Author's response to reviews

Title: Thromboelastographic Evaluation of the Effects of Recombinant Factor VIIa on Dilutional Coagulopathy

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Author's response to reviews: see over
Dear Editor-in-Chief

We appreciate the valuable comments from the reviewers regarding the manuscript 'Thromboelastographic Evaluation of the Effects of Recombinant Factor VIIa on Dilutional Coagulopathy'. The manuscript has been revised according to the comments made by the reviewers and we have responded point-by-point to the specific comments in the following pages.

There seems to be a slight misunderstanding regarding the aim of the study as two of the referees wanted the study to be expanded in order to study the subject more in depth. However, the aim of the study was to evaluate if the commercially available reagent kits provided by the manufacturer were able to give us an instrument that could be used to evaluate the effects of HES hemodilution and addition of recombinant factor VIIa (rFVIIa). The answer to that question is clearly NO.

In order to better specify the aim of the study we have changed the title of the manuscript to "An evaluation of standard rotational thromboelastography in monitoring of effects of recombinant factor VIIa on coagulopathy induced by hydroxy ethyl starch"

There are several outstanding questions as mentioned by some of the reviewers regarding why it does not work and how the effects could be better monitored. These issues are clearly relevant, but they are outside the scope of this specific study.

The reason for testing these kits was that there is a belief, at least in non coagulation specialist groups, that ROTEG can be used for monitoring of effects of rFVIIa. This is the case partly because there are several reports of monitoring of rFVIIa with ROTEG published in different journals, some of them authored by Dr Ingerslev. It may be obvious to coagulation specialists that the circumstances in hemophilia is very different when monitoring with ROTEG is performed, but it is certainly not obvious all the colleagues who are not coagulation specialists. We are ourselves specialists in Anesthesia and Intensive Care with a special interest in coagulation (e.g. senior investigator Ulf Schött has been an invited lecturer on colloid effects on haemostasis at the 21th international symposium on Intensive Care and Emergency Medicine, Brussels, Belgium March 20-23 2003) and in our daily discussions with less coagulation interested colleagues we often meet the belief that ROTEG can be used for general monitoring of rFVIIa. Therefore we wanted to study the issue and we chose HES hemodilution as a model.

Even though we cannot answer all questions regarding why ROTEG with standard reagent kits is inappropriate, we can conclude that it fails to monitor rFVIIa effects in this setting. This is an important finding that may help correcting a widespread misunderstanding regarding the usability of ROTEG for monitoring of rFVIIa. The reasons for this are obscure and probably will force the ROTEG manufacturer to come up with new EXTEG cuvettes, perhaps with lower TF concentrations. Studies by Dr Ingerslevs group have been performed with very diluted TF concentrations. Dr Ingerslev has not communicated the reason for using such low TF concentrations.
Personal communication (dr Schött at SCANTEM Meeting Gothenburg 11-12 November 2004) with Prof Ulla Hedner, vice president at NovoNordisk, indicates the presence of a study not yet in manuscript, where different tissue factor concentrations have been tried together with ROTEG. She believes a too high tissue factor concentration induces an immediate DIC-like situation in the ROTEG cuvette with consumption of fibrinogen and platelets, thus explaining the deterioration of the analysis in the EXTEG system after addition of rFVIIa.

On the following pages the specific comments made by the reviewers are responded to.

With Best Regards

Martin Engström        Peter Reinstrup        Ulf Schött
Response to comments made by Dr Michael Avidan (reviewer 1)

Dear Dr Avidan,

Thank you for your valuable comments regarding the manuscript. We understand that you found the manuscript not to cover what was expected according to the title. We have therefore changed the title of the manuscript in order to better specify the issues dealt with in the study. The new title is "An evaluation of standard rotational thromboelastography in monitoring of effects of recombinant factor VIIa on coagulopathy induced by hydroxy ethyl starch".

The aim of the study was to evaluate if ROTEG, with standard reagent kits, could be used to monitor effects of rFVIIa in a setting of a coagulopathy caused by hemodilution with HES. The answer to that question was no. There are several closely related issues that we could have studied as well, but we decided to study this well specified issue and to leave the analysis of why this was the case for later studies.

