Statistical Analysis Plan for the Danish Cardiovascular Screening Trial II

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Introduction

This document specifies the planned statistical analysis for the Danish Cardiovascular Screening (DANCAVAS) Trial II, as carried out following the protocol published at the Clinical Trials Registry: NCT03946410

Sample size considerations

According to the protocol: "A study with 4 controls per invited subject is planned based upon the mortality reduction in the VIVA study, a hazard ratio of 0.93 in the screening group is expected. If so, with a 0.05 significance level, and 90% power, we´ll need 5245 experimental subjects and 20980 controls."

Randomizations

Subjects were randomized 1:3.

Statistical principles

All analyses were performed as intention-to-screen and as superiority analyses. The endpoints were compared for the two groups using a Cox hazard regression for analysis of unadjusted hazard ratios (95% confidence intervals). Both relative and absolute risk estimates will be reported, as well as the number needed to invite (NNI) in order to save one life will be estimated using Newcombe's method (ref https://pubmed.ncbi.nlm.nih.gov/19519911/). Two-sided p-values of 0.05 or less are considered to indicate statistical significance.

Practical considerations

Screening data were collected using REDCap hosted by OPEN (Open Patient data Explorative Network, Odense University Hospital, Odense Denmark) with project number OP_122. Outcome data, including death, hospitalization and medical prescription, were collected from the Danish nationwide registries. Data were analyzed using Stata on OPEN's secure analysis server (OPEN Analyze).

Planned analyses for the primary publication: Benefits and harms of the randomized, clinical controlled Danish Cardiovascular Screening (DANCAVAS) II trial of 60-64 year old men

Inclusion criteria

All men, aged between 60 and 64 years, living in the involved communities at date of randomization.

Exclusion criteria

There were no exclusion criteria in the study.

Outcomes

Primary outcome

- All-cause mortality (time to event or censoring), assessed at December 31 2024.

Secondary outcomes

- Stroke; all, ischemic, hemorrhagic, and unspecified after randomization (time to event or censoring), assessed at December 31 2024.
- Myocardial infarction after randomization (time to event or censoring), assessed at December 31 2024.
- Amputation due to vascular disease after randomization (time to event or censoring), assessed at December 31 2024.
- Aortic dissection, any site after randomization (time to event or censoring), assessed at December 31 2024.
- Aortic rupture, any site after randomization (time to event or censoring), assessed at December 31 2024.

Explanatory outcomes

- Attendance rate
- Initiation and adherence to preventive medications after randomization: antithrombotic agents, anticoagulation, lipid-lowering agents, antihypertensive, and antidiabetics
- Elective aortic aneurysm repair after randomization

Safety outcomes

- Major intracerebral and gastrointestinal bleeding leading to hospitalization after randomization (time to event or censoring), assessed at December 31 2024
- Cardiac revascularization, peripheral vascular revascularization and aortic repair after randomization (time to event or censoring), assessed at December 31 2024
- Incident cancer from 6 months[1] after randomization (time to event or censoring), assessed at December 31 2024
- Mortality after cardiovascular surgery (30 days)
- Change in quality of life (QoL) after randomization

[1] Incident cancer is registered as a safety outcome to examine if the screening examination and intervention may induce cancer, and as cancer might be an incidental finding in the screening examination we will have a wash-out period consisting of the first 6 months after randomization.

Planned tables and figures and corresponding analyses

Table 1. Baseline characteristics

Characteristics of the participants will be reported separately for the two randomized groups: invited to screening versus control group, and within the invited to screening group: participants versus non-participants.

	Randomly assigned groups		**Within the invited to screening group**		
Characteristic	**Invited to screening (N=XX)**	**Control group (N=XX)**	**Participants (N=XX)**	**Non-participants (N=XX)**	**P value**
Age – years [numerical]					
Prescription of medical treatment the last year before randomization • Anti-thrombotic agents – no (%) • Anticoagulants – no (%) • Lipid-modifying agents – no (%) • Antihypertensive agents – no (%) • Antidiabetic agents – no (%)					
Hospital admission during the last five years before randomization • Stroke – no (%) • Ischemic heart disease – no (%) • Peripheral occlusive arterial disease – no (%) • Aortic aneurysms – No (%)					

