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Feasibility of mapping and cannulation of the porcine epicardial lymphatic system for sampling and decompression in heart failure research

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Handling editor:

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1st editorial decision

Date: 06-Apr-2018

Ref.: Ms. No. JCTRes-D-18-00005
Detailed illustration and cannulation of the porcine cardiac lymphatic system
Journal of Clinical and Translational Research

Dear authors,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by May 06, 2018.

To submit a revision, go to https://jctres.editorialmanager.com/ and log in as an Author. You will see a menu item called Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

EDITOR: Please address the following issues in the discussion section:
1) the cannulation system described can work in still hearts but I am not sure would work in live beating hearts so I am not sure this would work for fluid collection;
2) clearly explain the (clinical) significance of the observed variability in the drainage system.

Reviewer #2: The manuscript entitled illustration and cannulation of the porcine lymphatic system describes the anatomical position of the lymph collector vessels on the surface of the porcine heart using Indian ink injection. As a result of this method only the large vessels on the cardiac surface are visualized and the bed of capillaries on the surface and in the myocardium are not. I would like to suggest to down-tone the title of the manuscript.

In the figures, the course of the vessels is indicated in colour on the surface of a heart, which is very helpful. However, the chosen line thickness is very thin, a thicker line would increase the visibility and more easily highlight the course of each tract. In the results section also anatomical variations are described, which are shown in the images as dashed lines. It took a while before I could identify them on the images, this should be improved. Several of anatomical variations might be misinterpreted and could be the result of a better perfusion of the vessel bed during the visualization procedure. The authors should address this in the results section.

The introduction is rather archaic. Recent molecular biological analyses have enormously forwarded the knowledge on the cardiac lymphatic vasculature using immunohistochemical analyses and genetically modified mice. A comparison to the mouse lymphatic vasculature would be very helpful and increase the scientific value of this manuscript. I would like to suggest the following review as a starting point for the authors; Norman and Riley (2016) Clin. Anat. 29:305-315 and Abouelkheir et al., (2017) Exp Biol Med 242:884-895.

Finally, the manuscript needs to be corrected by a native English speaker.

Reviewer #3: This is a paper about the anatomy of the porcine cardiac system. The authors identify anatomic variability in the system and describe a cannulation technique. The findings are interesting and worth further exploration. The clinical significance of this has yet to be determined. The authors make several claims in the paper that are not proven or studied so those need to be modified.
1) In your abstract, the conclusion section you state "we propose a new technique for fluid collection" I am not sure this is a correct statement people have used cannulas to collect fluid from lymph vessels before and in any case

2) In the conclusion, you state "the present study states the importance of the cardiac lymph system and its possible impairment during heart surgeries"
this sentence is not supported by any data in this article and should be removed
3) In the conclusion, the last sentence "we claimed that cardiac…" Is an assumption and again not supported or discussed in this paper. Consequently, this sentence should be modified.
4) In drawings such as figure 1A the red line appears to cross the coronary. How were the color lines in the figures drawn based on what guide? Do the lymphatics really cross coronaries like that?
5) Table one needs to be modified to be made shorter and only with relevant data.
6) The caption of figure 4 is not clear please modify it.
7) In the discussion in the second paragraph the sentence "Thus, it is possible route along the artery should be considered in operational…" Is not clear. Currently, the cardiac lymphatics are not considered during such surgeries. Can the author explain why this is the case? And why we should potentially change our practice? What is the possible consequences of not considering these lymphatic channels?

Authors' rebuttal

Reviewer comments	Changes
Reviewer 1	
The cannulation system described can work in still hearts but I am not sure would work in live beating hearts, so I am not sure this would work for fluid collection.	We thank the reviewer for bringing up this point. We have revised the text and have added some additional explanations for clarity in page 14 line 253-262.
Clearly explain the (clinical) significance of the observed variability in the drainage system.	We added a better explanation of the clinical relevance in page 3 line 38-44 and in page 15 line 274-278
Reviewer 2	

The manuscript entitled illustration and cannulation of the porcine lymphatic system describes the anatomical position of the lymph collector vessels on the surface of the porcine heart using Indian ink injection. As a result of this method only the large vessels on the cardiac surface are visualized and the bed of capillaries on the surface and in the myocardium are not. I would like to suggest to down-tone the title of the manuscript.

We thank the reviewer for the advice. We agree with the suggestion and have therefore revised the title for clarity. The title is now:" Feasibility of mapping and cannulation of the porcine epicardial lymphatic system for sampling and decompression in heart failure research". Furthermore, we made additional changes related to the title through the entire document.

Several of anatomical variations might be misinterpreted and could be the result of a better perfusion of the vessel bed during the visualization procedure. The authors should address this in the results section.

However, the chosen line thickness is very thin, a thicker line would increase the visibility and more easily highlight the course of each tract. In the results section also anatomical variations are described, which are shown in the images as dashed lines. It took a while before I could identify them on the images, this should be improved.

We thank the reviewer for pointing out this issue. The lines thicknesses in all pictures have been increased and we hope that this increases the visibility of the courses and meets the expectations of the reviewer.

Thank you for raising this interesting point. We added an explanation in page 13 line 223-230.

