PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effects of Menaquinone-7 supplementation in patients with aortic valves calcification: study protocol for a randomized controlled trial
AUTHORS	Lindholt, Jes; Frandsen, Niels Erik; Fredgart, Maise; Øvrehus, Kristian; Dahl, Jordi; Møller, Jacob; Folkestad, Lars; Urbonaviciene, Grazina; Becker, Søren; Lambrechtsen, Jess; Auscher, Søren; Hosbond, Susanne; Alan, Dilek; Rasmussen, Lars; Gerke, Oke; Mickley, Hans; Diederichsen, Axel

VERSION 1 – REVIEW

REVIEWER	Marie-Annick Clavel Université Laval, Canada
REVIEW RETURNED	22-Feb-2018

GENERAL COMMENTS	The manuscript described the methods of a very interesting ongoing trial on the use of Metaquinone-7 supplementation to slow down aortic stenosis progression rate. The rational is clear and protocol well describe. The study is well planned. Regarding the hypothesis, the authors wrote: … “in patients with severe valve calcification, but without aortic valve stenosis.” Actually, severe calcification has been defined to identify severe aortic stenosis (ESC guidelines 2017). “significant” or “substantial” calcification would be better than “severe”. Regarding statistics, often aortic valve calcification, as well as the evolution of it are non-normal variables.

REVIEWER	Marcello Rattazzi Department of Medicine - DIMED, University of Padova, Italy
REVIEW RETURNED	25-Feb-2018

GENERAL COMMENTS	The study investigates the effect of Menaquinone-7 supplementation in slowing the progression of aortic valve calcification. The rationale of the study is solid and the methodological approach is adeguate. I don't have specific comments.

REVIEWER	Susan Xu Houston Methodist, USA
REVIEW RETURNED	28-Mar-2018

GENERAL COMMENTS	The authors stated that analysis of variance (ANCOVA) will be used. But there is also another factor, time, and I think it will be interesting to see the changes over time as well. Please incorporate this into the statistical analysis.

REVIEWER	Antonella Zambon University of Milan-Bicocca - Italy
REVIEW RETURNED	04-Apr-2018

GENERAL COMMENTS	I have only few issues for the authors. 1) In their sample size estimation they did not consider the drop-out. Why? 2) How did the authors face the problem of missing data? 3) Did the authors consider the loss of power due to unbalances among centres in the estimation of sample size?

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:
Reviewer: 1
The manuscript described the methods of a very interesting ongoing trial on the use of Metaquinone-7 supplementation to slow down aortic stenosis progression rate. The rational is clear and protocol well describe. The study is well planned.
Regarding the hypothesis, the authors wrote: … "in patients with severe valve calcification, but without aortic valve stenosis." Actually, severe calcification has been defined to identify severe aortic stenosis (ESC guidelines 2017). "significant" or "substantial" calcification would be better than "severe".
Authors' response: We agree, and have changed to substantial (p2 and p5).

Regarding statistics, often aortic valve calcification, as well as the evolution of it are non-normal variables.
Authors' response: Thank you for this comment. We agree that aortic valve calcification most likely follows a distribution which is skewed to the right, whereas differences over time become more symmetrically distributed. The main point, though, is that the Central Limit Theorem secures the test statistic to be roughly normally distributed, independently of the distribution of patients' individual scores, as long as N is 'large enough' (where 'large enough' means at least 30 observations in simple test settings, see for instance Bowerman: Business Statistics in Practice (8th ed.), McGraw-Hill). The application of general linear models will, if deemed appropriate, also enable the conduct of negative binomial regression analysis on the primary endpoint, considering it as a count variable.

Reviewer: 2
The study investigates the effect of Menaquinone-7 supplementation in slowing the progression of aortic valve calcification. The rationale of the study is solid and the methodological approach is adeguate. I don't have specific comments.
Authors' response: Thank you.

Reviewer: 3
The authors stated that analysis of variance (ANCOVA) will be used. But there is also another factor, time, and I think it will be interesting to see the changes over time as well. Please incorporate this into the statistical analysis.
Authors' response:
Thank you for this comment. We incorporated group x time point interaction into the analysis (p7).

Reviewer: 4
I have only few issues for the authors.
1) In their sample size estimation they did not consider the drop-out. Why?

Authors' response: Thank you for this comment. We have now adapted the sample size statement appropriately. Additionally, we have made a more precise power calculation. All participants in the trial are recruited from the huge population based screening trial, DANCAVAS (n>10 000). The participants were included in DANCAVAS from 2014 until 2017, and in this study a non-contrast CT scan has been performed. We have presently included 50 participants of the DANCAVAS participants in the AVADEC trial, and we have now compared the old DANCAVAS CT scan with the new AVADEC CT scan. Among these participants the aortic valve calcification score increased with 50 Units per year. In the AVADEC protocol we estimated a progression rate among the placebo treated participants at 32 Units in two years. We have now used an expected progression rate among the placebo treated participants at 100 Units per two years. (p7)

2) How did the authors face the problem of missing data?
Authors' response: Missing data will be treated as such without applying imputation techniques; however, we added supplementary sensitivity analyses using imputation techniques (under the missing at random assumption) for the primary endpoint if more than 5% of expected data points will be missing (p7).

3) Did the authors consider the loss of power due to unbalances among centres in the estimation of sample size?
Authors' response: No; the rationale for doing so was to recruit a representative, consecutive series of patients who are roughly proportionally distributed across the four centers compared to demand in daily routine. We do not aim at a comparable recruitment rate at all participating centers …..

VERSION 2 – REVIEW

REVIEWER	Marie-Annick Clavel Université Laval (Québec, Canada)
REVIEW RETURNED	15-May-2018

GENERAL COMMENTS	The authors have answer my questions. I have no further points.

REVIEWER	Susan Xu Houston Methodist Research Institute, USA
REVIEW RETURNED	03-May-2018

GENERAL COMMENTS	The primary endpoint is the change in AVC score from baseline to two years. However, the sample size calculation was based on testing the two group difference at the end of two years, it didn't reflect the change over time. Would it be better to calculate sample size based on repeated measurements analysis of variance?

REVIEWER	Antonella Zambon Department of Statistics and Quantitative Methods - University of Milan-Bicocca
REVIEW RETURNED	28-May-2018

GENERAL COMMENTS	No comments

VERSION 2 – AUTHOR RESPONSE

Reviewer comments to Author:

Reviewer: 3

The primary endpoint is the change in AVC score from baseline to two years. However, the sample size calculation was based on testing the two group difference at the end of two years, it didn't reflect the change over time. Would it be better to calculate sample size based on repeated measurements analysis of variance?

Authors' response:

We contrast an expected progression of 100 units over time (change from baseline to 24 months) in Placebo patients with an assumed reduction by 20%, meaning a progression of 80 units over the 2-year interval, in patients provided with additional MK-7 treatment. To this end, we actually do focus on change over time, but we acknowledge that the sample size considerations could alternatively be extended to also include assumptions on the 12-month time point. We refrained from doing so since input from the DANCAVAS study (see Section 3.5) was based on only N=37 subjects in the first place and the sample size assessment is more conservative when only employing 2 (instead of 3) time points (which we deem more appropriate taking the uncertainty of a priori knowledge into consideration).

We added more information on the data basis in Section 3.5: …The mean annual AVC progression is unknown, but based on data from 37 subjects of the DANCAVAS study. (p6)