Ischemic heart disease: myocardial infarction and coronary revascularization

Table 2. Findings by screening, in all and by specific condition	N (%)	95% CI
CAC SCORE		
0		
≥ 0– 99		
≥ 100		
≥ 400		
≥ 1000		
ANEURYSMS		
Ascending aorta ≥ 45 mm		
Aortic arch ≥ 40 mm		
Descending aorta ≥ 35 mm		
Abdominal aorta ≥ 30 mm		
Iliac arteries ≥ 20 mm		
PERIPHERAL ARTERIAL DISEASE (PAD)		
Lowest ABI < 0.90 or Highest ABI ≥ 1.4		
Lowest ABI < 0.90		
Highest ABI ≥ 1.4		
ATRIAL FIBRILATION		
Confirmed by ECG		
DIABETES MELLITUS		
HgBA1C ≥ 48 mmol/mol		
UNKNOWN HYPERCHOLESTROLAEMIA		
Total Cholesterol ≥ 8.0 mmol/l		
POTENTIAL UNKNOWN HYPERTENSION		
Systolic BP ≥ 160 mmHg and/or diastolic BP ≥ 100 mmHg		
Initiated antihypertensive treatment within 6 months after randomization		
TOTAL POSITIVE FINDINGS		

Table 3. Primary and secondary outcomes

Primary and secondary outcome will be analyzed and compared between randomization groups, all outcomes representing a time to event using a Cox proportional hazards model. Time of randomization defines the onset of risk time and exit from analysis is time of event or censoring on 12-31-2024 whichever came first. Deaths without secondary events are right censored. The model's assumption about proportional hazards will be assessed on the basis of the Schoenfeld residuals and visual inspection of log-log plots of outcome versus analysis of time. Only the first event of each category is counted.

Effects of the DANCAVAS screening on mortality and cardiovascular outcomes.

Outcome	**Invited to screening (N=XX)**			**Control group (N=XX)**			**Hazard Ratio (95% CI)**	**p value**	**NNI (95%)**
	Events No (%)	Years at risk Median (IQR)	no. of events per 1000 person-years	Events No (%)	Years at risk Median (IQR)	no. of events per 1000 person-years			
Primary outcome									
All-cause mortality									
Secondary outcome									
MACE*									
CVD-specific mortality									
Stroke									
• Ischemic									
• Hemorrhagic									
• Unspecified									
Myocardial infarction (AMI)									
MALE**									
Lower limb revascularization									
major lower limb amputation									

NNI; number needed to invite

*: Cerebro-coronary vascular-morbidity defined as a composite endpoint of Major Cardiovascular Events (MACE): CVD specific mortality, stroke, AMI.

**Peripheral vascular-morbidity defined as a composite endpoint of Major Lower Limb Events (MALE): Lower limb revascularization or major lower limb amputation

Table 4. Exploratory outcomes

Explanatory outcomes are reported as counts separately for each group and compared between groups by hazard ratio (95% CI). Individuals who had received a relevant prescription within 1 year before randomization were excluded from analyses. Time of randomization defines the onset of risk time and exit from analysis is time of event or censoring on 12-31-2024 whichever came first. Deaths without events are right censored.

Event	**Invited to screening (N=XX)**			**Control group (N=XX)**			**Hazard Ratio (95% CI)**	**p value**
	Events No (%)	Years at risk Median (IQR)	no. of events per 1000 person-years	Events No (%)	Years at risk Median (IQR)	no. of events per 1000 person-years		
Initiation* of antithrombotic agents								
Initiation* of anticoagulation								
Initiation* of lipid lowering agents								
Initiation* of antihypertensive agents								
Initiation* of antidiabetic agents								
Elective aortic aneurysm repair								

* No prescription the last year before randomization

Table 5. Safety outcomes

Safety outcomes are reported as counts separately for each group and compared between groups by hazard ratio (95% CI). Time of randomization defines the onset of risk time and exit from analysis is time of first event or censoring on 12-31-2024 whichever came first. Deaths without secondary events are right-censored.

	Invited to screening (N=XX)			**Control group (N=XX)**			**Hazard Ratio (95% CI)**	**p value**
	Events – no (%)	Years at risk Median (IQR)	no. of events per 1000 person-years	Events – no (%)	Years at risk Median (IQR)	no. of events per 1000 person-years		
Severe bleeding								
- Intracerebral bleeding								
- Gastrointestinal bleeding								
Cancer								
Cardiac revascularization								
Peripheral vascular revascularization								
Aortic repair								
Mortality after cardiovascular surgery (30 days)								

Table 6. Stratified analyses of the primary outcome

Analyses of the primary outcome (all-cause mortality) will be repeated stratifying for Educational age, cardiovascular disease, stroke, ischemic heart disease, heart failure, peripheral occlusive arterial disease, aortic aneurysms, hypertension, diabetes mellitus and lipid lowering therapy. The same model as for the primary outcome (Table 2) will be applied for these analyses.