The analyses were performed at 37°C after the HES had been heated in a heating block to 37°C and the ROTEG analyser was adjusted to run at 37°C. This has been clarified in the methods section.

We studied the effects of rFVIIa on a coagulopathy caused by hemodilution with HES. We did not try to study the effects of a pure hemodilution without colloids. This has now been more clearly expressed in the manuscript. We are aware of the difference between dilutional coagulopathy and a coagulopathy caused by colloids and of the fact that with limited crystalloid hemodilution may even a hypercoagulable state be found.

You have asked for tests of platelet count, platelet function and PTT. The reason for not performing these tests was that we wanted to use tests readily and rapidly available as point-of-care tests. Platelet function tests are complicated and not readily available at our institution. The platelet count will decrease as function of the level of dilution and the test is not available as a point-of-care test. Regarding PTT, you are right, we should have performed that as well, but unfortunately we did not. However, both clinically and academically PT/INR usually better reflect hemodilutive effects than PTT/APTT.

We have not studied the effects on pH exerted by the hemodilution in all our patients, but in a pilot volunteer (see below) we analysed both Ca and pH levels that were within normal levels with the dilution used. The direct effects of pH on the coagulation system are poorly examined. It is known that acidosis is a co-existing phenomenon in many severely bleeding patients developing a coagulopathy, but if it is a cause of the coagulopathy remains to be studied further. It has also recently been found by a group from Duke University in the US that the activity of rFVIIa is impaired at pH 7.0.

Regarding the statistical methods we have changed the wording. Initial Kruskal-Wallis tests were performed and Wilcoxon’s paired test was performed when Kruskal-Wallis indicated a significant difference.

We have also, in the discussion, mentioned that the CT was non-significantly prolonged in both INTEG and EXTEG analysis. It is a short prolongation, but the prolongation of PT after addition of HES was not very pronounced either even though it was significant.
One of the reasons why we expected an improvement of the coagulation after addition of rFVIIa was that HES induces a platelet dysfunction and rFVIIa is used for treatment of platelet disorders, e.g. Glanzmann’s thrombastenia. It is also in non coagulation specialist groups a common belief that ROTEG can be used for monitoring of rFVIIa in general. There have been publications stating that ROTEG can be used for monitoring of effects of rFVIIa in for example hemophilia. It may be obvious to you as a specialist in the field that this cannot be interpreted in the way that ROTEG can be used for monitoring of rFVIIa in general, but this is the belief of many colleagues less interested and knowledgeable in coagulation issues. What we have tried to do with this study is to correct this misunderstanding especially if standard EXTEG cuvettes are in use. It remains to be communicated in scientific literature why a lower tissue factor activator may be needed in the future (hopefully) commercial available new EXTEG cuvettes.

We have removed a section of the discussion discussing potential explanations to the problems with the method in order to make the message of the study more clear, and to avoid unnecessary speculations.

With Best Regards

Martin Engström  Peter Reinstrup  Ulf Schött
Response to comments made by Dr Andreas Hillarp (Reviewer 2)

Dear Dr Hillarp

Thank you for your valuable comments regarding the manuscript. We have responded to the comments in the section below and changed the manuscript accordingly.

1. Your comment is correct and the wording has been changed and doesn’t mention the lysis stage anymore.

2. The word ref had erroneously found a place where it shouldn’t be. The word has been removed.

3. The point in the discussion mentioning dysfunctional fibrinogen or platelets is referring to potential explanations for the further impairment of the ROTEG analysis. Likely explanations for an increase in CFT and impairment of A15 in general can be either platelet dysfunction or fibrinogen dysfunction. In this specific case it may be unlikely that the explanation is fibrinogen dysfunction.

   We have added a small section in the discussion addressing the fact that the most likely cause of the impairment of the coagulation is a platelet dysfunction.

4. We agree that it might be interesting, but we have not performed any titration studies with the EXTEG reagent as the aim of the study was to study the commercially available kit according to the manufacturer’s instructions. As told in comments above, there exists already such a study, but not yet in manuscript according to Prof Ulla Hedner (please see above).

