Author’s response to reviews

Title: Monitoring of argatroban and lepirudin anticoagulation in critically ill patients by conventional laboratory parameters and rotational thromboelastometry - a prospectively controlled randomized double-blind clinical trial

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Author’s response to reviews:

In reply to

BANE-D-17-00266

Monitoring of argatroban and lepirudin anticoagulation in critically ill patients by conventional laboratory parameters and rotational thromboelastometry - a prospectively controlled randomized double-blind clinical trial Martin Beiderlinden; Patrick Werner; Astrid Bahlmann; Johann Kemper; Tobias Brezina; Maximilian Schaefer; Klaus Goerlinger; Holger Seidel; Peter Kienbaum; Tanja Meyer-Treschan BMC Anesthesiology
Dear Sir or Madam, dear Dr. Bartels,

We thank the editors and the reviewers for their time and effort spent to improve our manuscript "Monitoring of argatroban and lepirudin anticoagulation in critically ill patients by conventional laboratory parameters and rotational thromboelastometry - a prospectively controlled randomized double-blind clinical trial" (BANE-D-17-00266).

We have revised our manuscript according to the reviewers’ comments.

A detailed step-by-step response is given below; all changes to the manuscript are highlighted in the revised version.

We would be very happy, if the revised version would be considered suitable for publication in BMC Anesthesiology.

On behalf of all authors,

With best wishes,

Tanja Meyer-Treschan

Editor Comments:

Please pay especially close attention to reviewer #2’s comment’s regarding sample size, lack of blinding and randomization, description of thrombotic complications, as well as the need to more robustly adjust for multiple comparisons.

In reply:

Sample size

In the revised version we added a new paragraph on sample size to the methods section. It reads: “This study was purely exploratory, thus no prospective sample size calculation had been done.”
Blinding

In the previous version of our manuscript, we explained, that this sub-study was part of the “larger double-blinded clinical trial (“Argatroban versus Lepirudin in critically ill patients (ALicia”) page 9 line 7-8 of the previous version. In the revised version, we provide more information about blinding in the methods section: “Study drugs were prepared by personnel not involved in data collection and delivered to the ward and applied in neutral syringes to facilitate blinding of patients and personnel involved in treatment.”

Randomization

In the revised version, we explain, that “Randomisation was based on a computer generated multi-block 1:1 group assignment.”

Thrombotic complications

Reviewer 2 asked us to describe clinical complications, such as thromboembolic events. However, this sub-study was specifically designed to explore, whether ROTEM is capable of monitoring direct thrombin inhibitors in a routine ICU setting. It was thus not powered nor was it planned to report on clinical outcomes. The latter are the main part of the parent trial and have been reported in great details already elsewhere (1). Therefore, we think it is not appropriate to provide clinical outcomes for the small population of this sub-study.

Adjustment for multiple comparisons

Reviewer 2 refers to Bland-Altman plots and requests other means to adjust for multiple comparisons. Our manuscript does not contain Bland-Altman plots.

Reviewer reports:

Nathan Clendenen (Reviewer 1):

In reply:

We thank Nathan Clendenen for his time and effort spent to improve our manuscript. We are happy to read, that he considers our data compelling to support the use of ROTEM and our manuscript well written and adequately powered to detect significant correlations between the clinical tests and drug levels.
Specific suggestions:

The manuscript would benefit from a comment on whether the patients' baseline coagulation parameters differ substantially from normal values before treatment with argatroban and lepirudin. This would also help as a frame of reference to appreciate the change in the lab values with treatment.

In reply:

We added a sentence to the results section of the manuscript to clarify this aspect: “Coagulation parameters at baseline were within the normal range except for aPTT, which was slightly elevated above the upper limit of 37 seconds in both groups after heparin infusion had been stopped.”

Similarly, a comment on whether the inter-assay variability differs substantially when analyzing samples from critically ill and anti-coagulated patients compared to typical variability when testing healthy controls. This would further support ROTEM as a testing modality with the potential to supplant conventional anti-coagulation monitoring.

In reply:

Done. We added this to the discussion section of the revised version of the manuscript, the new sentence is underlined: “Results of several trials indicate that monitoring argatroban by aPTT may not be appropriate in the specific patient population of critically ill. One reason is the high inter-assay variability, which means, that results of aPTT measurements differ greatly depending on the reagent used for analysis (2). Specifically in critically ill patients, variability is increased due to several factors that can lead to over- or underestimation of drug effects, such as low levels of clotting factors or elevated levels of factor VIII (3,4).”

The authors could also highlight potential limitations of ROTEM such as availability of the assay or cost.