	Invited to screening (N=XX)	**Control group (N=XX)**	**Hazard Ratio (95% CI)**	**P value**
	no. of events per 1000 person-years			
Psychiatric disease • Yes • No				
Cardiovascular disease* • Yes • No				
Stroke* • Yes • No				
Ischemic heart disease* • Yes • No				
Heart failure* • Yes • No				
Peripheral occlusive arterial disease* • Yes • No				
Aortic aneurysms* • Yes • No				
Hypertension at baseline • Yes • No				
Diabetes mellitus at baseline • Yes • No				
Lipid lowering therapy at baseline • Yes • No				

* Hospital admission during the last five years before randomization

Cardiovascular disease: stroke, myocardial infarction, coronary revascularization, heart failure, peripheral occlusive arterial disease and aortic aneurysms

Ischemic heart disease: myocardial infarction and coronary revascularization

Table 7. Adherence to preventive medications

Adherence to a medication is defined as medication possession ratio (MPR) of at least 80% from 1st redeemed prescription over a time period of 3 years. Values below 80% will be considered as non-adherence. Individuals who failed to redeem prescriptions during the first three years after randomization will not be eligible for analysis, and only individuals who redeemed at least one relevant prescription can be included in the analyses. Five medication groups will be considered: anti-thrombotic agents (A), anticoagulants (B), lipid-lowering agents (C), antihypertensive (D), and antidiabetics (E). The adherence results will be presented as relative risks with 95% confidence intervals.

Drug class (ATC)	Non-adherent patients in control population (n (non-adherent)/n (total) (%))	Non-adherent patients in screening population (n (non-adherent)/n (total) (%))	Relative risk of non-adherence (RR (95%CI))
Anti-thrombotic agents (B01AC)			
Anticoagulants (B01AA, B01AE, B01AF)			
Lipid-lowering agents (C10)			
Antihypertensive (C03A, C03B, C07, C08 excl C08DA, C09)			
Antidiabetics (A10)			

Figure 1. Enrollment, Randomization, and Follow-up.