5. The section discussing the potential TFPI generation has been removed from the manuscript as it could be confusing. This should make the message of the study clearer. The theory was that when rFVIIa was added to the test tube it could potentially initiate a coagulation process leading also to activation of TFPI. This TFPI production would then interfere with the initiation of clotting after addition of the TF containing EXTEG reagent and thus impair the ROTEG analysis. In the INTEG system clotting is initiated with a contact activator that would not be inhibited by the TFPI generated already at addition of rFVIIa. Therefore the INTEG analysis would not be impaired after addition of rFVIIa. But as mentioned earlier we have removed that section of the discussion. The reason for the deterioration of the EXTEG analysis graph after addition of rFVIIa might also be a too strong thrombin activation and induction of DIC prior to the start of the ROTEG graph. Platelet decrease or dysfunction more heavily affect the graph than fibrinogen decrease or dysfunctional fibrinogen. Whether the deterioration depends more on platelet dysfunction is however speculation in this setting. It is also possible that rFVIIa also induces a more loose clot (this has been described in treatment of haemophilia patients (personal communication dr Schött with Prof Erik Berntorp, University of Lund) that could explain the the deterioration of the ROTEG graph.

6. PK has been changed to PT in table 1.

7. We have added a new figure only to describe the ROTEG analysis graph better. Scales are incorporated into the new figure called figure 1. The figure called figure 1 previously has been renamed to figure 2.
With Best Regards

Martin Engström          Peter Reinstrup          Ulf Schött
Response to comments made by Dr Jorgen Ingerslev (Reviewer 3)

Dear Dr Ingerslev,

Thank you for your valuable comments regarding the manuscript. We note that you think the study should be expanded in order to better investigate the potential explanations for the inability to use ROTEG for monitoring of treatment effects on HES-induced coagulopathy. However, the aim of the study was to evaluate if ROTEG, with standard reagent kits, could be used to monitor effects of rFVIIa in a setting of a coagulopathy caused by hemodilution with HES. The answer to that question is, according to our study, no. The reason for the study was that there is a general misunderstanding among non coagulation specialists that ROTEG can be used for general monitoring of effects of rFVIIa. It may be obvious to you with your vast experience in the area that is not the case, but that is not the case among our colleagues working with for example severely bleeding trauma patients. You and your group have studied monitoring of rFVIIa with ROTEG and published some very interesting and successful reports on this subject e.g. in hemophilia patients. It is not obvious to other colleagues that these findings cannot be generalized to monitoring of rFVIIa in general.

We have studied the commercially available kit as that is the only opportunity for us working as specialists in Anesthesia and Intensive Care with a need for point-of-care analysis utilizing these commercially available kits. It is not possible to perform advanced titrations or dilutions in the ICU or in the operating theatre, as the staff is neither properly trained nor have the time to perform such tests. Thus, the aim of the study was to study the usability of the standard tests. We agree that there are several issues that would be interesting to study further in order to elucidate why our analyses turned out the way they did, but that is remaining for future studies find out. (See above personal communication with Prof Ulla Hedner)

We have made significant changes to the manuscript in order to more clearly describe the aim of the study and we have also changed the title.

You are perfectly right that we do not know of any report where rFVIIa has been used to correct HES-induced coagulopathy. However, rFVIIa has seemed to be effective in various settings and we therefore thought it was worth testing if it would be effective also in this setting. RFVIIa is also used for treatment of platelet disorders, e.g. Glanzmann’s thrombasthenia and also for that reason we found it reasonable that it would be effective in treating the coagulopathy induced by HES as HES is known to induce a platelet dysfunction. HES induces a readily detectable coagulopathy at a 33% dilution and we therefore did not find any reason to study different levels of dilution as that wouldn’t change the conclusions drawn. We have performed pilot tests also with 50% hemodilution and the effects were similar to the effects with 33% dilution, the only difference being that the effects were more pronounced. Further on, we checked Ca and pH effects and there were no unphysiologic changes in these parameters at the dilution chosen in this study (see above).

We hope these explanations have been satisfactory and that you are willing to accept that the aim of the study was limited and not to study the whole complex area of monitoring of rFVIIa.

With Best Regards

Martin Engström           Peter Reinstrup           Ulf Schött