Statistical Analysis Plan for the study: "The effects of Menaquinone-7 supplementation in patients with severe coronary calcifications: study protocol for a randomized controlled trial"

SAP authors: Anna Mejldal [a], Selma Hasific [b], Axel Diederichsen [a]

Affiliations:

[a] Open Patient data Explorative Network (OPEN), Odense University Hospital and Department of Clinical Research, University of Southern Denmark, Odense, Denmark

[b] Department of Cardiology, Odense University Hospital, Odense, Denmark

Introduction

This document specifies the planned statistical analysis for the study "The effects of Menaquinone-7 supplementation in patients with severe coronary calcifications: study protocol for a randomized controlled trial" as carried out following the protocol published at ClinicalTrials.gov Identifier: NCT05500443.

Sample size considerations

According to the protocol (1): "We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control per experimental subject. In the AVADEC trial, the mean (standard deviation) two-year CAC progression among 182 men with CAC score ≥ 400 was 380 AU (330 AU) in the placebo group and 288 AU (280 AU) in the intervention group. The joint standard deviation was 311 AU. If this is true in a population of men and women, inclusion of 180 experimental subjects and 180 control subjects are needed to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05 (two-sided). Accordingly, 360 subjects are needed. However, to comply with the uncertainty and to account for drop-out of 10%, 400 patients will be included. The sample size is based on two years of treatment."

Randomizations

Randomization is performed by the pharmacy at Odense University Hospital. Based on a computer-generated assignment scheme the tablets will have a random number according to the sequential order.

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 are collected using REDCap hosted by OPEN (Open Patient data Explorative Network, Odense University Hospital, Odense Denmark) with project number OP_1703. Data are analyzed using Stata on OPEN's secure analysis server (OPEN Analyse). A prespecified random seed of 319846 will be used for bootstrapping and/or imputation if these methods are deemed nescessary.

Planned analyses for primary publication

Outcomes

Primary outcome: Main analysis

The primary outcome is the change in coronary artery calcification (CAC) score (numerical) from baseline to 24 month for the full cohort of included patients.

Secondary outcomes

Secondary outcomes are:

- Change in CAC score from baseline to 24 months in men and women, respectively
- Change in CAC score from baseline to 24 months in two pre-specified subgroups (baseline CAC score <1000 and ≥ 1000)
- Change in coronary plaque composition by contrast CT from baseline to 24 months
- Change in calcifications in the aortic valve by non-contrast CT from baseline to 24 months
- Cardiac events (non-fatal myocardial infarction, coronary revascularization, and cardiac death) during the follow-up period
- Change in quality of life from baseline to 24 months.

An exploratory endpoint is:

- Change in MGP with different phosphorylation (p and dp) and carboxylation forms (c and uc).

Safety outcomes

Safety outcomes are:

- Death
- Cardiovascular events (myocardial infarction, coronary revascularization, heart valve surgery, stroke, significant aortic disease (dissection, rupture and surgery) and significant peripheral artery disease (thromboembolisms and surgery)
- Venous thromboembolism including pulmonary embolism
- Bleeding (including intracranial bleeding and hemorrhage associated with a drop in hemoglobin of ≥ 2mmol/l)
- Cancer, including solid and hematologic
- Significant deterioration in laboratory measurements (hemoglobin, creatinine (eGFR), sodium, potassium, calcium, magnesium, albumin, phosphate, alkaline phosphatase, parathyroid hormone or prothrombin time-international normalized ratio (PT-INR)).

Dichotomous safety outcomes are evaluated as occurred / not occurred between baseline and 24 months. Numerical safety outcomes are 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.

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.

Counts and proportions for categorical characteristics (with categories as specified in Table 1 below).

As the intervention is randomized, no differences between groups will be estimated. Number of missing observations in each group will be specified.

Characteristics	**Vitamin K +D group (N=XX)**	**Placebo group (N=XX)**
Age – years [numerical]		
Body-mass index – kg/m^2 [numerical]		
Coexisting condition – no (%) • Diabetes • Hyperlipidemia • Hypertension • Renal failure, eGFR < 60 mL/min1.73 m^2		
Family history of premature CVD - no (%)		
Smoking status - no (%) • Active smokers • Former smokers • Non-smokers		
Total cholesterol – mmol/L [numerical]		
HDL – mmol/L [numerical]		
LDL – mmol/L [numerical]		
Estimated GFR – ml/min/1.73 m^2 [numerical]		
dp-ucMGP – pmol/L [numerical]		
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] • 400-999 AU - no (%)		

• ≥ 1000 AU - no (%)		

ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, 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

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).

All available data will be used in the analyses in 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
Primary outcome				
CAC score (all patients)				
Numerical secondary outcomes				
CAC score (men)				
CAC score (women)				
CAC score (baseline CAC score 400-999)				
CAC score (baseline CAC score ≥1000)				
AVC score (all patients)				
dp-ucMGP				
Quality of life				

Table 3. Secondary outcome on plaque composition

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)				

Table 4. Dichotomous secondary outcome and adverse events

Dichotomous secondary outcomes 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.