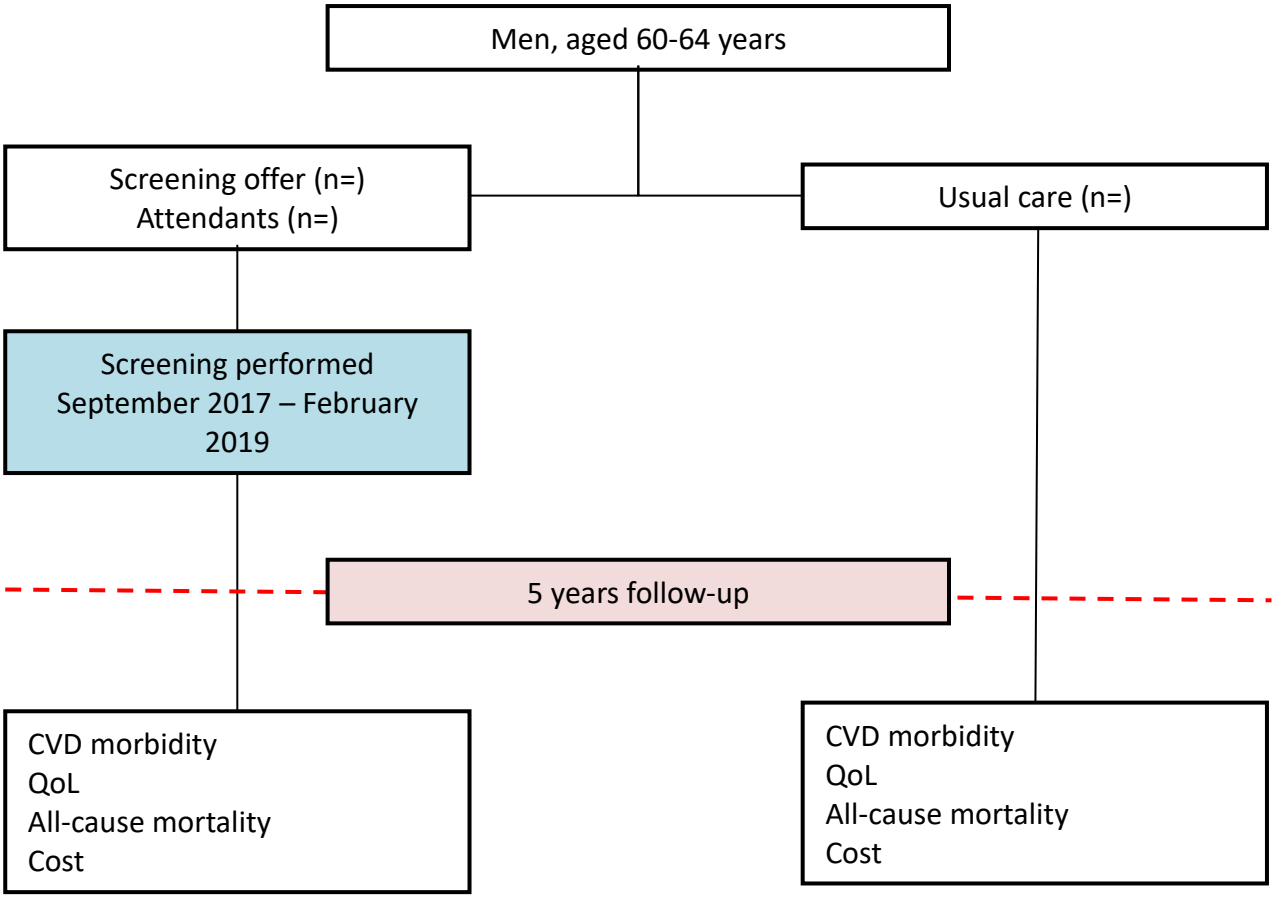

Figure 2. Effects of the DANCAVAS screening on primary and secondary outcomes.

Cumulative event curves from the two randomized groups will be generated with the use of the Nelsom-Aalen cumulative hazard estimates. The primary outcome will be shown in Panel A, while the composite secondary outcomes (MACE (B), MALE (C), will be shown separately in Panel B-C. See mockup Panel A below, remaining panels are similar.

