

Author's response to reviews

Title: Heart rate variability after radiofrequency ablation of epicardial ganglionated plexuses on the ovine left atrium

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Author's response to reviews:

We do appreciate the insights of all the reviewers and are especially thankful to Pangiota Flevari for a positive review. We tried to react to the issues addressed with our best. Answers to reviewers' questions are given in this letter and corrections are done to the manuscript as requested. Best regards to all from our research team.

To Lianjun Gao (reviewer 1):

1) RFA of GA was performed in one group, the results showed some parameters were back to before over time. But how to confirm the complete elimination of GA by RFA? Maybe surgical dissection of the tissue (for example, the fat pad) on LA epicardium. With the verification complete elimination of GP, the results could be more convincing.

Due to the network like structure of cardiac ganglionated plexus described in our previous work (3, 4), multiple GP's could be described as a single cardiac plexus with varying density of distribution throughout the heart, rather a sort of localised autonomic structures as ganglionated plexuses (subplexuses). This could explain why damage to significant part of the network leads to temporary shift in the autonomic balance. Thereby we did not aim to prove destruction of any specific part of the cardiac plexus (any of specifically named GP) and specifically targeted one of the most densely innervated parts of LA containing not only a significant amount of GPs but also a number of important neural tracts to increase the overall damage to intrinsic cardiac innervation. We believe number of applications of RF energy and visual verification of tissue damage was sufficient to assure any structures including neural were ablated in target area where thickness of sheep atrium wall is as thin as 1-2 mm. More over damage to neural structures using the same methodology was verified histologically in our previous anatomical studies (3, 4) which led to this physiological evaluation of the effects of RFA. Density of GP also varies greatly between the individuals in sheep as we found in the past studies.

2) Control groups might be not comprehensive. I think that a group of sheep just experienced pericardial space opened and dorsal LA area revealed is necessary.

This is a good insight worthy of future implementation to any following studies, unfortunately not available for this project anymore. On the other hand, thoracotomy and opening of pericardial sack alone would affect HRV in short term, most likely days until healing (sheep were free running in 20-30 minutes after the operation, and all sutures were removed in 4-5 days as fully healed), not weeks or months.

3) Pulmonary vein isolation also affects the GP densely situated around PV roots, in other words, endocardial ablation could injure the GP outside to some extent. If possible, the endocardial ablation should be performed in one added group.

Since most of the sheep ganglia are situated epicardially, endocardial ablation would not add to the extent of the damage to the neural structures. Due to thickness of sheep atrium wall of 1-2 mm, transmural ablation could be achieved both endocardially or epicardially.

4) The authors described the change of HRV after epicardial RFA, the clinical significance needs to be discussed more.

Our group of co-authors (including clinical electrophysiologists performing AFib ablations) decided not to make far reaching speculations about clinical significance and role in the treatment of AFib, because the purpose of our study was to find out how ablation influences HRV in a chronic 1-year experiment.

To Agnieszka Noszczyk - Nowak (reviewer 2):

1) There is no local ethics committee approval.

We received a permission from the National Food and Veterinary Department which is responsible for the licenses for the large animal (sheep, cow, etc.) studies (the same as local ethics committee is obligatory for other studies).

2) Introduction of the article is very poor, not presenting the current state of knowledge about the ablation of the ganglion plexus (GP). No recently published articles (2017) were cited.

We added knowledge and recent publications about GP ablation to discussion part of the article.

3) Why did the sheep were anesthetized with halothane? Isoflurane/sevoflurane would be much better and safer option.

We used unified complex anaesthesia for all our animal studies proposed and supervised by an anaesthesiologist. No adverse effects of anaesthesia were observed.

4) How did you check the effectiveness of the ablation you performed?

We had visual control of lesion on the atrial tissue at the site of ablation. RF parameter correlation with lesion transmural and visual changes of tissue were confirmed in pre-study experiments.

5) How was the position of the electrode controlled during ablation and the presence of GP in this area?

Position was controlled by direct visual-anatomical control. Presence of GP's in ablation area was confirmed in our previously published histological studies.

6) Lack of information about the number of applications performed during ablation.

We provided the numbers under the 'Methods' section at a line 14: 'Ten to fifteen RF energy applications of 20 W power, 500 kHz frequency were applied for 30 s under direct visual control with the +50°C tissue temperature safety cut-out.

7) Lack of atrial effective refractory period (AERP) before and after ablation of each GP site.

We did not have the aim to check other intraoperative EP parameters because they are incomparable in chronic study.

8) Lack of histopathological examination of the left atrium during the follow-up and after follow-up period.

Histopathological evaluation during the follow-up was not planned as the aim of the study was to evaluate changes in functional parameters reflecting ANS state after ablation of cardiac ganglionated plexus. Material for histopathological evaluation was taken after the follow up period and will be presented at the consequent study.

9) No information on the exact conditions under which the Holter monitoring took place.

Animals were monitored under conditions as close as possible to normal – they were mostly free roaming in a large pen in the pasture if weather conditions permitted, if not (snow or rain) - monitoring was performed in a barn.

10) Figures are unreadable. Number of animals and standard deviation (SD) are missing. HRV results during the follow-up period were not discussed. No explanation was given on the return of those parameters to the baseline values, what would present the regaining ANS function. Conclusions seem to be only a presumption. The hypothesis was not confirmed by histopathological study.

Conclusion section was modified.

11) I would like you to mention about the AFACT study in discussion (Ganglion Plexus Ablation in Advanced Atrial Fibrillation: The AFACT Study. Driessen AHG, Berger WR, Krul SPJ, van den Berg NWE, Neefs J, Piersma FR, Chan Pin Yin DRPP, de Jong JSSG, van Boven WP, de Groot JR. J Am Coll Cardiol. 2016 Sep 13;68(11):1155-1165).

We added AFACT study to the discussion.

12) No study limitations were included eg. there were no attempts to induce the AF afterwards.

The section "Limitations" was added to the article.

To Panagiota Flevari (reviewer 3):

I have only one question: Did the authors examine distal epicardial nerves, as they have previously done in their previous study? How this would combine with HRV changes following RF ablation?

Yes, we do examine them. Results are to be ready for publishing in a separate article.