In reply:

Done. We added the following paragraph to the limitation section of our discussion in the revised version of the manuscript: “Furthermore, our data indicate, that ROTEM parameters have the potential to supplement conventional anti-coagulation monitoring. Nevertheless, it has to be taken into account that the routine use of ROTEM is limited by several factors, as devices are not
routinely available on all ICUs, conduct of measurements is time consuming for ICU personnel and adds to the costs spent for laboratory assessment.”

Lachlan Miles (Reviewer 2):

In reply:

We appreciate the time and effort spent to improve our manuscript. We are happy to learn, that Lachlan Miles considers our findings interesting for the development of new theories of anticoagulation.

General comments

The overall argument of this small sub-study of a larger randomized controlled trial is that aPTT does not provide an effective estimate of relative lepirudin or argatroban level, despite being elevated in the presence of the drug in critically ill patients. In contrast, various ROTEM parameters provide a moderate correlation with drug effect, despite no change in maximum clot firmness. This is in keeping with our current understanding of coagulation, as these drugs should exert no effect on platelets or fibrinogen. This is an interesting finding, as the development of new theories of anticoagulation (Hoffman, Blood Rev, 2003) suggests that aPTT and PT are limited in the presence of multiple confounding factors.

There is no reporting of the process used for randomization or blinding. Whilst I appreciate that this has been reported as part of the aforementioned parent study, it is still not acceptable for a reader to search for information regarding these processes, especially when there are substantial trends towards patients in the argobatran group being older and heavier.

In reply:

We added the sentence: “Randomisation was based on a computer generated multi-block 1:1 group assignment.” to the methods section of the revised version to clarify this aspect.

The study is substantially limited by its small sample size. The substantial delay in publishing these data also means that one of the drugs tested (lepirudin) has been withdrawn from the market.
In reply:

The drug lepirudin was taken off the market by the manufacturer during the active recruiting period of the main trial.

The study is also limited by an absence of reporting of clinically significant outcome data. The study authors have made an oblique reference to loss of CVVH filters despite the increase in aPTT, but have not reported the incidence of this event, nor any other thrombotic complications. These events should at the very least be reported, as whilst poor correlation between aPTT and drug levels are of interest, some form of meaningful clinical outcome would increase the interest and generalisability of this paper.

In reply:

This sub-study was specifically designed to evaluate the ROTEM technology in a small group of critically ill patients treated with direct thrombin inhibitors and to test the applicability of the technique.

We agree with the reviewer, that we have a limited sample size in this ROTEM study.

Thus, it is not meaningful to report clinical outcomes for this sub-group of patients, as the results would be underpowered and would not be generalizable at all.

In contrast, the parent trial “Argatroban versus Lepirudin in critically ill patients (ALicia)” was powered to detect differences in a clinically relevant outcome parameter that is filter patency in continuous renal replacement therapy. Other clinically relevant endpoints, such as bleeding and thromboembolic events, have been also measured in the main trial and have been reported there (1).

From a statistical perspective, whilst an effort has been made to account for repeated measures using reduced p-values, a more statistically robust method, especially for the Bland-Altman plots used in figure 5 and 6 has been described previously by Myles and Cui (Myles, Br J Anae, 2007). Further statistical advice should be sought as to which of the techniques, those used by the authors here or those proposed by Myles and Cui, are more appropriate. I am not able to make this determination myself.

In reply:

Bland-Altman plots are used to compare results of different measurements by plotting the difference between two measures against the average of two measures (5). We do not display Bland-Altman plots nor did we perform the Bland-Altman method for our calculation.
As described in the method section of the previous and the current version of our manuscript, we used spearman correlation and accounted for multiple comparisons using a Bonferroni adjustment, which we consider to be the appropriate method.

Overall, this manuscript presents an interesting thesis, but the study is severely limited by:

1. The small sample size of the study and lack of information on how it was determined;

   In reply:
   The small sample size was due to the fact, that this was a pre-planned sub-study of the larger trial. However, as this sub-study was exploratory, no prospective sample size calculation had been done. The sub-study was established later than the main trial and was thus limited to the analysis of blood of 35 patients.
   In order to clarify this, we added a paragraph to the method sections, which reads:
   “Sample size
   This study was purely exploratory, thus no prospective sample size calculation had been done.”

2. The statistical methods used to correct for repeated measures;

   In reply:
   As pointed out in greater detail above, unfortunately the reviewer did not report correctly on the methods we applied.

