CAC score (baseline CAC score ≥ 400 AU)													

p-value[a] for the treatment effect from baseline to 12 months follow-up

p-value[b] for the treatment effect from 12 to 24 months follow-up

p-value[c] for the treatment effect from baseline to 24 months follow-up

p-value[d] for the difference in treatment effect from the first 12 months to last 24 months

Table 3. Analyses on plaque composition and – burden in the total cohort

Analyses on change from baseline to 24 months on per patient level in all participants will be carried out similar to Table 2.

	Vitamin K+D group	**Placebo group**	**Group difference**	
	Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	p-value
Plaque volume and composition				
Total plaque (mm^3)				
Calcified plaque (mm^3)				
Noncalcified plaque (mm^3)				
Low-attenuation noncalcified plaque (<30 HU) (mm^3)				
Plaque burden				
Total plaque burden (%)				
Calcified plaque (%)				
Noncalcified plaque (%)				
Low-attenuation noncalficied plaque (<30 HU) (%)				

Table 4. Analysis on coronary obstruction

Change in frequency of level of coronary obstruction (no/nonobstructive/obstructive) from baseline to 24 months will be compared between treatment groups by mixed-effects ordered logistic regression including a random intercept for each patient and a fixed effects interaction between treatment and time point. As baseline measurements are obtained before randomization, treatment at baseline will be modelled as a separate common treatment category, constraining baseline measurements to no systematic treatment effect between the two treatment arms, as suggested in literature (6).

	Baseline	**24 months follow-up**	**Group difference**

	Vitamin K+D group	Placebo group	Vitamin K+D group	Placebo group	Treatment effect OR (95% CI)	p-value
Coronary obstruction						
Normal – no (%)						
Nonobstructive – no (%)						
Obstructive – no (%) *Total* *1 vessel* *2 vessel* *3 vessel*						

Table 5. Analysis on cardiovascular events

Events from baseline to 24 months follow-up will be reported as counts and proportions and will be compared between groups by logistic regression estimating OR with 95% CI.

	Vitamin K+D group	**Placebo group**	**Group difference**	
	Number (%)	Number (%)	OR (95% CI)	p-value
Events				
Myocardial infarction				
PCI procedure				
CABG surgery				
All cause death				

Supplementary table S1. Adjusted analyses of the primary outcome

Results from an adjusted analysis of the primary outcome, mean change in CAC score from baseline to 24 months follow-up. The analysis will be performed as a regression adjusting for known cardiovascular risk factors (those included in Table S1) to further investigate the treatment effect.

	Coefficient change baseline to 24 months (95% CI)	**p-value**
Intervention (Vitamin K+D)		
Predictor variable		
Age, yrs		
BMI, kg/m^2		
Diabetes		
Hyperlipidemia		
Hypertension		
Atrial fibrillation		
Renal failure		
Family history of premature CVD		
Smoking status		

Former smoker Active smoker		
CAC score ≥ 400 AU		

Supplementary table S2. Analysis on compliant patients

Analysis on patients with compliance > 90% from baseline to 24 months.

		Vitamin K +D group	**Placebo group**	**Group difference**	
		Number (proportion)	Number (proportion)	Odds ratio (95 % CI)	P-value
Compliance of at least 90%				NA	
		Mean change from baseline (95 % CI)	**Mean change from baseline (95 % CI)**	**Treatment effect (95 % CI)**	**P-value**
Primary outcome (only including with compliance of at least 90%)					
Continuous CAC score					

Supplementary table S3. Stratified analyses of the primary outcome

Stratified analyses for mean change in CAC score for the total population from baseline to 24 months follow-up in this table are similar to the statistics used for table 5 in the primary publication.

