Reviewer's report

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

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Reviewer: Heleen M Oudemans-van Straaten

Reviewer's report:

The authors present a controlled study in critically ill patients on CRRT comparing the effect of different anticoagulation strategies on complement activation an neutrophil activation. The study is of interest, but the writing can be improved.

The major/minor classification of the comments is indicated at each comment)

Ad Abstract (Major)
1. The results are not clearly presented. With the present description they are difficult to understand for the reader. Please re-write the results.

Net production of C5a across the filter was significantly higher in the H group than in the other groups, but production decreased over time. The concentration of C5a in the ultrafiltrate decreased over time in all groups. Inlet (c.q. plasma) concentration was significantly higher before starting citrate in the C-groups, and increased most over time in the C group as well, especially the last 12-h. Inlet C5a remained stable in the other groups.

There was a net production of elastase and MPO across the filter during H anticoagulation, while production was minimal and absent in the NA and C group respectively. Inlet MPO increased in the first 10 minutes of CVVH in the H group (p=0.024), while elastase and MPO inlet concentrations increased over time in the NA group, especially in the last 12-h.

Ad Introduction
2. P3 (Minor)

Please ad ‘as’ before ‘observed’ to line 7 of paragraph 2.

Please change ‘lacked a control group’ into as compared to heparin’ in line 12 of paragraph 2.

Ad Methods
3. P4 (Major)

Please describe the criteria for using either no anticoagulation or citrate in patients with an increased risk of bleeding.

4. P5 (Minor)

Please change ‘available’ in line 2 into ‘used’. 
Please describe the criteria for the use of lactate or bicarbonate replacement.

5. P7 (Major)
   Ad C5a “The concentration in the inlet plasma was higher in Group 3”. Please mention explicitly that this was already the case at the start of CVVH (as suggested elsewhere), because from the figure it seems that this difference developed during CVVH, especially in the last 12-h of treatment. I wonder by which test the authors determined a significantly higher baseline concentration, because neither in the methods section nor in the results section a comparison between individual time points is mentioned. This could be of importance, also for other pattern differed between groups. Please explain.

6. P8 (Major)
   Please report the calculated sieving coefficient for C5a at the different time points and for the different groups in stead of only reporting p-values.

7. P8 (Major)
   “C5a directly correlated with elastase and MPO in the inlet plasma”. This is remarkable given the different patters between the mediators over time (increase of MPO in the first 10-min in the H-group; increase in C5a in the last 12h in the C-group and an increase in MPO and elastase in the last 12-h in the NA group. Could the authors please comment on this?

8. P9 (Major)
   “We also suggest that citrate prevents endothelial release of MPO by heparin”. Please change this sentence into “We also suggest that heparin triggers endothelial release of heparin, while CVVH with citrate or no anticoagulation do not.” Or something alike. However, to my opinion the authors cannot conclude this from the present data, because they took no sample after the heparin bolus but before connection of the circuit. They can discuss this a finding from other studies, that this may be the case in the present study, but that unfortunately they cannot conclude this from the present data. At least this is my interpretation.

9. (Minor) Please reformulate the sentence starting with “These findings..” (line 2).

10. “At initiation of CVVH and throughout the study, inlet levels of C5a were highest in the citrate group”. See above

11. Major: The authors first suggest that elastase and MPO release was possibly associated with complement activation in the filter. However, this suggestion is not supported by the data of the present study nor by data from hemodialysis patients. Therefore, this is a theoretical association not supported by clinical data. Please reformulate this argumentation.

12. Minor: Same paragraph: “Conversely, there was no evidence for neutrophil degranulation in the filter in the other groups.” I would suggest saying that there was not evidence for neutrophil degranulation in the citrate group and minimal degranulation in the NA group. This makes the later argumentation more consistent.

14. Major: Please delete from the conclusion that citrate prevents MPO release from the endothelium. Although this may be the case, this cannot concluded from the present study. Inlet concentrations were not different between groups.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I have no other competing interests than being interested in the field.