Author’s response to reviews

Title: Polymorphisms on PAI-1 and ACE genes in association with fibrinolytic bleeding after on-pump cardiac surgery

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Author’s response to reviews: see over
Response to Dr. Spiezia

Dear Dr. Spiezia,

Thank you for spending your time and expertise on reviewing our manuscript. We apologize for late response because the editorial office delayed the forwarding of your review until recently. Below we respond to your critiques and comments point-by-point referring to page (P) and line (L) number, and if changes should be required, they will be marked up in the manuscript by using the track changes function in Word.

**Reviewer:** “The study is a small appendage of a previous paper published in this journal (Ozolina A et al. BMC Anesthesiol. 2012;12:27). The results of this study are, to me, minimal and could be condensed in a "Letter to the Editor" or in a "Short report"

**Authors:** As the Reviewer correctly states, we partly have based the present study on material from a previously published article. We have clarified this on three places in the manuscript, 1) in the Abstract: please, see Methods, (P 2, L10): “We studied PAI-1 -844 A/G, and ACE intron 16 I/D polymorphisms by means of polymerase chain reaction technique and direct sequencing of genomic DNA from the same cohort of patients (n=83) that we have presented earlier.” 2) in Background (P4, L 26): “Consequently, by examining the same patient cohort as referred to above [14], our primary end-point was to address the associations between PAI-1 -844 A/G and ACE Intron 16 I/D polymorphisms and fibrinolytic bleeding after CPB surgery”; 3) in Methods (P 4. L3 0): “Methods has been presented previously and will only be shortly described” (Ozolina A et al. BMC anesthesiology 2012, 12:27; Ozolina A et al. Medicina (Kaunas, Lithuania) 2012, 48(10):515-520).

**Authors:** “Since we already had DNA from a cohort of patients, in whom we could combine translational factors with thoroughly obtained clinical data, we found it appropriate to search for further polymorphisms. We consider as utmost important to identify polymorphisms that might increase the potential for augmented postoperative blood loss. We assume that such tests soon will be among those routinely assessed before major surgery”.

**Reviewer:** “As far as the message of the paper is concerned the authors stated that the mechanism of action of the two polymorphisms should be related to an increase in the fibrinolytic potential but no specific assays neither in this nor in the previous publication (i.e. euglobulin lysis time, whole bloodthromboelastometry/thromboelastography) was made to evaluate the contribution of the real increase of the fibrinolytic system in the clot lysis”.

**Authors:** “We agree that euglobulin clot lysis time and thromboelastography (TEG) or maximum clot lysis by using thromboelastometry (ROTEM) would have provided valuable information and strengthened our message. Dr. Molnar also raised the same concern. On the other hand, according to recent studies, TEG/ROTEM can only detect severe fibrinolysis in 5% of cases as compared to 57% of the cases of moderate fibrinolysis diagnosed with fibrinolytic markers, such as antiplasmin-plasmin complex (Raza I et al. J Thromb Haemost. 2013;11). Lower thresholds have been suggested for detecting 30-minute fibrinolysis (LY30) by TEG (Chapman MP et al. J Trauma Acute Care Surg. 2013 Dec;75(6)). We admit this point in Limitations section (P12,L5). Moreover, for cardiac surgery patients it is hard to
detect hyperfibrinolysis because they receive antifibrinolytics during the surgery. Therefore, we limited our methods to determination of fibrinolytic markers since these agents reflect fibrinolytic potential more sensitively early before the surgery. Because grant for research was scanty and kits for genomic studies are expensive, we kept the number of samples as low as possible.”

**Reviewer:** “I don't understand why patients with surgical bleedings were excluded by the analysis? The hyperfibrinolysis could justify surgical bleedings, isn't it?”

**Authors:** “We agree. Excessive fibrinolysis can amplify surgical bleeding if there is a site of insufficient surgical hemostasis. We exempted patients with increased postoperative blood loss originating from a surgical source at the reopening, and focused mainly on changes in hemostasis after the use of CPB. We consider surgical bleeding as the result of poor surgical hemostasis and based the indication for reopening on evaluation of clinical and hemodynamic changes. We diagnosed a surgical bleeding as such, only if we could identify one or more bleeding sites. If not, the case counted as non-surgical bleeding. If the patient was reopened, the CTD volume until reoperation, and 24 hours afterwards was registered. From our point of view, hyper-fibrinolysis per se can count as a cause of bleeding”.

**Reviewer:** “An article of limited interest”

**Authors:** “We humbly respect the Reviewer’s opinion, but disagree. There is abundant number of studies available showing associations between genetics and bleeding, thrombosis and diabetes mellitus etc. To our knowledge, our findings are new and important. Out of the study population, nearly 30 % were carriers of PAI-1 844 GG or ACE Intron 16 I/I polymorphisms, respectively, that both resulted in lower PAI-1 preoperatively and higher blood loss postoperatively (Ozolina A. Thesis. Riga Stradins University, Riga, Latvia 2013). We are looking forward to see confirmatory evidence from other centers with larger groups of patients. In a not too far future, we feel confident that information about increased risks of postoperative bleeding due to genetic polymorphisms will be highlighted in the list of precautions, as soon as the digital journal is downloaded at the pre-anesthetic visit”.

**Reviewer:** “Quality of written English: Needs some language corrections before being published”.

**Authors:** “Since none of us writes and speaks English as our native language, we consider that contention as highly likely. We have shortened the manuscript as you suggested and revised it with special emphasis on improving the language”.

**Reviewer:** “Statistical review: No, the manuscript does not need to be seen by a statistician”.

**Authors:** Thank you. We appreciate your comments.
Response to Dr. Molnar

Dear Dr. Molnar,

Thank you for spending your leisure time on reviewing our manuscript. We are sorry for late response because the editorial office, due to misunderstanding, delayed the forwarding of your review until recently. Below we respond point-by-point to your comments referring to page (P) - and line (L) numbers in the manuscript if required to make changes.

Reviewer: “The idea fits nicely into current trends of aiming to individualize critical care. Methods are sound, results are convincing and the limitations are more-or-less pronounced in the appropriate section of the manuscript”.

Authors: “Thank you for this positive and forward-looking statement”.

Reviewer: “My only concern is that although the authors blame increased fibrinolysis for increased bleeding due to the found gene type, but apart from D-dimer measurements there is nothing else to prove it. It is bit disappointing to me, that such sophisticated laboratory measurements are not accompanied/reinforced by objective measurements, such as thrombelastography (TEG or Rotem), which has become a routine POCT diagnosis in most European centers. Would you please comment on that and a sentence or two would be required in discussion as well”.

Authors: We understand this concern, which was also raised by Dr. Spiezia and we commented as follows:

We agree that euglobulin clot lysis time and thromboelastography (TEG) or maximum clot lysis in thromboelastometry (ROTEM), would have provided valuable information and strengthened our message. The same concern also was raised by Dr. Molnar. On the other hand, according to recent studies, TEG/ROTEM can only detect severe fibrinolysis in 5% of cases as compared to 57% of the cases of moderate fibrinolysis diagnosed with fibrinolytic markers, such as antiplasmin-plasmin complex (Raza I et al. J Thromb Haemost. 2013;11). Lower thresholds have been suggested for detecting 30-minute fibrinolysis (LY30) with TEG (Chapman MP et al. J Trauma Acute Care Surg. 2013 Dec;75(6)). We admitted this point in Limitations section (P12,L5). Moreover, for cardiac surgery patients it is hard to detect hyperfibrinolysis because they recieve antifibrinolytics during the surgery. Therefore, we limited our methods to determination of fibrinolytic markers since these agents reflect fibrinolytic potential more sensitively early before the surgery. Because grant for research was scanty and kits for genomic studies are expensive, we kept the number of samples as low as possible.”

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

Authors: We agree. Thank you.

Reviewer: Quality of written English: Acceptable

Authors: We have revised and shortened the manuscript and have put special emphasis on correcting linguistic mistakes.
Ozolina A et al. “Polymorphisms on PAI-1 and ACE genes in association with fibrinolytic bleeding after on-pump cardiac surgery”

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

Authors: The statistics was also commented on and found sufficient by two other referees.
Response to Dr. Vaage

Dear Dr. Vaage,

Thank you for spending your time on reviewing our manuscript. We are sorry for the late response because the editorial office (due to misunderstanding) delayed the forwarding of your review until recently. Below we respond to your comments point by point, and if required to make changes in the manuscript, we have marked them up. Below we refer to page (P) - and line (L) numbers for changes.

Reviewer: This is a manuscript that already has been reviewed and the reviewers have asked a series of detailed questions that critically questioned several parts of the manuscript. According to this reviewer, the Authors have answered all questions very thoroughly and they have outlined the limitations of the study.

Authors: The reviewer is correct and after his review, we have shortened and revised the manuscript and made a few linguistic improvements.

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

Authors: We are convinced that outlining genetic polymorphisms in the coagulation – and fibrinolytic systems are of great importance for patient safety and will be a part of future pre-anesthetic routines.

Reviewer: Quality of written English: Acceptable

Reviewer: Statistical review: Yes, and I have assessed the statistics in my report

Authors: Thank you for these positive statements.