	Vitamin K+D group	**Placebo group**		
	Mean change from baseline (95 % CI)	**Mean change from baseline (95 % CI)**	**Treatment effect (95 % CI)**	**p-value**
Age < 70 years				
Age ≥70 years				
No diabetes				
Diabetes				
No hyperlipidemia				
Hyperlipidemia				
No hypertension				
Hypertension				
No atrial fibrillation				
Atrial fibrillation				
No renal failure				
Renal failure				
Non-smoker				

Active/former smoker					
No statin therapy					
Statin therapy					
dp-ucMGP (below median)					
dp-ucMGP (above median)					

Supplementary table S4. Analyses on difference in the two groups at baseline, 12 and 24 months

	Baseline		**12 months**		**24 months**		**p-value[a]**	**p-value[b]**	**p-value[c]**
	Vitamin K+D group	**Placebo group**	**Vitamin K+D group**	**Placebo group**	**Vitamin K+D group**	**Placebo group**			
Plaque volume and composition									
Total plaque (mm^3)									
Calcified plaque (mm^3)									
Noncalcified plaque (mm^3)									
Low-attenuation noncalcified plaque (<30 HU) (mm^3)									
Plaque burden									
Total plaque burden (%)									
Calcified plaque (%)									
Noncalcified plaque (%)									
Low-attenuation noncalcified plaque (<30 HU) (%)									

p-value[a] for the difference in change from baseline to 12 months follow-up

p-value[b] for the difference in change from 12 to 24 months follow-up

p-value[c] for the difference in change from baseline to 24 months follow-up

Supplementary tables S5, S6. Analyses on plaque composition and – burden in the two subgroups

Participants with CAC score < 400 AU	**Vitamin K+D group**	**Placebo group**	**Group difference**	
	Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Plaque volume and composition				
Total plaque (mm^3)				
Calcified plaque (mm^3)				
Noncalcified plaque (mm^3)				
Low-attenuation noncalcified plaque (<30 HU) (mm^3)				
Plaque burden				
Total plaque burden (%)				
Calcified plaque (%)				
Noncalcified plaque (%)				
Low-attenuation noncalcified plaque (<30 HU) (%)				

Participants with CAC score ≥ 400 AU	**Vitamin K+D group**	**Placebo group**	**Group difference**	
	Mean change from baseline (95 % CI)	Mean change from baseline (95 % CI)	Treatment effect (95 % CI)	P-value
Plaque volume and composition				
Total plaque (mm^3)				
Calcified plaque (mm^3)				
Noncalcified plaque (mm^3)				

Low-attenuation noncalcified plaque (<30 HU) (mm^3)				
Plaque burden				
Total plaque burden (%)				
Calcified plaque (%)				
Noncalcified plaque (%)				
Low-attenuation noncalcified plaque (<30 HU) (%)				

Figure 1. Enrolment and Randomization of Patients.