3. The failure to report information regarding inclusion, randomisation and blinding for the parent study;

   In reply:
   Inclusion criteria are reported in short in both, the previous and the current version of the protocol: “critically ill patients with suspected HIT were treated with argatroban (the argatroban-group) or lepirudin (the lepirudin-group), aiming at a target aPTT of 1.5 to 2 times baseline.”
   We added more information about the randomisation process to the revised version of the protocol, as explained above.
Blinding was achieved as follows: Blood samples for coagulation monitoring were taken by personnel not involved in drug application. Drugs were delivered to the ward and applied in neutral syringes. This information on blinding has been added to the revised version of the protocol.

4. The failure to report clinically significant outcomes with respect to thrombotic complications.

In reply:

As pointed out above, the sample size of this sub-study would be underpowered to generalize clinical outcomes. Clinical outcomes of 66 patients in the parent trial have previously been published (1).

Abstract

Page 5, line 14: The authors outline the taking of blood at various time intervals "after argatroban or lepirudin infusion". It is unclear to the reader if this refers to commencement or cessation.

In reply:

We added the word “initiation” to the sentence, which now reads as: “Before as well as 12, 24, 48 and 72 hours after initiation of argatroban or lepirudin infusion, blood was analysed…”

Page 6, line 1: The authors state that they have shown "in critically ill patients, TT and ROTEM parameters provide better correlation to argatroban and lepirudin plasma concentrations than aPTT". The information presented in the abstract shows no evidence to justify this statement. Include additional information comparing aPTT to ROTEM/TT that justifies this statement or revise.

In reply:

Line 21 to 22 on page 5 of the previous version states: “For both drugs, there was no significant correlation between aPTT and aPTT ratios and plasma concentrations.” This statement is then followed by: “INTEM CT, INTEM CT ratios, EXTEM CT, EXTEM CT ratios, TT and TT ratios correlated significantly with plasma concentrations of both drugs.” (lines 22 to 24 page 5). These results are summarized to conclude:” In critically ill patients, TT and ROTEM parameters provide better correlation to argatroban and lepirudin plasma concentrations than aPTT.”
Background

Page 7, line 5: The authors outline the excretory pathways for both agents, and state that elimination is hepatic for argatroban and renal for lepirudin. This is followed by the sentence: "Thus both drugs have to be carefully titrated in patients with reduced kidney or liver function". This sentence is internally inconsistent with the prior statement (i.e. if lepirudin is completely renally excreted, why must hepatic function be taken into account?). Please clarify.

In reply:

We changed the sentence to “Thus, argatroban both drugs has to be carefully titrated in patients with reduced liver function. Lepirudin needs to be carefully adapted to those with reduced kidney function.”

Page 7, line 7: The authors use the term "critically ill" when referring to a paper by Doepker et al. Please provide a less nebulous definition for "critically ill".

In reply: In the pdf built after submission of our material, on page 7 in line 7 there is no citation. The citation in line 9 refers to papers by Tschudi et al. and by Williamson et al.

Page 7, line 18: The authors make reference to the Child-Pugh classification system when defining hepatic impairment, and in the subsequent sentence, state that many critically ill patients "do not meet the criteria of hepatic cirrhosis". Cirrhosis is a diagnosis made on imaging or histopathology, not the diagnostic criteria of Child-Pugh. Patients with acute hepatic failure may not have macroscopic or microscopic features of cirrhosis. Please remain internally consistent with these terms. A better phrasing would be "do not fulfil the Child-Pugh criteria for hepatic impairment".

In reply: Done. We changed the sentence as requested.

Page 8, line 17: The authors assert that viscoelastic coagulation assays have "been proven beneficial as part of goal-directed bleeding management in patients with major haemorrhage". The article cited refers to cardiac surgery, and not major haemorrhage.

In reply:

The publication by Deppe et al., which we cite, is reasonably young, published in June 2016 and a meta-analysis of all trials on ROTEM and TEG… “aimed to determine the current evidence
for or against POCT-guided algorithm in patients with severe bleeding after cardiac surgery.” (cited from the abstract of Deppe (6))

Furthermore, it could be argued that this systematic review has been supplanted by more recent evidence questioning the role of VCAs in this scenario. A more cautious statement supported by more recent evidence as well as a more specific reference to the patient population in question is in order.

In reply:

To further strengthen our statement, that ROTEM is beneficial, we added a new reference that summarizes the findings of a systematic review of the Cochrane database, published in August 2016, which comes to conclusion, that “ROTEM-guided transfusion strategies may reduce the need for blood products …in patients with bleeding”. (7)

The most recent systematic review and meta-analysis on this topic was published in June 2017 and now also cited in the revised version of the manuscript. It summarizes: “Pooled effect estimates showed that TEG - or ROTEM-guided algorithms for management of coagulopathic haemorrhage reduced the number of patients requiring transfusion…” (8). We consider this a beneficial effect. Thus, we believe that the statement we made in the previous version is valid and did not change it in the current version of the manuscript.

