Author's response to reviews

Title: Citrate prevents heparin induced complement activation and neutrophil degranulation, when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients.

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Author's response to reviews: see over
Dear Dr. Henderson and reviewers,

Thank you very much for the comments regarding our manuscript for which we suggest the new title “Citrate confers less filter-induced complement activation and neutrophil degranulation than heparin when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients.”

We feel the suggestions and points raised have been of value and added to the quality of the manuscript.

In this letter we provide a detailed point-by-point response to each of the referees concerns. During the revision, in addition to comments made by the reviewers, we made some other minor, mostly textual changes to the manuscript to improve the quality. We have highlighted all changes made.

On behalf of the authors,

Louise Schilder

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Reviewer:
Somchai Eiam Eiam-Ong
Reviewer’s report:
Dear The Editor in Chief

Although the authors have responded nearly all the questions, they still did not adequately reply regarding the design of the study. In my view, to combine different studies which were conducted in different situations would result in less reliable conclusion. Furthermore, the second reviewer of this manuscript also seems to be the PI of one study the authors utilize in their study. Taken together, to keep the standard of the journal I have to reject this manuscript. However, the final decision depends on the Editor justice.

The reviewer raises the issue that the design of the study, in which patients from two different studies are combined to form the citrate group, would result in less reliable conclusions. However, the patient characteristics in the two citrate groups are similar. Furthermore, this is not a patient outcome study and we therefore feel pooling of the data is legitimate. However, to give more insight in the characteristics of the two groups of citrate anticoagulated patients, we have added the following table (table 2) to the manuscript:

Table 2. Characteristics of patients anticoagulated with citrate from two different studies.

<table>
<thead>
<tr>
<th></th>
<th>Observational study (n=10)</th>
<th>Randomized study (n=7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 (32-79)</td>
<td>56 (42-74)</td>
<td>0.54</td>
</tr>
<tr>
<td>Sexe, male</td>
<td>5 (50)</td>
<td>6 (86)</td>
<td>0.30</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75 (60-100)</td>
<td>80 (60-110)</td>
<td>0.48</td>
</tr>
<tr>
<td>Reason of admission:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>5 (50)</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>Circulatory failure</td>
<td>1 (10)</td>
<td>1 (14)</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>1 (10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Post-resuscitation</td>
<td>0</td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>3 (30)</td>
<td>4 (57)</td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>6 (60)</td>
<td>1 (14)</td>
<td>0.13</td>
</tr>
<tr>
<td>SAPS II</td>
<td>50 (32-60)</td>
<td>62 (38-86)</td>
<td>0.06</td>
</tr>
<tr>
<td>SOFA</td>
<td>13 (8-18)</td>
<td>14 (9-15)</td>
<td>0.81</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>9 (90)</td>
<td>7 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Vasopressor dependent</td>
<td>8 (80)</td>
<td>5 (71)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mortality in ICU</td>
<td>6 (60)</td>
<td>6 (86)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Median (range) or number (percentage) where appropriate. SAPS II: Simplified Acute Physiology Score II; SOFA: Sequential Organ Failure Assessment score; ICU = Intensive care unit.
The reviewer furthermore suggests that the second reviewer, prof. Oudemans-van Straaten, was the primary investigator of a study that was utilized in the current study. We would like to emphasize that we did not use any data other than data from the studies described in the manuscript and performed in our center. Data from studies of prof. Oudemans-van Straaten were certainly not used in our analysis and she was not involved in any way in our study.

Editorial Comments:
The authors have done a post hoc secondary analysis of samples collected for other studies. So the methodology is limited since the study was not designed to look at this outcome. However, the authors have been clear about what they did. They have not tried to pass this off as a primary study. They say in their revision letter that they thought the two citrate groups (from the two studies) were comparable, so the combined them and they think this was valid. So, they have rebutted the reviewer. However, they have not presented any data on the characteristics of the two citrate groups.

1. Further information
In light of referee 1’s concerns, we may have to seek further advice from an external editorial adjudicator to confirm whether the authors’ assertion that it is valid to combine the groups is correct. However, it would be beneficial for us if the authors could please present data on the demographic and clinical characteristics of the two citrate groups they have combined. We would also encourage the authors to include a limitations section to discuss how the fact the samples were collected for two different studies might limit their results.

The concerns of reviewer 1 regarding the methodology are discussed above. Also, data of patients from the two different citrate groups are now presented in the manuscript in table 2. Furthermore, as the Editor suggested, we have added the following to the limitation section in the Discussion:

Page 11: “Furthermore, the citrate group consisted of patients from two different studies, and although patient characteristics between groups did not differ this could have affected results.”