* Excluded due to known CVD, including MI, PCI and CABG procedure

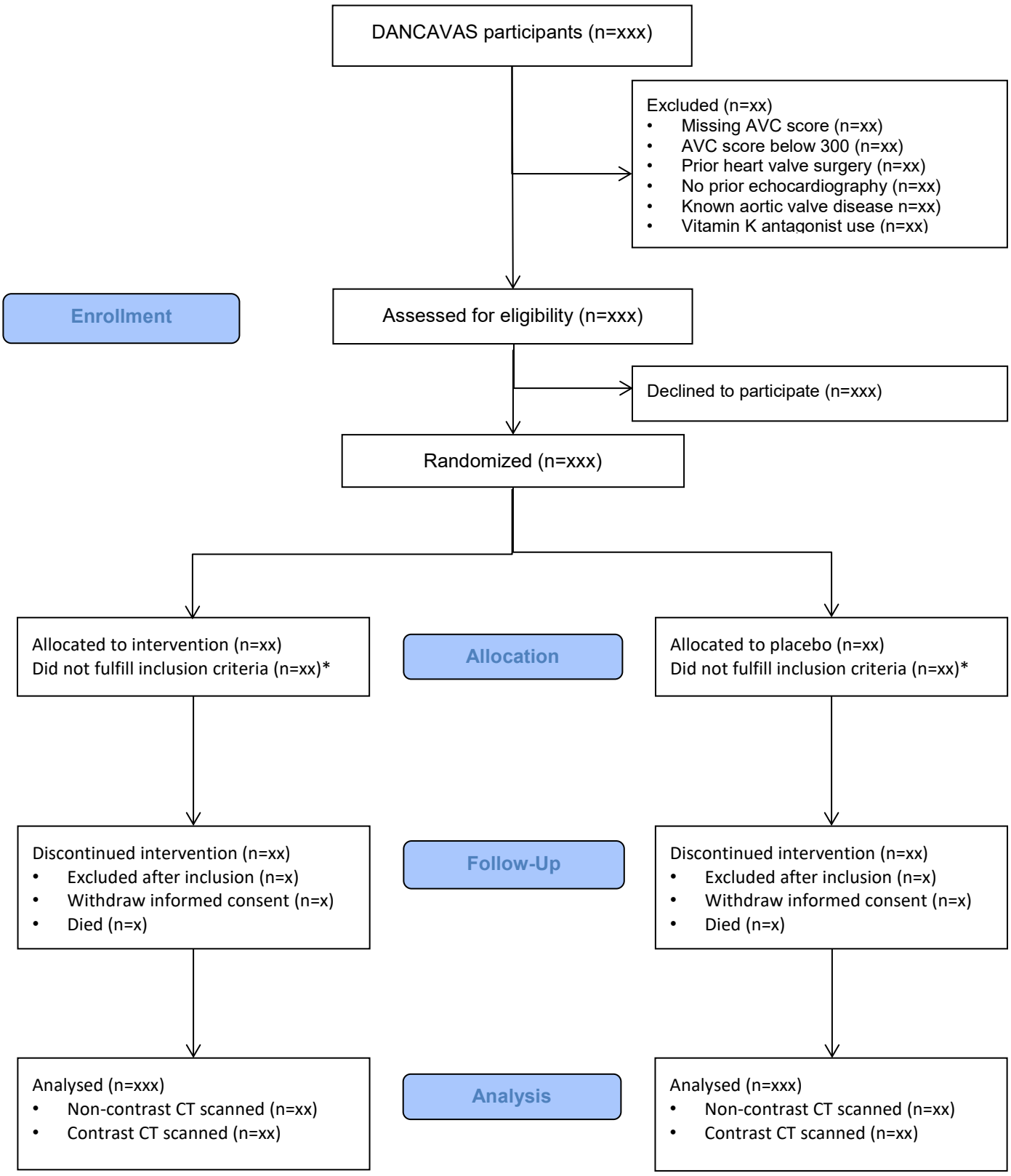


Figure 2. CAC score progression according to treatment allocation.

CAC score at baseline, 12, and 24 months will be presented as line graph for the two treatment groups presenting mean with 95% confidence interval (as obtained from the linear mixed models applied for Table 3). Moreover it will be presented for the two subgroups (CAC score < 400 and ≥400 AU). (See mockup figure 2A below, 2B and 2C are similar)

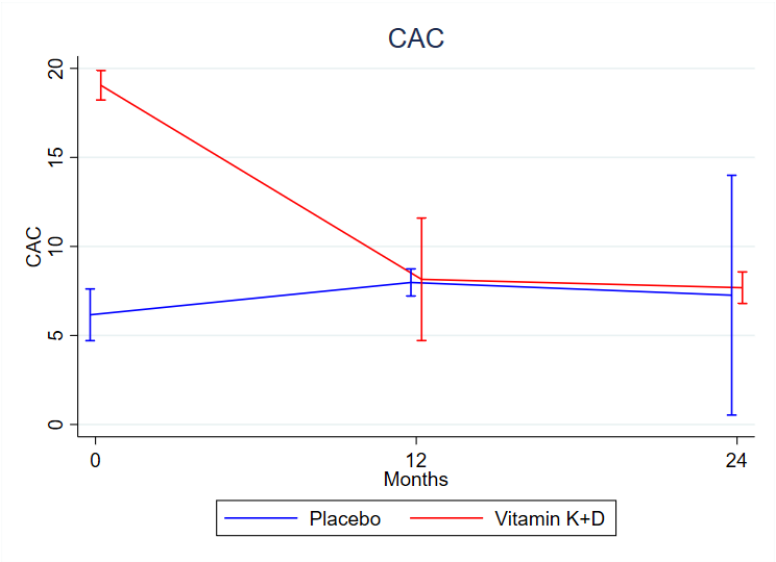


Figure 3. Change in plaque volume from baseline to 24 months

Change in plaque volume (mm^3) with the 4 groups (total, calcified, noncalcified, low-attenuation noncalcified plaque) from baseline to 24 months follow-up will be presented as a box plot to show difference between the treatment group and placebo group.