All available data will be used in the analyses in Table 2, and number of missing observations in each group will be specified.

Event	**Vitamin K +D group (N=XX)**	**Placebo group (N=XX)**	**p-value**
Secondary outcome			
Cardiovascular events (combined) – no (%) • Non-fatal myocardial infarction • Coronary revascularization • Cardiac death			
Adverse Events			
Any event – no (%)			
Death – no (%)			
Cardiovascular events (combined) – no (%) • Myocardial infarction • Coronary revascularization • Heart valve surgery • Stroke • Significant aortic disease (dissection, rupture and surgery) • Significant peripheral artery disease (thromboembolism and surgery)			
Venous thromboembolism – no (%)			
Severe bleeding – no (%)			
Incident cancer – no (%)			

Table 5. 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**	**Group difference**	
	Mean change (95 % CI)	Mean change (95 % CI)	Treatment effect (95 % CI)	P-value
hemoglobin				
creatinine (eGFR)				
carbamide				
sodium				
potassium				
calcium				
magnesium				
albumin				
phosphate				
Alkaline phosphotase				
parathyroid hormone				
prothrombin time-international normalised ratio (PT-INR)				

Supplementary Table S1. Stratified analyses of the primary outcome

Analyses of the primary outcome (progression in CAC score) will be repeated stratifying for age (below or above mean age at baseline), diabetes (yes / no at baseline), hypertension (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 CAC score 400-999 and ≥1000. 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
Below mean age				
Above mean age				
No diabetes				
Diabetes				
No hypertension				
Hypertension				
No statin therapy				
Statin therapy				
No renal failure				
Renal failure				
Baseline MGP < median				
Baseline MGP >= median				

Supplementary Table S2. 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 mixed effects linear regression with all baseline characteristics included in Table 1 as covariates generating 100 imputations. Results will be reported in a table structured as Table 2.

	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 with imputation				
CAC score (all patients)				

Supplementary Table S3. Reasons of abandoning the study

	Vitamin K+D group (n=)	Placebo group (n=)
Excluded after inclusion		
Withdraw informed consent		
• Significant comorbidity		
• Lost interest		
• Experienced side effect		
• Personal reasons		
Died		

