Title: Activated coagulation time vs. intrinsically activated modified rotational thromboelastometry in assessment of hemostatic disturbances and blood loss after protamine administration in elective cardiac surgery: analysis from the clinical trial (NCT01281397)

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Author's response to reviews: see over
Editors in chief
Mr Vipin Zamvar, Royal Infirmary of Edinburgh, United Kingdom
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Zagreb
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Dear Editors in Chief,
On behalf of all co-authors allow me to thank you for the extraordinary review, which has contributed to the improvement of our manuscript.
Our working group has carefully examined the reviewers’ comments, and herein we provide point-by-point answers in response to these comments. Please note that all the questions and concerns raised by reviewers have been addressed, and changes to the manuscript have been done so the manuscript is now completely in line as suggested by reviewers. Changes within manuscript are marked with bold characters.

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Research article
“Activated coagulation time vs. intrinsically activated modified rotational thromboelastometry in assessment of hemostatic disturbances and blood loss after protamine administration in elective cardiac surgery: analysis from the clinical trial (NCT01281397)”
Authors: Mate Petricevic, Bojan Biocina, Davor Milicic, Lucija Svetina, Marko Boban, Ante Lekic, Sanja Konosic, Milan Milosevic and Hrvoje Gasparovic

AUTHORS RESPONSE TO REVIEWS:
REVIEWER 1:
Petricevic and colleagues present results of their study on assessing two point of care devices for assessment of hemostatic disturbances and blood loss after protamine administration in elective cardiac surgery, a topic of interest in everyday clinical practice. The manuscript is well organized and written (with minor spelling errors). However there are several issues I think the authors should address before a decision for publication could be made:
MAJOR COMPULSORY REVISIONS:

1. The title states that the article analyzes patients from a clinical trial NCT01281397. At least a brief description of the trial should be presented in the methods section, particularly with clear exclusion criteria.
   Also, a trial was supposed to include 400 patients and this study includes 148. Was that a portion of the study cohort and during which period of the trial were the patients investigated (initial, mid, or late)?

AUTHORS: This manuscript presents exploratory analysis from a clinical trial NCT01281397. Trial was designed in prospective observational fashion. The aim of trial was to assess possibility of point-of-care hemostatic devices (rotational thromboelastometry and multiple electrode aggregometry) to predict excessive bleeding in elective cardiac
surgery. Parameters of rotational thromboelastometry and multiple electrode aggregometry were obtained perioperatively respective values were correlated with observed key endpoints such as chest tube discharge and transfusion requirements. Patients (n=148) undergoing elective cardiac surgery procedures requiring CPB between July 2010 and January 2011 were prospectively studied. Criteria for excluding patients from the group of subjects were: age younger than 18 years, urgent procedure, patients with off-pump cardiac surgical procedure, administration of antiplatelet agents other than and clopidogrel, patients with inaccurate antiplatelet therapy administration documentation, urgent surgery, and patients requiring surgical exploration for excessive bleeding due to obvious surgical bleeding with a bleeding vessel identified. Initially, a trial was supposed to include 400 patients. However, during study period our research group decided to perform interim analysis after approximately every 50 patients. After 148 patients enrolled, interim analysis revealed positive results in regard to primary hypothesis that point of care tests for assessment of platelet function and viscoelastic blood properties may predict bleeding in cardiac surgery patients. Thus, considering positive results we decided to terminate study earlier since our primary hypothesis was confirmed much earlier than our primary estimation was.

2. Chest tube output (CTO) is an obvious end point choosen. Excluded from the study were patients "requiring surgical exploration for excessive bleeding due to obvious surgical bleeding with a bleeding vessel identified". The number of such patients (if any) in the cohort was not given in the results section and 148 consecutive patients recruited leave the impression that no surgical exploration was neccessary in this cohort. That should be pointed out (if that is the case in fact).