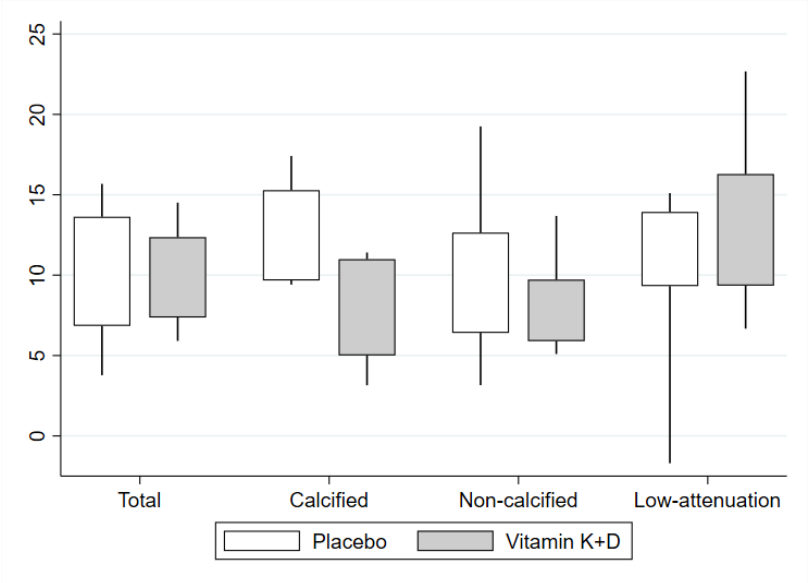
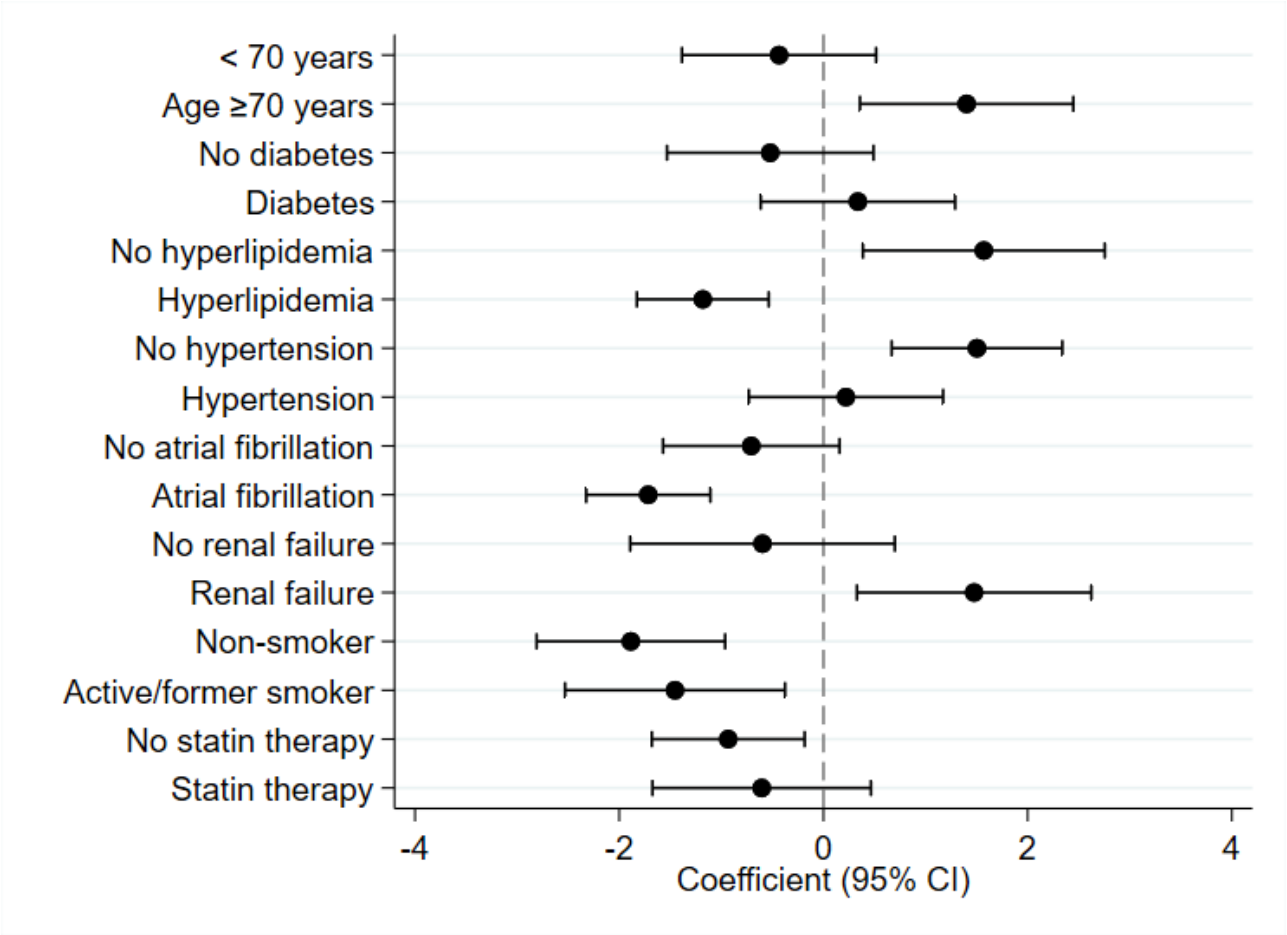