Figure 1. Enrollment and Randomization of Patients

Flow chart

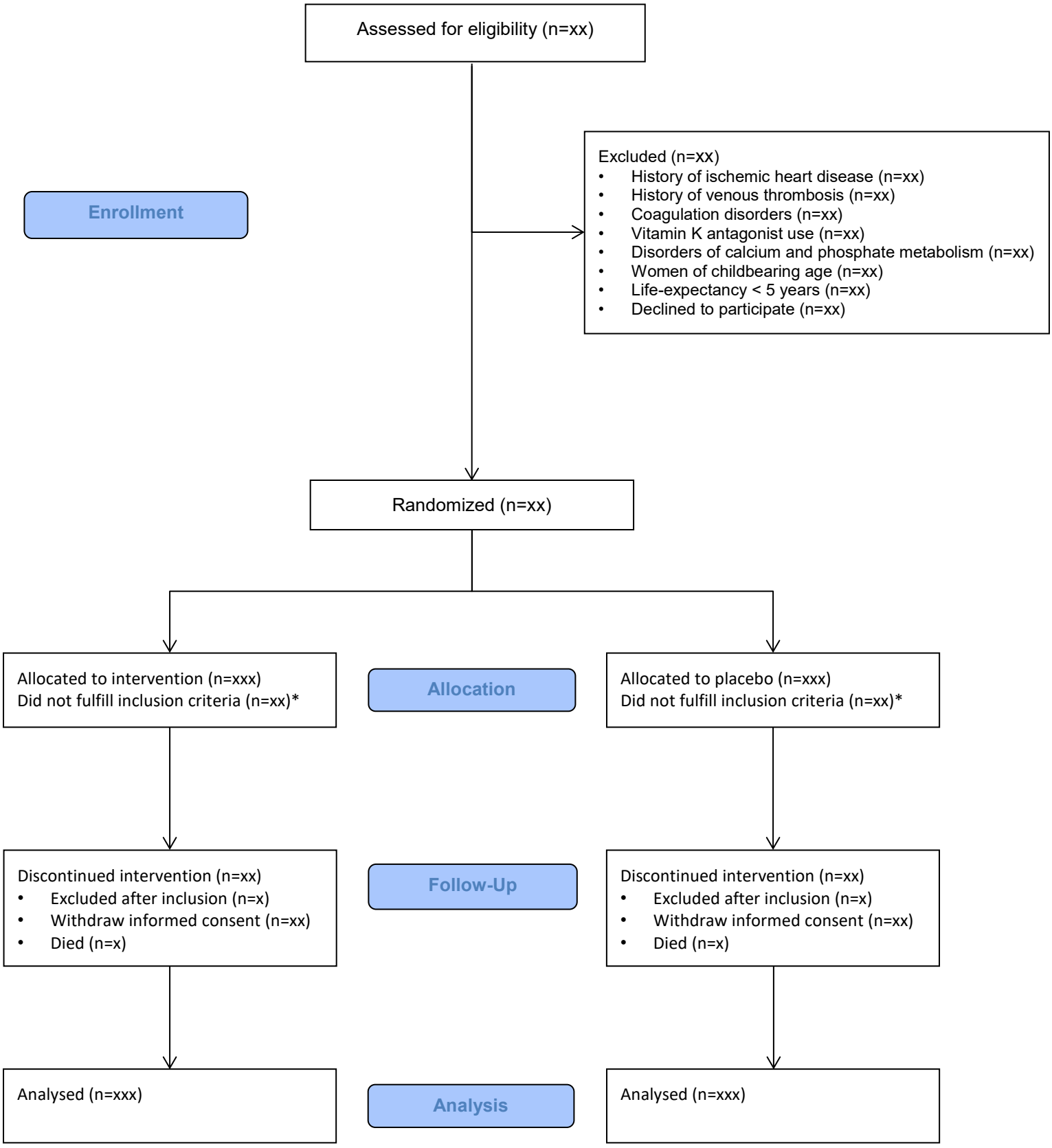

Figure 2. CAC progression according to treatment allocation

CAC score at baseline, 12, and, 24 months will be presented in a graph, where the two treatment groups are plotted with their means and 95% confidence intervals (as obtained from the linear mixed models applied for Table 2). The figure will be presented for all patients (Figure 2A) as well as stratified by the baseline CAC score subgroups 400-999 (Figure 2B) and ≥1000 (Figure 2C). (See mockup figure 2A below, 2B and 2C are similar)

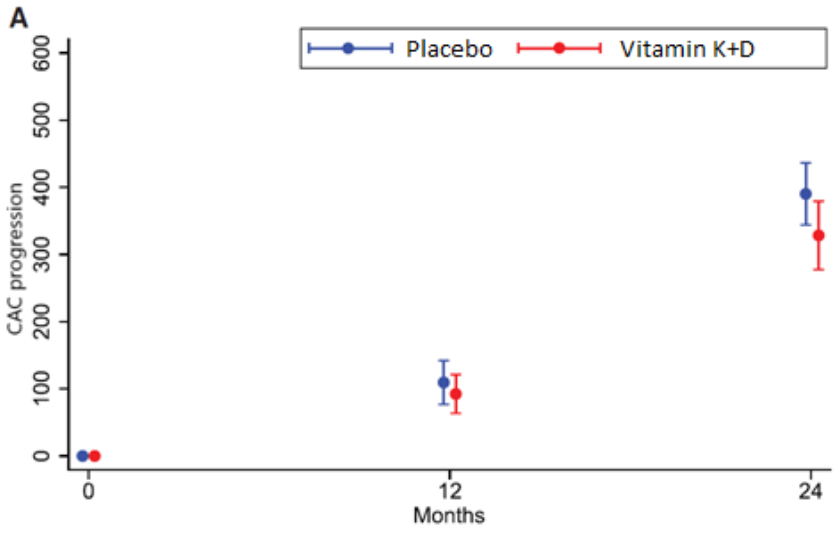

Figure 3. Change in plaque volume from baseline to 24 months

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.

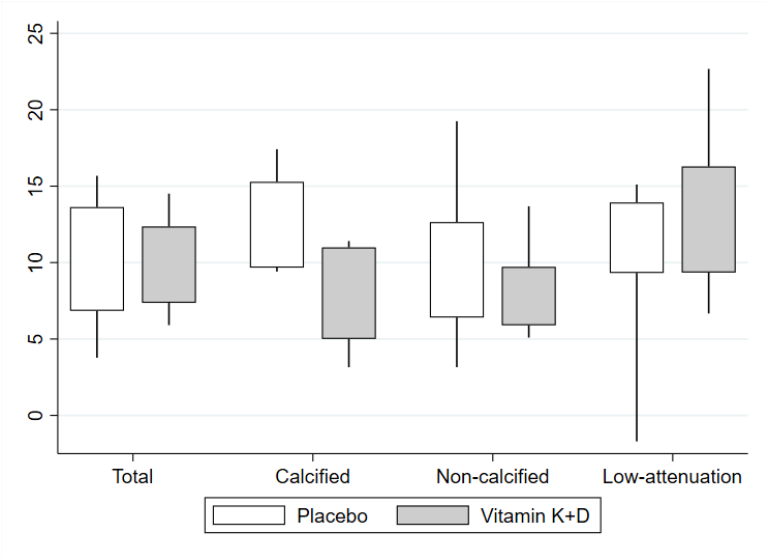

Figure 4. Forest plot of stratified analyses of the primary outcome

Coefficients with 95% confidence intervals from stratified analyses (as reported in Supplementary table 1) 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.)