Response letter (*invited resubmission with minor revisions* ***jcm- 1083627)***

Dear Reviewers,

we would like to thank you for your valuable comments on our paper *"Soluble Pecam-1 as a Biomarker in Periprosthetic Joint Infection".*
With this blinded response letter, we would like to take the chance of resubmission for potential publication of our article in The Journal of Clinical Medicine.

We appreciate all of the reviewers' comments and are pleased to address all raised issues after the minor revision process with the attached detailed point-by-point response. With gratitude, we'd like to thank the reviewers for their specific comments which helped us to further improve our manuscript. All of the below mentioned comments, corresponding remarks and corrections were implemented in the revised manuscript.
The manuscript text has been edited accordingly and, as clearly stated in your submission guidelines, track-changes were used.

We confirm that all authors have read the revised manuscript and approved its resubmission. With this minor revision, we hope to meet the reviewer's expectations and would like to thank you once again for your time and effort regarding the substantial improvements of our manuscript.

Please do not hesitate to contact us if you have further questions.

Yours sincerely,



The Corresponding Author
On behalf of all authors

Point-by-point response to the comments of **Reviewer 3** on the manuscript *"Soluble Pecam-1 as a Biomarker in Periprosthetic Joint Infection"*

*(invited resubmission with minor revisions **jcm- 1083627**)*

Specific comments **Reviewer 3:**

Reviewer 3:

1.) **Reviewer 3:** Unfortunately, the reasons for elevated sPecam-1 remain unclear and it cannot be stated if sPecam-1 is produced by the cells to fight infection or if it is a by-product with potential unfavorable reactions. As an advantage of the study, additional testing was performed in an in vitro cell culture study, where sPecam-1 did not reveal cytotoxic effects.

Response: *Thank you for pointing at this very relevant aspect regarding the underlying mechanism of action (MoA). As mentioned in the conclusion, we are aware that this question is of immense importance when considering potential therapeutic strategies involving sPecam-1. However, in this study we meant to demonstrate that sPecam-1 can be extremely valuable in a diagnostic set up in the first place, not yet explicitly anticipating a therapeutic role. Since our results show that sPecam-1 does not have a cytotoxic effect on the entity of PBMCs, even in high concentrations, we do not believe that it acts as a simple byproduct, triggering unfavorable reactions. However, you are completely right by pointing out that this underling mechanism needs to be further addressed when expanding the study to aspects that go beyond a diagnostic implementation. For reasons of clarity, we added the following statement within the limitations in the discussion section and thank you for pointing out this issue (Line 290-294: According to the obtained results,* it cannot be reliably determined if sPecam-1 is produced to fight pathogens or if it can be seen as a by-product with potential unfavorable reactions. However, according to *the fact that sPecam-1 does not have a cytotoxic effect on the entity of PBMCs, we do not assume that it acts as a simple byproduct.)*

2.) **Reviewer 3:** It might be, however, problematic to combine highly different surgical approaches (DAIR, 1-stage revision, 2 stage revision) in one treatment group ("infected group" n=22). This group will be highly heterogeneous from a clinical point of view as this will have caused different surgical strategies, which will affect the results. To solve this, more clinical data and perhaps subanalyses regarding the 3 different surgical

groups (DAIR, 1-stage revision, 2 stage revision) would be favorable to allow further subgroup analyses.

Response: *We clearly see the reviewer's point and appreciate the straight message. We also agree on the fact that the PJI group shows substantial differences with regard to the treatment strategy and probably the respective short-, middle – and long-term results. However, the goal of this study was primarily to assess a new biomarker with regard to the PJI-related clinical workup. On the basis of the obtained results, we were clearly able to demonstrate the diagnostic potential of sPecam-1 in this context. Nevertheless and in accordance with the reviewer's suggestions, a multi-center study, which we currently aim to establish, should clarify the mentioned issues by differentiating the diagnostic potential of sPecam-1 according to different treatment groups in a larger patient cohort. Due to the limited number of patients and the main focus on PJI diagnostics, we decided not to perform further subgroup analyses. With regard to your concerns, the authors of the present study hope that the above mentioned issues might explain our approach. By addressing the raised issues, we hope to meet your expectations. Finally, we thank you for this valuable comment.*

3.) Reviewer 3: The aseptic group would also benefit from further clinical details, as it is often difficult to distinguish infected from non-infected endoprosthesis. Did those patients with higher sPecam-1 levels in the non-infected revision group reveal clinical differences compared with those patients with lower sPecam-1 levels in this group? I would recommend to include some more clinical details from the treatment groups.

Response: *Thank you for this comment – we completely agree on the fact that this information is highly relevant. However, when setting up this study we strictly assigned our groups using the EBJIS definition. By this, we were able to define a valid cut-off value for diagnostic purposes as a potential add-on to current strategies. We point out that the PJI-diagnostic using sPecam-1 as a marker molecule can potentially be fast, cheap and easy, unlike current standard practice. Further addressing your valid point, we would like to pass on the information that we are currently investigating a larger group of patients in the "aseptic" group that showed slightly elevated quantities of sPecam-1. With regard to the different sPecam-1 levels in the non-infected patient cohort, we assume that the number of previous interventions might have an impact on the biomarker concentration. As mentioned above, we are currently investigating a larger group of the aseptic patient cohort to clarify this issue. However, at this stage we felt that it was already of high value for the scientific community to reveal the potential of sPecam-1 in diagnostics.*

Finally, we are pleased to notice that our study seems to contain some valuable information in the reviewer's opinion. Once more, we'd like to thank you for raising some critical issues, which helped us further to improve our manuscript.