AUTHORS: Patients requiring surgical reexploration for excessive bleeding were supposed to be excluded from study if bleeding vessel would be identified during reexploration. Reexploration per se was not exclusion criteria, in particular if no exact surgical cause of bleeding was present suggesting hemostatic disorder. Please note that study was prospective observational and during study period hemostatic management was based on consensus opinion between consultant anesthesiologist and cardiac surgeon. At our center, surgical reexploration is being performed for each case with suspicion to surgical cause of bleeding. During study period, no surgical reexploration for bleeding was performed. It is obvious that chest tube output is consisted of both “surgical” and “hemostatic disorder” origin. However, without surgical reexploration performed it is impossible to detect whether some proportion of patients was actually bleeding predominantly due to surgical cause. A more detailed explanation is included in manuscript.

3. Patients categories for analysis were determined according to the CTO where the "bleeder" category was determined as the amount of CTO above the 75th percentile which turned out to be >1500 mL. No bleeding dynamics was reported or any criteria on how was this (fairly significant amount of CTO) distinguished from surgical bleeding requiring reexploration (criteria on the amount of CTO for surgical bleeding were not mentioned either). This should be better elaborated.

AUTHORS: CTO was determined as study’s primary outcome. To estimate blood loss, we meticulously documented CTO, in first 24 postoperative hours and divided it by patient’s weight. Drainage loss was assessed after completion of a 30-min stabilization period. Blood
loss during the stabilization period was not included in the definition of postoperative hemorrhage. Such loss may be caused by postural changes when transferring the patient from the operating room table to the bed or because of fluid in the pleural or mediastinal cavity, which may have arisen from the rinsing with water as an attempt to achieve surgical hemostasis. Intraoperative and postoperative transfusion requirements (PRBC in mL, FFP in mL, fibrinogen concentrate in grams and platelet concentrates in units) were determined as study's secondary outcome. Surgical reexploration of the mediastinum for excessive bleeding was noted, along with any surgical explanation for the bleeding. Although some authors offer definitions of abnormal blood loss, we decided to make our own definition in order to adapt the volume of postoperative CTO to our study cohort. We believe that such a definition makes the most reliable correlation, and is not distorted to different perfusionistic, surgical and anesthetic techniques described by other authors. Postoperative CTO was recorded and divided by the patient’s weight. Patients were characterized as bleeders if their 24 h CTO (ml/kg) exceeded 75th percentile of distribution. In addition to insufficient surgical hemostasis, bleeding after CPB may be induced by many abnormalities in the coagulation system. Blood loss through these tubes is the sum of coagulopathic bleeding and surgical bleeding from wound edges. Unfortunately, it is impossible to differentiate bleeding volume according to surgical or coagulopathic cause. We didn’t have possibility to measure hourly dynamics of chest tube drainage. Furthermore, we did not have any pre-specified volume criteria for chest tube drainage in certain amount of time that would suggest surgical cause of bleeding. Hourly dynamics with pre-specified criteria for surgical bleeding would certainly reduce the number of patients bleeding due to surgical cause. A more detailed explanation is included in manuscript.

4. Since tranexamic acid was administered routinely at the induction of anesthesia and after protamine administration, it's potential effect on results should be discussed.

AUTHORS: Antifibrinolytics are routinely used at our center at two time points, (1) at the induction of anesthesia and (2) after protamine administration. There are conflicting data published recently on this issue. (Al-Lawati et al recently showed that antifibrinolytics were not able to prevent excessive postoperative bleeding in group of patients who underwent CABG with preoperative aspirin administration. In contrast to, Shi et al showed tranexamic acid to be beneficial in terms of reduced blood loss, major bleeding and reduced transfusion outcome in patients who were exposed to CLO within 7 days before surgery. The same authors obtained the same results in patients undergoing CABG without preoperative clopidogrel and aspirin cessation.). Since all patients received tranexamic acid in the same dose and at the same time we assume that all patients were well balanced in respect to possible effects of tranexamic acid. Even if correlations between POC hemostatic tests and bleeding outcome might be distorted in some degree by tranexamic acid, the possible role of tranexamic acid should not be overestimated. Aside from tranexamic acid, there are several factors that influence correlations between POC hemostatic devices and chest tube outcome such in prospective noninterventional studies such as ours. Despite the fact that patients were recruited in study, patients were regularly treated according to our center transfusion management. All procoagulant blood
components administered perioperatively, certainly affected correlations in way that correlations were probably attenuate as well as sensitivity/specificity values in bleeding prediction estimation model. This is ubiquitous shortcoming of all prospective observational studies. Please note that ideal research setting would not be ethically accepted since patients were supposed to receive the best current available hemostatic protocol in respective center. The use of tranexaminic acid is now commented in manuscript.