However, we would like emphasize this was a proof-of-principle study, not a patient outcome study. We therefore also added the following to the Discussion:

Page 12: “These baseline differences, however, may not have affected the proof of principle in these pathophysiological studies.”
Reviewer:
Heleen M Oudemans-van Straaten

Reviewer's report:
The authors have addressed most of the comments. However, in the revised version raises some new issues.

1. The new title “Citrate prevents heparin induced complement activation and neutrophil degranulation, when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients” is not precise. The same concerns the conclusion “Regional citrate anticoagulation prevents potentially harmful complement activation and neutrophil degranulation in the filter induced by heparin, during CVVH in the critically ill” The study does not show that citrate prevents heparin-induced complement activation because citrate and heparin were not used together. I would suggest Use of citrate anticoagulation for CRRT confers less complement activation and neutrophil degranulation than heparin or something alike.

The reviewer raises the issue that the title and conclusion are not precise, since they implicate that citrate prevents complement activation en neutrophil degranulation induced by heparin. Indeed, our results show that there was less filter-induced complement activation and degranulation of neutrophils when citrate was used compared to heparin. However, to avoid any misunderstandings about the title or conclusion, we have adjusted the title as follows:

Page 1: “Citrate confers less filter-induced complement activation and neutrophil degranulation than heparin when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients.”

Also, the conclusion (abstract and discussion) has been modified:

Page 2: “Conclusion. Citrate confers less filter-induced, potentially harmful complement activation and neutrophil degranulation and less endothelial activation than heparin when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients.”

Page 12: “In conclusion, citrate confers less filter-induced potentially harmful complement activation and neutrophil degranulation than heparin when used for anticoagulation during continuous venovenous haemofiltration in critically ill patients.”

Also adjusted:

Page 10: “Our finding that citrate may cause less neutrophil degranulation by heparin during CVVH, is
in concordance with studies during intermittent haemodialysis and a single study on CVVH [9,10,13, 12,15].”

2. “The sieving coefficient was low and lower in group 3 than the other groups (no anticoagulation 0.49 (0.05-9.45), heparin 0.35 (0.12-11.2), citrate 0.10 (0.02-0.62), P<0.001) nd decreased in time in all groups (P<0.001).” The difference in sieving coefficient between groups is remarkable because the same filter was used. A lower sieving coefficient could be due to more clogging, but this is likely not the case with citrate. The higher sieving coefficient might also indicate higher production at the filter membrane and subsequent direct removal by filtration. To my opinion this is the most likely explanation. Could the authors please comment on this? The sentence in the discussion referring to this result: “Due to higher ultrafiltration flows, concentrations of C5a in the ultrafiltrate were lowest in the citrate group, as was the sieving coefficient,” should be adjusted. The lower C5a concentrations in the ultrafiltrate of the citrate group can have several causes: 1. less production at the membrane (most likely), 2. dilution due to higher UF flows (marginal difference), 3. lower permeability of the membrane due to more clogging (very unlikely).

The reviewer raises the issue that the difference in sieving coefficient found between groups is not adequately discussed in the current manuscript. We agree and we have made substantial changes to the Discussion:

Page 9/10: “Levels of C5a in the ultrafiltrate were lowest in the citrate group, possible partly due to due to higher ultrafiltration flows and subsequent dilution. However, it is possible that the higher sieving coefficient of C5a observed in the no anticoagulation and heparin group, resulted from generation of C5a in the filter and subsequent removal in the ultrafiltrate. The lower sieving coefficient observed when citrate was used, may then argue in favour of less complement activation in the filter and thus improved biocompatibility. Also, the clearance of C5a was low and negligible in the citrate group. This finding is in concordance with another study, where there was hardly any C5a found in the ultrafiltrate and thus virtually no removal by convection of C5a during CVVH particularly when high volumes were used [17]. Possibly, removal of C5a by convection, especially in the citrate group with higher ultrafiltrate rates, was delayed due to incomplete membrane saturation and adsorption of C5a by the filter. Another factor in the low clearance by convection could be that C5a, even though a small molecule, could have been bound to other substances, thereby exceeding size limits for convective clearance.”

Consequently, one reference has been added and the references have been rearranged in chronological sequence.
3. The suggestion of the first reviewer to replace the annotations of group numbers into group names is not consequentially performed. Please adjust.

_We have performed the annotations of group numbers into group names throughout the manuscript, as suggested._

4. Please add "in" before "study" in the following sentence of the discussion: “The time course of the release of elastase and MPO seems to be dissimilar our study” (page 10)

_We have made the following adjustment:_

_Page 10: “The time course of the release of elastase and MPO seems to be dissimilar in our study,...”_

5. Please adjust the conclusions of abstract and main text (see 1.)

_We have adjusted the conclusion of the abstract and main text (see 1.)_