Author's response to reviews

Title: Impaired renal function impacts negatively on vascular stiffness in patients with coronary artery disease

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Author's response to reviews: see over
Reply to Reviewers

We would like to thank both Reviewers for their thorough evaluation of our paper. We would like to apologise for the delay in submitting a revised version of this paper. This was related to the need to acquire additional data on calcium and phosphate from samples at the time of endothelial function testing. All changes in the manuscript are highlighted yellow.

Reviewer: Tomasz Zapolski

In the article entitled: “Impaired renal function impacts negatively on vascular stiffness in patients with coronary artery disease” Authors have demonstrated that even in patients with advanced atherosclerotic disease, concomitant renal impairment is associated with a further increase in vascular stiffness. The manuscript is well written. This is an original and interesting paper providing the reader to new issue of pathogenesis of impaired renal function in arterial stiffness. Nevertheless, to become suitable for publication, the Authors should raise the manuscript profile adding some relevant additional details and comments.

We would like to thank Dr Zapolski for his favourable evaluation of our paper and the thorough review.

Discretionary Revisions

1. Atherosclerosis is regarded as a combination of two separate diseases: atherosclerosis and sclerosis. The arterial stiffness reflects sclerotic component and means mechanical properties of arterial wall. Calcium overload, which is characteristic feature for deterioration of renal function, is associated with arterial stiffening. Vascular calcification is detected either in the tunica intima or in the tunica media. Calcification in the intima is characteristic of most stages of atherosclerosis. Medial calcification is particularly common in patients with renal dysfunction and may occur independently of atherosclerosis. Medial wall calcification increases vascular stiffness and reduces arterial compliance. That’s why the analysis including markers coexisting with renal impairment as calcium level, calcium phosphorus score etc. is also recommended. This may probably support the finding of the present study that osteopontin is independently associated with vascular stiffness.

Thank you for these important comments. We fully agree that we have not discussed calcification adequately in our paper. We have probably over-simplified our paper in that we acknowledged the role of vascular calcification in later stages of renal disease but have not analysed its role in our patients with mild-to-moderate CKD. Embarrassingly this is against our own thinking, as much of our ongoing research is looking into the role especially of phosphate on vascular function in patients with CKD.
We have now extended our analysis and assessed calcium and phosphate levels in our patients at the time of their vascular studies.

In keeping with our other work, we have found a significant correlation between phosphate levels and PWV. Calcium levels and the Ca x PO4 product were not significantly associated with PWV in our cohort. We have therefore added phosphate to our regression models but after adjustment for age and the other cofactors it was not a significant determinant of eGFR in this study.

Thank you also for pointing out that these additional data could help to show the calcium-independent association between osteopontin and PWV. We were indeed able to show this.

We have, in reply to the Reviewer's comments, added data on calcium and phosphate to tables 1 and 4, added calcium and phosphate to the regression analysis in the results section and added a sentence on the independence of the association between osteopontin and vascular stiffness of calcium and phosphate in the discussion. We have also added scatterplots on phosphate to table 2.

2. Endothelial dysfunction is a crucial precursor of the development of cardiovascular disease. The endothelium maintains the balance between vasoconstriction and vasodilatation. The role of the endothelium in controlling the vascular tone, especially vasodilatation, has been shown via the endothelial-derived nitric oxide (NO). Decreased NO production has also been linked to progression of renal dysfunction. Accelerated vascular damage and defective vascular repair have been proposed as a mechanism for premature atherosclerosis and arterial stiffness, common findings among patients with deterioration of renal function. Impairment of NO biosynthesis (e.g., by ADMA) or NO bioactivity (as with oxygen-derived free radicals) causes endothelial vasodilatation dysfunction. Thereby, the correlations between arterial stiffness and oxygen-derived free radicals promoters such as T-chol, LDL-chol, hs-CRP, should be also included into present study. The deleterious effects of T-chol, LDL-chol and hs-CRP in part a consequence of decreased availability of endothelium-derived NO, include smooth muscle proliferation, collagen synthesis, and deterioration of elastin which may impair arterial compliance. Surprisingly the total cholesterol and LDL-cholesterol levels were lower among patients with CAD when compared to control group. Why? Did CAD patient treat with statins? If yes, this may influence the inflammatory status in this group. Similarly in CAD group there were much more patients with diabetes, known inflammatory factor.

Thank you also for these comments. We fully appreciate a number of paradoxical findings in our data that the Reviewer mentioned. In particular, the lower lipid levels in patients with CAD is counterintuitive and clearly, as suggested by the Reviewer, related to statin therapy. We have encountered this problem previously (e.g. Delles C et al. Atherosclerosis 2010;211:271-7). As long as we do not strictly control drug therapy in these patients, which would be associated with major ethical challenges, we cannot directly analyse the contributions of lipids and statin treatment.

We have now acknowledged this limitation in the discussion.

With regard to the comment on CRP we apologise for our oversight. Clearly CRP should have analysed in the same way as other biomarkers in table 4 that reach significant univariate correlation
factors in table 4. When adjusted for other determinants of PWV, however, CRP did not contribute to vascular stiffness in our study.

We have now added scatterplots on CRP to Figure 2. We have also mentioned that CRP did not contribute to the PWV in the results section on regression models.

Reviewer: Ziyad Al-Aly

This is a study by Rossi and collaborators that examined whether renal function is associated with vascular stiffness in patients with CAD. The authors conclude that renal function is a determinant of vascular stiffness even in patients with severe atherosclerotic disease.

We would like to thank Dr Al-Aly for his favourable evaluation of our paper and the thorough review.

I have few comments:

The hypothesis underpinning the research question is not sufficiently clear. Also, the research question is not very clear to me either. The authors state in the introduction that the aim is to examine whether renal function and cad have additive effect, but the design of the statistical analyses does not seem suitable to address the research question satisfactorily.

The authors may consider undertaking formal interaction analyses to 1). Examine whether cad status modified the association between eGFR and PWV and 2) examine whether eGFR variable modifies the association between cad and pwv. The analyses currently undertaken and the results as presented simply do not rigorously support the conclusion of the authors.

Thank you very much for this comment. We fully appreciate that our aims were not described precisely enough and that the statistical analysis that we employed do not currently address "additive effects". We have addressed the Reviewer's comments by (a) rewording the introduction slightly; and (b) by adding formal interaction analysis.

We have modified the abstract and the introduction slightly, added a description of the analysis to the methods section and added a paragraph to the first section of results.