Author’s response to reviews

Title: Coagulation activation, depletion of platelet granules and endothelial integrity in case of uraemia and haemodialysis treatment.

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Version: 2 Date: 18 January 2013

Author’s response to reviews: see over
Date: 17 January 2013

RE: MS 1925035413839149 revised version (MS/12.016.R1)
Coagulation activation, depletion of platelet granules and endothelial integrity in case of uraemia and haemodialysis treatment

Dear dr. Henderson,

We appreciate your positive comments and statements with respect to our manuscript MS 1925035413839149. The text of the original manuscript has been modified in line with the valuable comments from the reviewers. Corrections and remarks are listed below along the enumeration of comments from the reviewers.

Reviewer 1 (TE):
1. The article has to be re-read carefully for correcting language and some type and linguistic errors.
   - Linguistic errors are corrected and the ‘Word change mode’ has been applied to indicate new and deleted text respectively.

2. In the title it should be clarified that the term platelet depletion is referred to PLT granules.
   - Title of the manuscript has been changed to Coagulation activation, depletion of platelet granules and endothelial integrity in case of uraemia and haemodialysis treatment.

3. It would be better if the analysis among the 3 groups (ESRD, CKD and healthy subjects) was performed by means of ANOVA.
   - ANOVA-statistics are performed and results are incorporated into the manuscript.

4. Discussion / Conclusion section
   - Discussion and Conclusion sections are rewritten according to the instructions for changing.
   - The Conclusion section is shortened to the specified results of the study.

5. Discussion section: LMWH contribution to PLT aggregation
   - Subjects on regular HD-treatment:
     The Material & Method section has been changed. During this study blood samples from the subjects’ group on regular HD treatment were taken from the fistula (t0) before the administration of LMWH. Therefore, in this study it is not possible to establish a correlation between LMWH and PLT activation, depletion of PLT granules staining density or activation of coagulation.
     In former studies, however, we have investigated PLT activation during HD treatment. Indeed the kind of anticoagulant, such as LMWH or trisodiumcitrate is related to the amount of activation (Schoorl et al in Ned Tijdschr Klin Chem Labgeneesk 2006; 31:236-8, Gritters et al in Nephron Clin Pract 2007;106:c9-16, Schoorl et al Scand J Clin Lab Invest 2008;68:335-42).
     Although we also believe that the amount of LMWH plays an important role in the degree of PLT activation, we could never establish a statistically significant correlation between the amount of
LMWH and PLT activation. In the above mentioned studies Fragmin® is administered as a bolus injection at t=0 according to the protocol for prevention of coagulation activation. Concentrations concerning anti-Xa in plasma were 0 U/mL before the start of HD treatment and ranged from 0.8 – 1.1 U/mL at t=5 minutes after starting HD treatment. In our subjects’ group with HD treatment correlations between anti-Xa concentrations and PLT activation markers (CD62p and PF4) or PLT granules staining density were not statistically significant at the 0.05 level.

Reviewer 2: (ME):

6. Statistical corrections
   - To establish the association between the different parameters correlation coefficients were calculated and expressed as Pearson’s coefficients. The section Statistical evaluation in the Material & Methods and the section Association between proET-1 and modifications in PLT morphology or markers indicating activation of coagulation in the Results are both modified.

7. Discussion: how coagulation being activated in HD patients although TAT are within normal value
   - During this study blood samples from the subjects’ group on regular HD treatment were taken from the fistula (t0) before the administration of LMWH. Mean results for TAT and fibrinogen concentrations in the group with end-stage CKD were within the reference range and did not statistically differ from the results of the reference group. However, in the group with end-stage CKD increased TAT or fibrinogen concentrations were established in respectively 15% and 40% of the subjects, indicating activation of the coagulation pathway or an acute phase response already before the beginning of a new HD session. In former studies it was demonstrated that during a HD session activation of FXII was induced and subsequently the intrinsic coagulation pathway. The increased concentrations of TAT as well as prothrombin fragment 1+2 indicated that thrombin was generated during HD treatment. (Bartels et al Scand J Clin Lab Invest 2000,60:283-290, Bartels et al Scand J Clin Lab Invest 2003,63:417-424, Schoorl et al Scand J Clin Lab Invest 2011,71:240-247)
   The text in the Discussion section has been adjusted accordingly.

8. Add some ref. to explain why the results are –ve, and other that have the same result
   - With respect to different biomarkers of activation of PLTs and the coagulation pathway dissimilar results could be obtained. Although activation of PLTs and the coagulation pathway is present, concentrations of activation and release products staying within the reference values could also be detected as a result of different release times, presence of neutralizing agents and removal by the dialyzer membrane.(Gritters et al Nephrol Dial Transplant 2009,24:3461-8, Schoorl et al Scand J Clin Lab Invest 2011,71:240-7)
   As an example:
   During HD on a low flux dialyser with LMWH as anticoagulant both Platelet Factor 4 (PF4) and β-thromboglobulin (β-TG) increase over time. Using the haemodiafiltration (HDF) method PF4 increases, but β-TG does not change, neither in the ECC, nor over time. As the molecular weights of PF4 and β-TG are 27kD and 36kD, it is suggested that these substances are removed by convective transport during HDF, which is obviously not the case in low-flux HD. (Gritters et al Nephrol Dial Transplant 2009,24:3461-8) The same applies for serotonin release from the PLT dense granules as a marker for PLT activation. It appeared that the released serotonin in plasma is removed by the dialyzer membrane. Schoorl et al Scand J Clin Lab Invest 2011,71:240-7)

9. Method section:
   - Specifications of the local ethic committee have been added: local Medical Ethical Committee (METC Noord-Holland, The Netherlands)

10. Results: trend must be replaced by correlation
    - The section Association between proET-1 and modifications in PLT morphology or markers indicating activation of coagulation in the Results has been modified: trend has been replaced by correlation.
10. b Add for comparison two Tables: one for correlation and one for the groups with proE1>250 and proET-1>250 pMol/L
   - Table I PLT granules staining density, coagulation parameters and endothelial integrity in end-stage CKD subjects with proET-1 concentrations of <250 and >250 pMol/L has been added.
   - Table II Pearson correlation coefficients (r) and statistical significance (p) between biomarker proET-1, PLT granules staining density and biomarkers indicating activation of coagulation has been added.

11. Figures
   - The legend to the Figures have been rewritten:
     o Titles have been added to the Figures
     o Statistical significance between the groups are indicated in the Figures

12. Abbreviations in the Discussion
    - Abbreviations in the Discussion have been clarified.

13. Update Reference list
    - The reference list has been updated

14. Conclusion
    - The Conclusion section is shortened to the specified results of the study

We hope that the revised manuscript is suitable for publication in BMC Nephrology.

With kind regards,

Marianne Schoorl