PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The Effect of Magnesium Supplementation on Vascular Calcification in Chronic Kidney Disease - A Randomised Clinical Trial (MAGiCAL-CKD): Essential Study Design and Rationale
AUTHORS	Bressendorff, Iain; Hansen, Ditte; Schou, M; Kragelund, Charlotte; Brandi, Lisbet

VERSION 1 - REVIEW

REVIEWER	Markus Ketteler Klinikum Coburg GmbH, Coburg, Germany
REVIEW RETURNED	04-Apr-2017

GENERAL COMMENTS	This study addresses an important clinical research topic, and the authors must be congratulated to have achieved funding for this project. Magnesium supplementation indeed has the potential to become a cost-effective remedy in order to reduce the cardiovascular risk burden in CKD patients, but so far no RCT has addressed this question. I just have a few remarks: - As far as I understand the protocol, the authors intend to also include subjects with a CAC score of 0. This will probably lead to a dilution of the results, as these subjects are very unlikely to develop calcifications within one year. Most studies use a cut-off score of > 30 or > 100 (volume score), because these individuals are likely progressors. So my recommendation would be to limit randomization to pre-calcified patients and recalculate the recruitment numbers. - Are patients on vitamin K-antagonists excluded? If not, these individuals should be distributed equally in both arms. - Does atrial fibrillation interfere with the CAC readings? - FGF23 is briefly discussed - I would like to recommend including FGF23 measurements, because such results could help interpreting the phosphate associated risk for calcification in this particular study setting. Another test of some potential importance would be measuring urinary phosphate excretion. The T50 test will be an excellent biomarker of for the purpose described in this protocol.

REVIEWER	Mariano Rodriguez Hospital Reina Sofia IMIBIC, University of Cordoba Spain
REVIEW RETURNED	06-Apr-2017

GENERAL COMMENTS	This manuscript describes a clinical trial designed to evaluate the effect of Mg supplementation o the progression of vascular calcification in patients wit CKD (GFR between 45 and 15 ml/min.

	The rational and the justification of the study is solid. I do not know whether the authors will be able to demonstrate an effect but certainly the study deserves to be performed. The design is appropriate but I would suggest examination (by CT) of the aorta down to the abdomen. The evaluation of compliance of big vessels should correlate better with calcification in large vessels that in coronary arteries. It is known that an increase in serum Mg reduces PTH secretion which in turn affects phosphaturia. This should be taken into consideration. Patients without evidence of vascular calcification are less likely to progress that those with moderate amount of vascular calcification. Wonder if the authors should have a minimum number of patients with some vascular calcification to enter the study. Wonder if patients with anticoagulant therapy should be excluded Provide some funds to generate a biobank. Provide mechanisms to assure a follow up of patients after the termination of the study, it would be on¡f interest to know the incidence of cardiovascular events and mortality .

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

• As far as I understand the protocol, the authors intend to also include subjects with a CAC score of 0. This will probably lead to a dilution of the results, as these subjects are very unlikely to develop calcifications within one year. Most studies use a cut-off score of > 30 or > 100 (volume score), because these individuals are likely progressors. So my recommendation would be to limit randomization to pre-calcified patients and recalculate the recruitment numbers.

We agree with the reviewer's comments regarding progression of calcification, and we debated this issue during the development of the trial. We came to the conclusion that it would be too costly to use CT-scans during the enrolment process and instead adjusted the sample size calculation to account for this. We estimated that approximately half of all screened patients would have coronary artery calcification at baseline (i.e. patients likely to have progression of calcification) and that we would therefore need twice as many subjects as would be necessary if only "calcified" patients were enrolled.

• Are patients on vitamin K-antagonists excluded? If not, these individuals should be distributed equally in both arms.

Subjects are stratified based on site and diabetes (yes/no), but not for use of vitamin K-antagonists. We have assumed that with given the moderately large sample size the distribution of subjects with vitamin K-antagonists would even out between the two treatment groups.

• Does atrial fibrillation interfere with the CAC readings?

In general, atrial fibrillation is an issue when performing CT-scans for CAC, however, this is due the risk of the flash scan being run during systole such that motion artifacts complicate CAC measurements. We have already experienced a few subjects with atrial fibrillation (which was first discovered when the subjects were ECG-monitored in conjunction with the CT-scan) who had low enough heart rates that it was possible to perform the CT-scan during diastole with no motion artifacts. Therefore, we have not specified atrial fibrillation as an exclusion criterion, but rather let it be up to the local site investigators to judge, whether it would be possible to perform the CT-scan and get good CAC measurements.

• FGF23 is briefly discussed - I would like to recommend including FGF23 measurements, because such results could help interpreting the phosphate associated risk for calcification in this particular study setting. Another test of some potential importance would be measuring urinary phosphate excretion. The T50 test will be an excellent biomarker of for the purpose described in this protocol.
We have added FGF23 and urinary phosphate excretion to the list of measurements, see figure 3 in the revised manuscript.

Reviewer 2:
• The design is appropriate but I would suggest examination (by CT) of the aorta down to the abdomen. The evaluation of compliance of big vessels should correlate better with calcification in large vessels that in coronary arteries.
Unfortunately, we have already included almost a third of the study population and it is therefore not possible to scan the full aorta in these subjects. We will, however, have segments of the abdominal aorta (levels L1 to L3) available in a approximately 1/3 of subjects, and will be able to measure changes in aortic calcification in these subjects.
• It is known that an increase in serum Mg reduces PTH secretion which in turn affects phosphaturia. This should be taken into consideration.
We have added urinary phosphate excretion to the list of measurements, see figure 3 in the revised manuscript.
• Patients without evidence of vascular calcification are less likely to progress that those with moderate amount of vascular calcification. Wonder if the authors should have a minimum number of patients with some vascular calcification to enter the study.
See comments for reviewer 1 above.
• Wonder if patients with anticoagulant therapy should be excluded.
See comments for reviewer 1 above.
• Provide some funds to generate a biobank.
Blood samples for serum and plasma as well as samples from a 24-hour urine excretion will be collected at week 0 and week 52 and stored at -80° for future use, see figure 3 in the revised manuscript.
• Provide mechanisms to assure a follow up of patients after the termination of the study, it would be of interest to know the incidence of cardiovascular events and mortality.
Subjects will be followed-up after 5 years, in order to assess incidence of cardiovascular events, death and ESRD. This has been added to the revised manuscript.