The introduction is rather archaic. Recent molecular biological analyses have enormously forwarded the knowledge on the cardiac lymphatic vasculature using immunohistochemical analyses and genetically modified mice. A comparison to the mouse lymphatic vasculature would be very helpful and increase the scientific value of this manuWe understand the reviewer's concern regarding the "archaic" introduction and would like to point out that we were aware of the suggested publication and achievements of Norman and Riley. However, as we used porcine hearts in the current study, publications regarding small animals were thought to be out of the scope. Consequently, we were focusing on large animal studies script. I would like to suggest the following review as a starting point for the authors; Norman and Riley (2016) Clin. Anat. 29:305-315 and Abouelkheir et al., (2017) Exp Biol Med 242:884-895.

with similarities to human cardiac anatomy which in turn are less studied compared to smaller animals. This aspect has stimulated us to write this manuscript. Nevertheless, we have added a short text segment in page 5 line

Finally, the manuscript needs to be corrected by a native English speaker.

Thank you for the suggestion, the manuscript has been corrected by a native English speaker.

80-83.

Reviewer 3

In your abstract, the conclusion section you state "we propose a new technique for fluid collection" I am not sure this is a correct statement people have used cannulas to collect fluid from lymph vessels before.	We thank the review for this comment. We agree with the comment of the reviewer. “New technique” was referring to the usage of venflon needles, which could have led to irritations. We have revised this paragraph in page 2 line 3-
In the conclusion, you state "the present study states the importance of the cardiac lymph system and its possible impairment during heart surgeries" this sentence is not supported by any data in this article and should be removed	conclusions in page 2-3 line 31-44 and page 14-15 line 267-278.
36 accordingly.	In the conclusion, the last sentence "we claimed that cardiac…" Is an assumption and again not supported or discussed in this paper. Consequently, this sentence should be modified.
We appreciate the reviewer for the useful comment and think that it has improved the manuscript. We changed the	Thank you. Similar to the previous comment, we changed the conclusions in page 2 line 31-44 and page 14-15 line 267-278.
In drawings such as figure 1A the red line appears to cross the coronary. How were the color lines in the figures drawn based on what guide? Do the lymphatics really cross coronaries like that?	We appreciate the reviewer's questions. Yes, the AVT and other collectors run from the apex towards the base and sometimes crossover coronary arteries. The lines are a representation of the general routs of the 40 investigated
Table one needs to be modified to be made shorter and only with relevant data.	We thank the review for the comment. We shortened the Table in page 11.
hearts exemplary shown on one heart. Lines were used for better visibility and description of different parts of the network which would not be possible otherwise.	The caption of figure 4 is not clear please modify it.
In the discussion in the second paragraph the sentence "Thus, it is possible route along the artery should be considered in operational…" Is not clear. Currently, the cardiac lymphatics are not considered during such surgeries. Can the author explain why this is the case? And why we should potentially change our practice? What is the possible consequences of not	We appreciate the comment of the reviewer. With regards to the caption, we
considering these lymphatic channels?	important enough (swollen myocardium, cardiac lymph nodes or vessel, etc.) that sutures, stitches, cutting or even clamping areas could be reevaluated to protect the function of some of these small structures. We are sorry that this sentence can be misunderstood, but we did not mean to change clinical practice at all.
are not sure which aspects of the caption were meant. However, as far as we understood we changed some minor aspects of the caption in page 12 line 201-206.	
We thank the reviewer for the remark and questions. During cardiac operations larger structures are repaired but smaller ones impaired. As descripted in the manuscript, lymph impairment could lead to cardiac disfunction and edema. Therefore, we think it is important to be at least aware of these structures and their paths. Additionally, we think that under some circumstances these structures are	

We found the comment very useful and think that it will improve the manuscript. Consequently, we added some

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2nd editorial decision

Date: 20-Jun-2018

Ref.: Ms. No. JCTRes-D-18-00005R1
Feasibility of mapping and cannulation of the porcine epicardial lymphatic system for sampling and decompression in heart failure research
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Before we move to production, there are a few small items that I would like you to address:

1) Remove all trademark symbols from the text, which includes the abstract (e.g., Venflon).
2) Line 27/28: should read 'compared to' instead of 'with.'
3) Line 29: should read 'only 57%.'
4) Line 33: should read 'To improve cannu-….'
5) Line 34 should read: …success rate, we proposed two sites for cannulation based on these findings and the use…'
6) Line 37: Relevance for patients should be boldface.
7) Line 46: please add the keywords 'heart decompression; perioperative sampling.'
8) Table 1, top entry: should read 'Investigation of endocardial, myocardial, and pericardial…' – missing the Oxford comma.
9) Table 1 should also feature a list of all abbreviations written out in full below the table so that it is comprehended in a stand-alone manner.
10) Table 1, second to last entry should read 'AVT joins the LPT, which originates at the CXT.'
11) Line 203: insert space behind equal sign and spell 'Dotted' with lower case 'd.'
12) Line 258: should read …the study…'
13) Line 259: remove hyphens in ex-vivo-working and the TM designation.
14) Please remove all the redactions.

I kindly ask you to send the final draft to our managing editor, Dr. Yao Liu (y.liu@jctres.com), so that your proofs can be rendered.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger

Journal of Clinical and Translational Research
Peer review process file 04.201802.002

Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:
aspects in the paragraph of the discussion in line 234 and 241-24