Figure 4. Forest plot of stratified analyses of the primary outcome

Coefficients with 95% confidence intervals from stratified analyses (as reported in Table 4) for differences in primary outcome (change of CAC score from baseline to 24 months) between treatment groups will be presented as a forest plot. (See mockup figure below.)



Planned analyses for secondary publication: Changes in volumetric bone mineral density in the lumbar spine and total hip

Exclusion criteria

Participants with use of prednisolone, anti-osteoporotic drugs, and patients with metabolic bone diseases (such as: prostate cancer, bone metastatic cancers, myelotomies, rheumatoid arthritis, primary hyperparathyroidism), patient with immobilization during observation will be excluded.

Outcomes

Primary outcome

- Co-primary outcome 1: change in BMD in spine from baseline to 24 month (numerical)
- Co-primary outcome 1: change in BMD in hip from baseline to 24 month (numerical)

Secondary outcomes

The secondary outcomes are as follows:

- Trabecular vBMD; total hip, femoral neck, inter- and subtrochanteric regions
- Cortical vBMD; total hip, femoral neck, inter- and subthrocanteric regions
- Cortical bone geometry and microstructure:
    - Cortical area; total hip, femoral neck, inter- and subtrochanteric regions
    - Mean Cortical thickness femoral neck; femoral neck is evaluated in 16 sectors, and mean cortical thickness is calculated and the sum of all sectors divided by 16.
- Trabecular bone geometry
    - Trabecular area; total hip, femoral neck, inter- and subtrochanteric regions
- Estimated bone strength:
    - Hip Cross sectional Moment of Intertia (CSMI)
    - Hip Section Modulus

Planned tables and figures and corresponding analyses

Table 1. Characteristics of patients at baseline

Statistical methods applied for table 1 are similar to the statistics used for table 1 in the primary publication.

Characteristics	Vitamin K +D group (N=XX)	Placebo group (N=XX)	p-value
Age – years [numerical]			
Body-mass index [numerical]			
Height [numerical]			
Weight [numerical]			
Coexisting condition – no (%) Diabetes Renal failure, eGFR < 60 mL/min1.73 m^2 Cancer Thyroid disease			
Fracture history (yes/no) – no (%)			
Calcium intake (mg) [numerical]			
Smoking status Active smokers - no (%) Former smokers – no (%) Non-smokers – no (%)			
Estimated GFR – ml/min/1.73 m^2 [numerical]			
dp-ucMGP – pmol/L			
Vitamin D			
Calcium			
Medications – no (%) Prednisolon Eltroxin Thycapzol			

dp-ucMGP dephosphorylated-uncarboxylated Matrix Gla-Protein, GFR glomerular filtration rate, † The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Main analyses

