Author's response to reviews

Title: Novel Association Patterns of Cardiac Remodeling Markers in Patients with Essential Hypertension and Atrial Fibrillation

Authors:

Andreas S Kalogeropoulos (andkalog@gmail.com)
Sotirios Tsiodras (tsiodras@med.uoa.gr)
Angelos G Rigopoulos (angelos.rigopoulos@otenet.gr)
Eleftherios A Sakadakis (elsakad@yahoo.gr)
Andreas Triantafyllis (andtridoc@yahoo.gr)
Dimitrios Th Kremastinos (kremastinos@gmail.com)
Ioannis Rizos (ioannis.c.rizos@otenet.gr)

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Author's response to reviews: see over
Dear Professor Gelpi,

We have carefully examined all the issues that were kindly raised by the referees regarding the manuscript that we recently submitted for consideration for publication to the *Cardiovascular Disorders Journal*, entitled “Novel Association Patterns of Cardiac Remodeling Markers in Patients with Essential Hypertension and Atrial Fibrillation” by Kalogeropoulos et al.

With respect to the manuscript content we proceeded in some elucidatory changes (typed in blue colour in the manuscript).

- In the paragraph of **statistical analysis**, we added further information regarding the exact method we used to confirm that the patients with atrial fibrillation (AF) had no intervening periods of sinus rhythm (SR). All patients had several electrocardiograms (ECGs) that were carried out by their supervising physician during their follow up. Accordingly, no documented period of sinus rhythm (SR) was observed during this follow up period.

- In the **discussion** section, we further analysed the possible pathophysiological pathways that are implicated behind the higher serum levels of matrix metalloproteinase-9 (MMP-9) in the subjects with permanent AF.

- In the **study limitations** section we clearly stated that further assessment of cardiac remodeling and oxidative markers in atrial tissue samples is necessary in order to confirm our results. Furthermore, our immunoassay
approach that did not have the capacity of differentiating the pro-forms and active forms of matrix metalloproteinases subtypes is considered as a limitation of our study. However, the later does not affront the value of our findings given that the primary target of our research effort was to reveal any potential associations of AF subtypes with different patterns of cardiac remodelling markers and our results to be further confirmed in other prospective research efforts or by assessing the aforementioned markers in tissue samples.

Please find in the next pages the specific reply to each comment of each referee. I would be more than willing to respond should you have any questions regarding the aforementioned changes and comments.

Yours sincerely,

Andreas S. Kalogeropoulos, MD
Department of Cardiology
Hammersmith Hospital
Imperial College of Healthcare, NHS Trust
Du Cane Road, W12 0HS,
London, United Kingdom
Phone number: 00447548669965
Fax number: 00442035658348
E-mail: andkalog@gmail.com
Response to referee #1

1. We agree with the perception that the measurement of MMPs with an immunoassay approach that could differentiate the pro- and active forms of MMPs subtypes would enhance the significance of our findings and is considered as one of the limitations of our study as it is stated in the corresponding segment of our manuscript. Nonetheless, the latter does not reduce the scientific value of our results. More specifically, our primary target was to study the interrelation patterns of MMPs with different types of AF (paroxysmal and permanent), in a specific and homogeneous AF population involving patients with essential hypertension as the sole cardiovascular disorder responsible for AF triggering. Indeed, we clearly showed that paroxysmal AF is associated with higher levels of MMP-2, whilst permanent AF with higher levels of MMP-9. Furthermore, in our retrospective analysis we showed that higher MMPs levels are associated with an increased incidence of AF in patients with essential hypertension. We believe that our findings could serve as a significant pool of informative data for the conduction of further prospective research protocols, as well as for studies in tissue samples of patients with AF, aiming to confirm and enlighten the aforementioned results. Finally, we would be more than willing to proceed to the re-assessment of the active forms of the MMPs, however the majority of our samples have already been used for other research protocols.
2. We agree that the additional assessment of the oxidative stress by measuring the serum levels of malondialdehyde would also add supplementary information with respect to the higher MMP-2 levels in patients with paroxysmal AF. We believe that this could be the subject of a future study and more interestingly of a study in tissue samples of patients with AF. However, the latter is extremely difficult to be done given the acute and unpredictable nature of paroxysmal AF. Furthermore as we previously mentioned the majority of our samples have already been consumed in other research protocols of our group and subsequently is not feasible to proceed to the additional measurement of serum malondialdehyde as it was kindly requested.
Response to referee #2

1. We agree with the conception that angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) have anti-inflammatory properties and would subsequently alter the inflammatory and fibrotic profile of the AF subjects in our study. Therefore, all the study participants were in anti-hypertensive treatment with ACEIs and ARBs for at least a year, in order to avoid as much confounding as possible. Additionally, we deliberately did not include ACEIs and ARBs doses given that the latter was exclusively determined by the supervising physician and could had been changed over the time. Accordingly, it was impossible for us to reliably document the doses and to determine the dose effect of the aforementioned drugs in our results.

2. We clearly stated in the statistical analysis section that there was no documented intervening period of sinus rhythm in patients with permanent atrial fibrillation and that was confirmed with regular basis electrocardiograms that the patients underwent during their follow up.

3. In the discussion section of the manuscript we further analysed the possible reasons for the increased matrix metalloproteinase-9 (MMP-9) levels in patients with permanent AF. Numerous experimental and clinical studies (reported in the manuscript’s reference’s segment) have shown the higher load of inflammation, tissue stretch and pro-fibrotic processes in patients with permanent AF. All these factors have been implicated in
triggering the expression and activation of matrix metalloproteinases including MMP-9 in patients with permanent AF. Additionally, angiotensin has also been shown to potentially induce a higher expression of MMP-9 and that its blockade could inhibit this process. However, our patients were all under ACEIs and ARBs treatment suggesting that other additional pathophysiological mechanisms could be involved behind the increased levels of MMP-9 in patients with permanent AF.