5. As for the management of postoperative bleeding, it appeared to be left to the clinical judgement of anesthesiologist. As the results show, all blood products were applied. But again, only PRBC and FFP had predetermined criteria for application. A more clear insight might be warranted.

AUTHORS: The study was designed as prospective observational trial. Transfusion management of procoagulant blood components was not changed during the study period and patients received procoagulant blood components either according to predetermined criteria or according to attending anesthesiologist preference. Administration of fibrinogen concentrate and patelet concentrate was left to anesthesiologist discretion. All patients were treated by the same group of consultant anesthesiologists. Text addressing this issue has been included in the manuscript.

6. Patients in the bleeder category had significantly longer CPB times and lower body temperature on CPB. Effects of both on postoperative bleeding should be discussed.

AUTHORS: It is well known that CPB alters the hemostatic balance and predisposes cardiac surgery patients to increased risk of excessive bleeding. Pathophysiology of excessive bleeding after CPB has been described by Green et al. There are several factors related to CPB that contribute to onset of hemostatic disorder such as: foreign surface contact, consumption of clotting factors, platelet activation and dysfunction and fibrinolysis. In addition to, hemostatic impairment during CPB arises in some extent from systemic hypothermia that induces kinetic slowing of coagulation, kinin and kalikrein activation, platelet function and fibrinolysis. The fact that hypothermia tends to increase bleeding in the cardiosurgical patients has been intuitively recognized by cardiac surgeons despite scarce evidence available at the beginning. Canine studies have shown that hypothermia causes thrombocytopenia and activates fibrinolytic system. Those results were confirmed in normal volunteers both in vitro and in vivo suggesting that adequate rewarming strategy may reduce the need for less safe alternative such as transfusion of procoagulant blood components. The role of CPB and hypothermia in hemostatic disorders onset has been included into manuscript.

7. Part of the discussion in which he authors propose a TEM guided algorithm of postoperative blood components administration should be left out completely as it is not substantiated by the parameters investigated in this study. Similarly parts of the conclusion section not pertaining to the topic of investigation e.g. " Concomitant use of InTEM and HepTEM tests enables precise detection of low to moderate heparin concentration [13].
Therefore, TEM test also enables physician to detect or prevent protamine excess resulting from empirical or ACT-based additional protamine administration. Hemostatic disturbances after adequate heparin-protamine neutralization management may be easily detected with additional use of ExTEM and FibTEM assays. This may be very useful, especially in patients who developed the coagulopathy due to other CPB associated factors. In such a cases, detection of coagulation factor depletion, platelets and/or fibrinogen dysfunction may lead to appropriate, “targeted” hemostatic therapy and more efficient hemostatic management. Such an approach may help to improve clinical outcome with lower incidence of excessive bleeding, lower transfusion requirements with lower incidence of transfusion related adverse outcomes. Appropriate cut-off’s for transfusion management guided by TEM should be directed according to cut-off’s defined through prospective non-interventional studies" or " In our study the attending clinicians, both the anesthesiologists and surgeons were blinded to InTEM results, therefore administering procoagulant blood products mainly on the clinical grounds. The use of procoagulant blood products certainly affected the amount of bleeding. This decrease in amount of bleeding would reduce the degree of correlation between blood loss and both ACT and InTEM parameters by reducing the sensitivity of each parameters. Although regularly used in clinical practice, ACT lacks to provide prediction of bleeding events. TEM InTEM test was shown to be superior over ACT in assessment of bleeding extent. In addition to, it may be used for heparine-protamine management by concomitant use of Heptem and titration of clotting time parameters which are influenced by either heparin and protamine. MCF parameters significantly correlate to amount of chest tube discharge." should be in discussion section although I think they are out of scope in this investigation. Furthermore, it is unusual for a Conclusion section to include references.

**AUTHORS:** The part of discussion section describing possibilities for transfusion management has been left out. Extensive changes have been done in “Discussion” section with aim to adjust it to reviewer suggestions. We decided to include references whenever we found it appropriate.