Primary and secondary numerical outcomes will be analyzed by mixed effects linear regression. The mixed effects linear regression models will include a fixed effect for treatment, a fixed effect for time point (baseline and 24 months) and a fixed effects interaction between treatment and time point. As baseline measurements are obtained before randomization, treatment at baseline will be modelled as a separate common treatment category, constraining baseline measurements to no systematic treatment effect between the two treatment arms, as suggested in literature (6). All mixed effects models will include a random intercept for each included patient. Normality assumptions on residuals and random effects will be evaluated by normal quantile-quantile plots. In case deviations from normality assumptions are detected analyses will be repeated with nonparametric bootstrapping with 1,000 bootstrapping samples (handling individual patients as bootstrapping clusters, and treatment groups as bootstrapping strata).

Missing data will be excluded from analyses in Table 2 (but see sensitivity analyses below).

Table 2 might be split into multiple tables in the resulting manuscript to ease dissemination.

		Vitamin K +D group	Placebo group		
		Mean change (95 % CI)	Mean change (95 % CI)	Treatment effect (95 % CI)	P-value
Primary outcomes					
Volumetric BMD in spine	0 – 24 months				
Volumetric BMD in hip	0 – 24 months				
Secondary outcomes					
Volumetric bone mineral density (mg/cm3)					
Trabeculært vBMD total hip	0 – 24 months				
Trabeculært vBMD femoral neck	0 – 24 months				
Trabeculært vBMD inter-trochanteric regions	0 – 24 months				
Trabeculært vBMD sub-trochancheric regions	0 – 24 months				
Cortical vBMD total hip	0 – 24 months				
Cortical vBMD femoral neck	0 – 24 months				
Cortical vBMD inter-trochanteric regions	0 – 24 months				
Cortical vBMD sub-trochancheric regions	0 – 24 months				
Bone geometry (cm2)					
Trabecular area sub-trochancheric regions	0 – 24 months				
Trabecular area femoral neck	0 – 24 months				
Trabecular area inter-trochanteric regions	0 – 24 months				
Trabecular area sub-trochancheric regions	0 – 24 months				
Cortical area sub-trochancheric regions	0 – 24 months				
Cortical area femoral neck	0 – 24 months				

Cortical area inter-trochanteric regions	0 – 24 months				
Cortical area sub-trochancheric regions	0 – 24 months				
Bone microstructure (cm)					
Cortical thickness sub-trochancheric regions	0 – 24 months				
Cortical thickness femoral neck	0 – 24 months				
Cortical thickness inter-trochanteric regions	0 – 24 months				
Cortical thickness sub-trochancheric regions	0 – 24 months				
Estimated bone strength					
CSMi (cm4)	0 – 24 months				
Section Modulus (cm3)	0 – 24 months				

Supplementary Table S1. Sensitivity analysis: Primary outcome with imputation

If more than 5% of observations are missing at 24 months for the main analysis of the primary outcomes reported in Table 2 the analysis will be repeated using multiple imputation (assuming missing at random) by linear regression with all baseline characteristics included in Table 1 as covariates generating 100 imputation sets. Results will be reported in a table structured as Table 2.

Supplementary Table S2. Main analyses adjusted for baseline characteristics

Main analysis as included in Table 2 will be repeated adjusted for D vitamin level and calcium intake at baseline as numerical covariates. The adjusted results will reported in a table structured as Table 2.

Supplementary Table S3. Mean and standard deviation of outcomes at baseline and 24 months

Mean and standard deviation (SD) for all outcomes stratified by treatment group will be reported at baseline and at 24 months.

	Baseline		24 months	
	Vitamin K +D group	Placebo group	Vitamin K +D group	Placebo group
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Primary outcomes				
Volumetric BMD in spine				

Volumetric BMD in hip				
Secondary outcomes				
Volumetric bone mineral density (mg/cm3)				
Trabeculært vBMD total hip				
Trabeculært vBMD femoral neck				
Trabeculært vBMD inter-trochanteric regions				
Trabeculært vBMD sub-trochancheric regions				
Cortical vBMD total hip				
Cortical vBMD femoral neck				
Cortical vBMD inter-trochanteric regions				
Cortical vBMD sub-trochancheric regions				
Bone geometry (cm2)				
Trabecular area sub-trochancheric regions				
Trabecular area femoral neck				
Trabecular area inter-trochanteric regions				
Trabecular area sub-trochancheric regions				
Cortical area sub-trochancheric regions				
Cortical area femoral neck				
Cortical area inter-trochanteric regions				
Cortical area sub-trochancheric regions				
Bone microstructure (cm)				