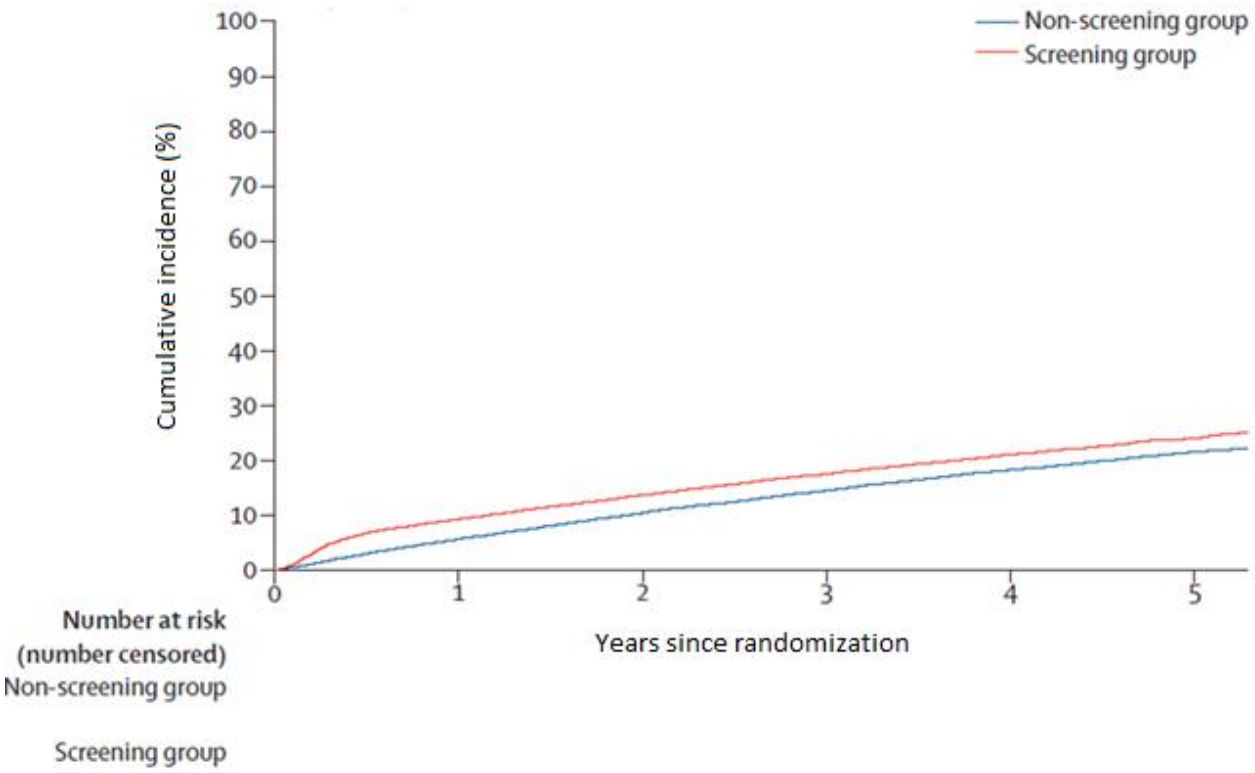

Figure 3. Initiation of preventive actions

Six plots demonstrating initiation of preventive actions in the two randomized groups will be generated. Baseline numbers will be individuals who have not redeemed a prescription for anti-thrombotic agents (A), anticoagulants (B), lipid-lowering agents (C), antihypertensive (D), and antidiabetics (E), respectively, the last year before randomization. Panel F illustrates elective aortic aneurysm repair. See mockup Panel A below, remaining panels are similar.

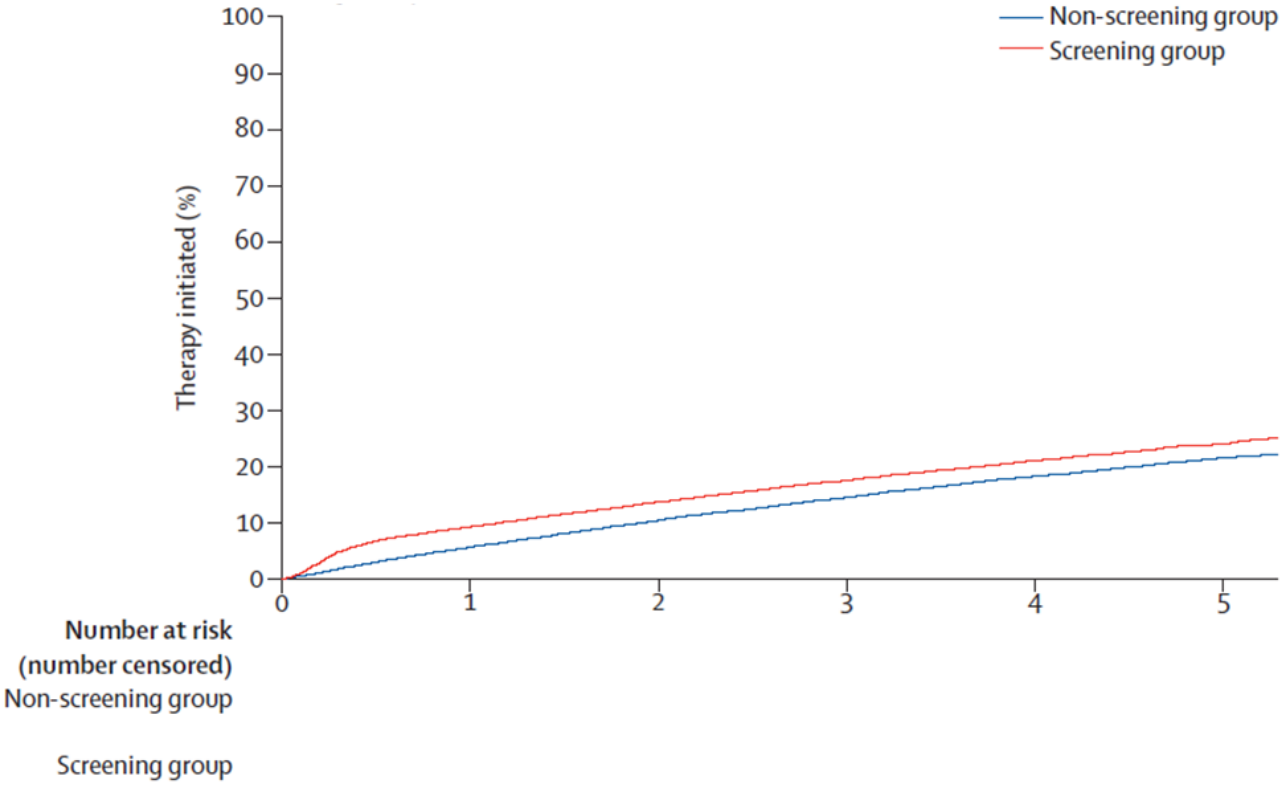

Figure 4. Forest plot of stratified analyses of the primary outcome

Hazard Ratio with 95% confidence intervals from stratified analyses (as reported in Table 5) for differences in all-cause mortality between randomization groups will be presented as a forest plot. See mockup figure below.