8. In the Conclusion section of the Abstract I find the statement " With aim to predict and prevent excessive postoperative CTO, as well as excessive transfusion requirements, hemostatic interventions with timely and targeted blood component therapy according to TEM results should be considered" beyond the scope of the investigation, at least as the objectives are set.

**AUTHORS:** We agree with reviewer’s comment. Conclusion section within Abstract is shortened with aim to stay focused on the scope of investigation.

**MINOR ESSENTIAL REVISIONS**

1. In the results section of the Abstract results pertaining to the topic of investigation should be elaborated (within word limits). CPB time or temperature should be left for the results section of the text.

**AUTHORS:** Abstract/Results section has been changed completely in line with reviewer suggestions.
2. Tables look very busy! In Tables 1a and 1b, data should be reorganized in such a matter to show continuous data as average with STDEV or median, and categorical data should be presented with number and percentage (just the positive value). In Table 2. groups should be more clearly labeled than < and >75 perc (eg: bleeder, non-bleeder). Data should be presented as average with STDEV.

**AUTHORS:** We appreciate reviewer's comment regarding tables. We have done changes in tables, so now they look like as suggested by reviewer. Please note that data are expressed according to normality of distribution. Even though some variables have had normal distribution, in order to provide uniform presentation of data, we decided to use non-parametric tests. This way of data presentation was confirmed by professional statistician directly involved in drafting the manuscript.

3. Figures could be merged into one with legend on the side.

**AUTHORS: Figures are merged into one.**

4. Abbreviations should be used consistently throughout the text. Eg. "ROC curves were constructed to assess the ability of ROTEM....". This was the first mention of ROTEM. It should be TEM as throughout the text.

**AUTHORS: done**

5. There are some typos in the Results section: "Table 1a and 1b" Should read "Tables"; "thus cut-off" should be "thus a cut-off"; "Median 210 sec vs 192 sec" should obviously be "192 sec"

**AUTHORS: Minor typewriting mistakes have been changed.**

**REVIEWER 2:**
Thank you for submitting this excellent article to the Journal of Cardiothoracic Surgery. In this paper, Petricevic and colleagues conducted prospective observational study with aim to compare activated coagulation time vs. thromboelastometry parameter (INTEM test) in assessment of excessive bleeding. INTEM, but not ACT correlated significantly with amount of chest tube output. This paper presents an interesting and important topic. Objectives of the paper and importance of the research question is clearly stated. Optimal hemostatic management remains to be challenging. Early intraoperative prediction of excessive bleeding risks seems to be very important because of possibility to timely treat hemostatic disorder and to timely prevent excessive bleeding. The study was conducted in prospective observational fashion enrolling 148 consecutive patients undergoing elective cardiac surgery procedures. Prospective observational study is appropriate research setting for this particular research question. Outcome measures are clearly defined. Post hoc definition of excessive bleeding according to the distribution of chest tube output among study participants is very interesting and precise. There are several ways in defining excessive postoperative bleeding, however, this approach presented by authors is adjusted to the study cohort as well as to anesthetic, perfusionistic and surgical techniques. ROC analysis is appropriate model of testing diagnostic tests accuracy. Results are clearly present. ROC analyses have had significant model. However, although statistically significant, the correlations are „moderate“ and authors should state that. We can expect moderate correlations due to several reasons: 1) chest tube output consists of both „surgical“ bleeding and bleeding due to hemostatic disorder, 2)
hemostatic disorder is multicausal, and assessment of one part of hemostasis may not provide strong correlations. Reported correlations are clinically relevant and useful i.e. sensitivity of 82.9% (ROC test for INTEM A 10 less or equal to 38mm) enables early (10 min) detection of patients who are not in risk of excessive bleeding. Therefore, it is possible to educate transfusion requirements in some proportion of patients. Discussion is well researched. Very recent paper by Galeone et al (JCVA 2013) should be considered for inclusion into discussion since that paper has a similar research question.

AUTHORS:
We appreciate comments provided by reviewer. Paper by Galeone et al (JCVA 2013) addresses very similar question. Manuscript published by Galeone et al has been included into discussion.

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