Cortical thickness sub-trochancheric regions				
Cortical thickness femoral neck				
Cortical thickness inter-trochanteric regions				
Cortical thickness sub-trochancheric regions				
Estimated bone strength				
CSMi (cm4)				
Section Modulus (cm3)				

Figure 1. Enrollment and Randomization of Patients.

Flow chart

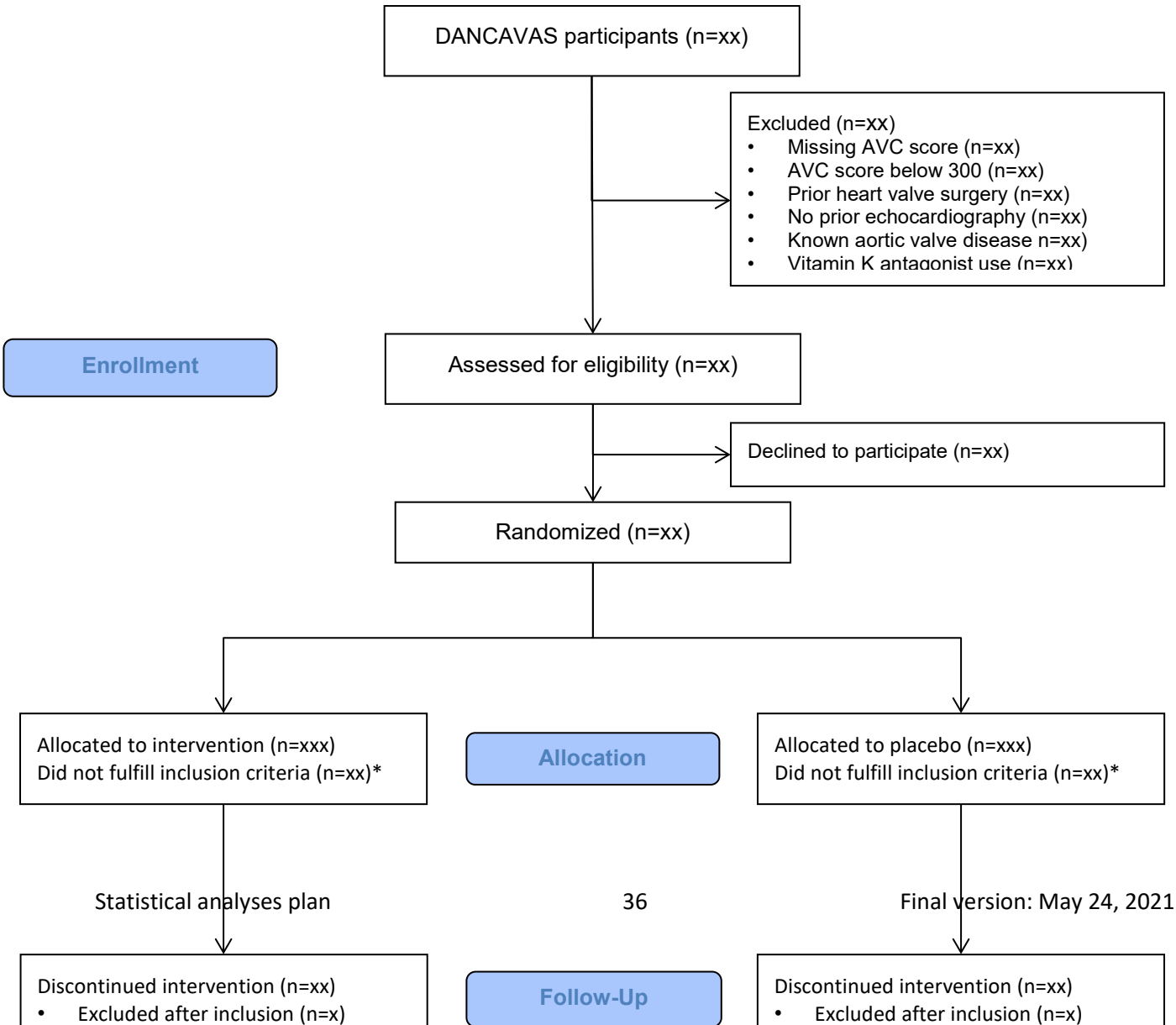


Figure 2. Change in BMD (spine and hip) from baseline to 24 months

Change in BMD spine and BMD hip from baseline to 24 months follow-up will be presented as a box plots to show difference between the treatment group and placebo group.