Page 8, line 19: The authors reference the roles of INTEM and EXTEM in the assessment of "intrinsic coagulation activation" and "extrinsic coagulation activation". It could be argued that on the basis of improved understanding of coagulation through the cell-mediated theory that terms such as "intrinsic" and "extrinsic" are outdated. At the very least, some reference to the historic "common pathway" should be included.

In reply:

Done. The revised version of the manuscript contains the following sentence This step is partially reflected by measurement of the thrombin time (TT), which is typically referred to by the historic “common pathway of coagulation”.

Page 8, line 23: For an English-language journal, the term "platelets" would be preferred to "thrombocytes".
In reply:

Changed to” platelets”, as requested.

Methods

Page 8, line 7: Despite the inclusion criteria for the ALicia study being published elsewhere, I would feel more comfortable as a reader if it could be included here so that I can review them for myself.

In reply:

Done, we added the inclusion criteria to the methods section of current version of the manuscript, which states: “We included surgical intensive care unit patients with expected ICU treatment >24 hours, age ≥18 years and suspected HIT (decrease in platelet count >50% from baseline, persisting for more than 24 hours, 4 T-Score >3 or positive PF4/heparin enzyme-linked immunosorbent assay).”

Page 10, line 12: The authors state that sample analysis occurred between February 2010 and November 2011. This manuscript has been submitted for peer review in August 2017. Is there an explanation for the extended delay?

In reply: We agree, that time has passed since the ROTEM measurements, taken in 2010 and 2011. However, this sub-study as a project was mainly managed by a young colleague (PW) as his doctoral thesis and took the time.

Page 10, line 20: Some further exposition is required for why patients on CRRT received such a low dose of lepirudin. One would assume that the use of CRRT somewhat restores drug excretion. Why is this not the case for lepirudin (i.e. molecular charge, molecule size, etc.)?

In reply:

The answer to this question is highly interesting, but far beyond the scope of the trial presented here.

Page 12, line 7: A company and location reference for SPSS is required.

In reply:
Done, we added the location of IBM (New York, USA) to the revised version of the manuscript.

Page 12, line 12: Please see general comments regarding statistical correction for repeated measures. I feel that further statistical input is required to confirm the approach used by the authors is the correct one.

In reply: We have provided a detailed answer to this aspect above.

Results

Page 13, line 9: Some reference to the therapeutic drug ranges of argatroban and lepirudin would be of use, especially as some clinicians will be unfamiliar with lepirudin now that is has been withdrawn from the market. At first glance, it is hardly surprising the argatroban group had a higher concentration as it was infused at a higher dose.

In reply:

“So far there is no recommendation for a plasma level, that should be targeted in order to provide adequate concentrations of direct thrombin inhibitors.” …an important information, which we added to the discussion section of the revised version of the manuscript.

Page 14, line 8: The authors state that 25 of the 478 ROTEM measures were assessed as invalid. How was invalidity defined?

In reply:

As stated in the methods section of both versions of the protocol “Results of ROTEM measurements, for which automatically generated failure codes occurred, were sent to the TEM®-Support department (TEM Innovation GmbH, Munich, Germany) for evaluation and only analysed further if assessed as valid”

“Most invalid cases were due to drying effects of the sample and missing deflection in the TEMogramm.” We added this information to the revised version of the manuscript.

Page 16, line 11: The authors state that lepirudin is licenced for anticoagulation in HIT. IS this still the case even though it has been withdrawn from the market?
In reply: We changed the sentence to “Argatroban is and lepirudin was licenced for anticoagulation of patients with HIT and HIT suspect.”

Conclusion

Page 20, line 3: The authors state that monitoring argatroban or lepirudin with aPTT is "not appropriate" due to the poor correlation with plasma levels. This is a strong statement in the absence of clinically significant outcome data. The rates of bleeding and thrombosis have not been presented, and even if they were, an observational trial is required (one arm aPTT monitoring, one arm TT or ROTEM monitoring) to prove this. Moderation of the statement is required.

In reply: We changed the sentence to “Monitoring of alternative anticoagulation with argatroban or lepirudin in critically ill patients with heparin-induced thrombocytopenia or suspect of heparin-induced thrombocytopenia by aPTT is not ideal, as it does not correlate with plasma levels.”

References:


