Author’s response to reviews

Title: The changes in clot microstructure in patients with ischaemic stroke and the effects of therapeutic intervention: a prospective observational study

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Author’s response to reviews: see over
9th January 2015

Dear Editor,

RE: Title: The changes in clot microstructure in patients with ischaemic stroke and the effects of therapeutic intervention: a prospective observational study

Thank you for considering our manuscript for publication following submission of the revised draft.

We would like to thank the reviewers for their time and valuable comments. We have addressed all the points raised and made changes as necessary as you can see below.

We look forward to hearing from you in due course.

Yours sincerely
P. A. Evans

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Editor's comment:

1. Please state that you examine clot-microstructure ex-vivo, especially in the abstract.

This has been stated.

2. Are the authors able to provide some clinical data: clinical outcome such as modified Rankin scale or NIHSS? It would be interesting.

We would have liked to report these data but these scores were not available at the time patients were recruited in this study. We have recently started recording modified Rankin scale in our hospital, which will be useful for any future studies.
Reviewer's report of Yasuhiro Suzuki:

1) Line 56, in conclusion of Abstract, Although you described “--- the new biomarkers such as predicting successful recanalization and bleeding risk following thrombolysis ---”, I think it is most important that the biomarkers is predicting the recrudescence of stroke. Although the means of the parameters in patients with stroke were within the range of the mean and standard deviation in healthy volunteers, do you mean they can be the prediction of recanalization and the bleeding risk when they are down from baseline?

Yes we meant this as a potential clinical implication, which needs to be explored in future studies. Nonetheless we agree with the reviewer this may be a premature assumption to be highlighted in the abstract and hence this sentence has been removed from the abstract.

2) Although you discussed in Line 203, these biomarker would be unsuitable for clinical use because the changes in these biomarker was small. However, if as described the line 207, you should show the additional data of 1.74 and 1.76 in Fig. 2. If we can tell the difference just by looking, df will be suitable.

Thank you for pointing this. The figure has been amended to include these values.

3) On Fig. 1, The differences in aspirin treatment recovered to the baseline at 24 hours after treatment. However, there was no explanation for the time point in the result section. Perhaps, it looks like that authors only described the results at 2-4 hours after the first administration of aspirin. As you described in Line 225, aspirin irreversibly binds to COXs, which results in the continuous inhibition of platelet aggregation until the life-span. Was the value of 24 hours the trough level? You should discuss that the parameters in Fig. 1 recovered to the baseline levels. This point is important as predicting biomarkers.

It is correct that the 24 values returned to baseline values. We agree with the reviewer this is an important finding, which needs further investigation. This has been discussed as some previous studies reported a stronger inflammatory response following reperfusion or it could be due to the ongoing neuroinflammatory/ischaeemic process during the subacute phase and hence these values may have been even higher than baseline values without treatment. The tests were done 2 hours after the morning dose so it’s not the trough level.

4) On Fig. 3, There was no explanation for Fig. 3 in the result section. It looks like that the surface of fibrin net was smooth in panel A and was rough in panel B. What is the humps of the surface in panel B? Were a lot of platelets or neutrophils adhered to the surface of fibrin? It would be helpful for readers to provide the in-depth explanation with Fig. 3. If they were the adhered platelets after aspirin treatment, you would need to discuss it.
Several proteins bind to fibrin and we discussed some possibilities. The rough surface of the fibers in panel B could be attributed to plasma proteins (plasminogen, tPA, albumin, a2AP, PAI-1, etc) that bind to fibrin. The larger knobs look more like microparticles.

Minor Essential Revisions:

1) Would you mind many typographical errors in this manuscript carefully? (e.g. Ref. 17 and so on)

All references revised and corrected where necessary and manuscript revised for any typographical errors.
Reviewer's report of Anetta Undas:

1. Patient characteristics should be detailed. The definitions of all the clinical states, e.g. hyperlipidemia were not provided. The term “antiplatelets” is unclear. Are any medications other than “antiplatelets or statins used? At least antihypertensive agents were probably used; ACE inhibitors, for instance, have been reported to alter plasma fibrin clot properties. A few basic laboratory tests are missing. Given available data, apart from fibrinogen, some other laboratory test are likely to alter clot parameters, such as creatinine, glycemia, C-reactive protein (see the latest reviews on fibrin clot properties in thromboembolism). Given the topic I would expect at least D-dimer levels.

The requested data have been included in table 1.

2. Stroke severity should be presented. Were there any associations between for example the modified Rankin scale and rheometric tests?

Please see point 2 above under editor’s comment.

3. Aspirin exerts several antithrombotic effects other that of COX-1 acetylation, including decreased thrombin generation. Given the fact that a small effect of rtPA on clots is surprising compared with a more potent effect of aspirin this issue needs more attention in the Discussion.

This has been considered in the discussion. The unexpectedly lower effect of tPA could be attributed to the fact that blood sampling was collected 2 hours after Alteplase infusion, which has a short half life.

4. In the Introduction more information on the factors that determine clot structure and function in acute ischemic stroke is needed. Are there any differences between patients with acute ischemic stroke and those with hemorrhagic stroke? Could we expect any differences between acute myocardial infarction versus acute ischemic stroke in terms of fibrin parameters? Please comment on it in the Discussion.

More information added

Minor comments

“real-time clot formation time” – this parameter has not been mentioned in the abstract. Data on the age, sex and mode of therapy are needed in the Abstract. Moreover, it should be clearly stated which clot parameters were changed following stroke therapy with rtPA. In the data presentation, stroke patients should be highlighted, not healthy controls. The last sentence of the Abstract could be deleted.

The suggested changes to the abstract made and the last sentence deleted.