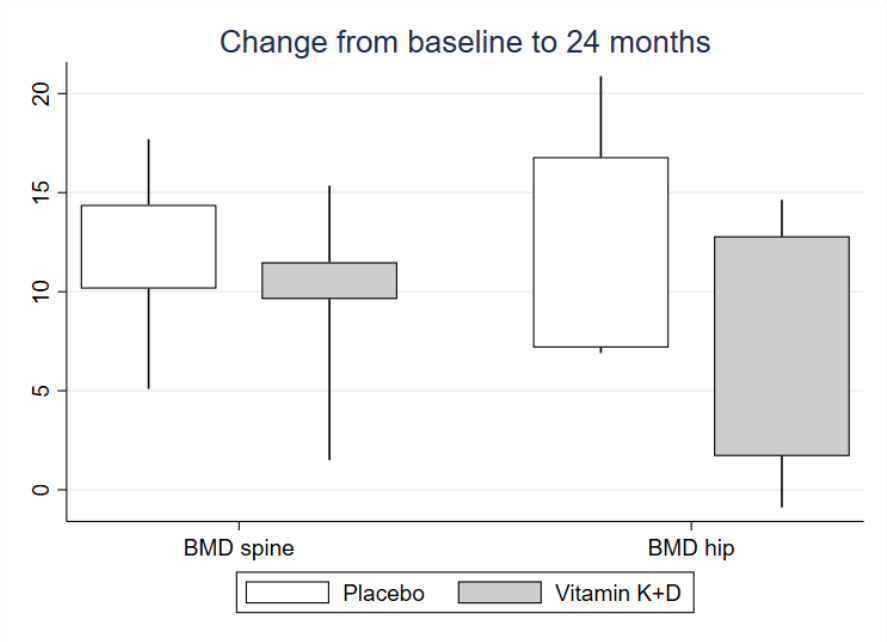
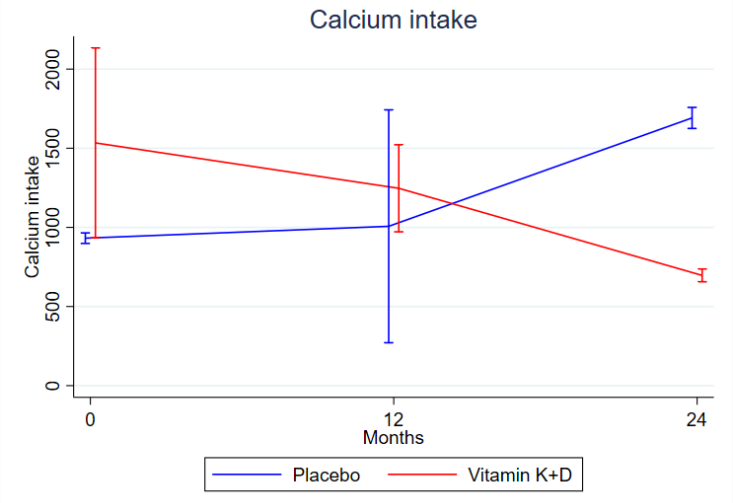


Figure 3. Calcium intake from baseline to 24 months

Calcium intake from baseline to 24 months follow-up will be presented as line graph for the two treatment groups presenting mean with 95% confidence interval obtained by fitting a linear mixed model specified as those applied for numerical outcomes in the main publication. Calcium intake is calculated as:

200 + (number of units milk x 200)+(number of units chees x 200)+(number of units fermented milk x 200)



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