Author’s response to reviews

Title: Hypercoagulability and the risk of recurrence in young women with myocardial infarction or ischaemic stroke: a cohort study

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Author’s response to reviews:

Dear Editor,

We thank you for the opportunity to revise our manuscript and respond to the comments raised by you and the peer reviewers. Our responses can be found below, including the changes in the new version of the manuscript.

(Reviewer 1):

1) R: Major comments RATIO patients presented with MI or ischaemic stroke in 1990-95 (P4, L24) and were included in 1995-98 (P4, L43), with blood samples for coagulation markers drawn after a median of 69 and 95 months for MI and ischaemic stroke cases, respectively (P4, L58-60). Accordingly, it seems that patients who died in the period 1990-95 after their index event were not part of the follow-up analysis.

A: We appreciate that the reviewer read carefully the paper and we thanks him/her for this comment. As pointed out by the reviewer, patients have been enrolled in the RATIO follow-up study after a median time of 1.5 years from the event and, for the present study, patients must have survived till the time of the blood drawn. Therefore, by design, this study focusses on the long-term risk of survivors of young stroke and myocardial infarction. Even though this selection does limit the external validity of our study, we believe that the practical implications for the interpretation of our study are limited. This is primarily based on the idea that the question answered by our data (does hypercoagulability affect the long-term cardiovascular risk in young survivors) is still a relevant clinical question. In fact, it might even be a more relevant question as compared with the question derived from a study in which short effects and long-term effects are
mixed. We do not think that this selection of survivors actually introduced a bias, as the data collection for both case groups (i.e. stroke and myocardial infarction) was similar. (see also our answer to question #2).

Nonetheless, we believe that the point raised by the reviewer merits some additional description in the manuscript, and we have decided to add the following text to the manuscript in the discussion section (page 10 line 12):

“Patients enrolled in our study a few years after the event, and therefore, patients suffering from early fatal ischaemic stroke or myocardial infarction are not included in the analyses. This implies that our results should be applied to survivors of both ischaemic stroke and myocardial infarction. In fact, including patients from the moment of their stroke would mix long-term and short-term effects, potentially hindering their interpretation”

2) R: Also, the apparent 2 year difference in the (median) time of blood sampling between the 2 groups seems a bit peculiar and the authors may want to clarify these matters.

A: The original RATIO study included 248 participants with myocardial infarction, 203 with ischaemic stroke and 925 controls. In a subset of these women blood samples were collected for blood measurement of clotting factors and DNA analyses (205 participants with myocardial infarction, 125 with ischaemic stroke and 638 controls). To compensate for the loss of statistical power in the ischaemic stroke group, we additionally recruited 50 women who presented with an ischaemic stroke at the University Medical Center Utrecht between 1996 and 2001. This approach had the unintended consequence that the time between event and blood sampling differed between the two case groups.

We added this explanation in the Methods section of the manuscript as follow (page 5 line 6):

“Blood samples and buccal swabs were collected for blood measurement of clotting factors and DNA analyses in a subset of women who initially participated in the first phase of the RATIO study (205 participants with myocardial infarction, 125 with ischaemic stroke and 638 controls). To compensate for the loss of statistical power in the ischaemic stroke group, we additionally recruited 50 women who presented with an ischaemic stroke at the University Medical Center Utrecht between 1996 and 2001, leading to a small difference in median time between event and blood sampling between the two case groups (69 months, range 38 to 117 months, for myocardial infarction cases and 95 months, range 23 to 146 months, for ischaemic stroke cases). The interval between the initial event and blood sampling minimises the risk of reverse causation as well as the mixing of short and long-term effects of hypercoagulability on cardiovascular risk.”

3) R: In addition, they may comment on the potential for differential effects of long-term different treatment of MI vs. ischaemic stroke, e.g. antithrombotic drugs, statin dose etc., to affect some of the (non-genetic) coagulation markers included in their genetic score, and the authors may also comment on whether markers of inflammation may have added prognostic value is this context.
We thank the reviewer for this interesting comment and suggestion, raised also by reviewer #2. Secondary prevention is of the utmost importance after a vascular event. We address several classes of medications separately:

Anticoagulant drugs are invariably associated with changes in the coagulation markers. However, since ischaemic stroke from cardioembolic origin had been excluded from the RATIO study, anticoagulant drugs (VKA) were not used in this group of patients and thus could have not impacted the calculation of the prothrombotic score.

Regarding antiplatelet drugs, their impact on measurable levels of prothrombotic markers, such as tissue factor, plasminogen activator, intrinsic coagulation factors, ADATSM13, VWF and anti-phospholipid antibodies, is minimal.

Other cardiovascular drugs such as statins may slightly affect measurable levels of some coagulation factors, such as tissue factor, thrombin, fibrinogen, factor XIII and factor Va (Undas et al, 2013).

In order to influence our main conclusions the prescription patterns for all drugs must have been dependent on either the prothrombotic score or differ between the two case groups in general. However, as most the measurements included in the prothrombotic score were not measured in clinical routine and prescription patterns for secondary prevention did not differ substantially between the two case groups, we believe that no substantial bias arose because of medication use that would negate our findings.

Probably inflammation has a link with some of the coagulation factors in our prothrombotic score and potentially could explain part of the association observed in our analyses. Unfortunately, we do not have measures of inflammation with which we could construct an inflammatory score in a similar fashion in order to test this hypothesis.

To highlight above issues, we have added the following sentences in the discussion section (page 11 line 12):

“Some medications, such as anticoagulants, antiplatelets and statins may have influenced the prothrombotic score. However, the ischaemic stroke patients did not use anticoagulant as their stroke was of non-cardioembolic origin. Antiplatelets and statins at baseline have at most a marginal effect on measured coagulation factors, but given that prescription of these drugs as such is not dependent on the prothrombotic score, it is unlikely that this would have caused a strong bias. Finally, we cannot exclude that our results partly reflect a different involvement of inflammation in the relationship between hypercoagulability and recurrences. Unfortunately, we do not have data in order to investigate this hypothesis.”

Minor comments

4) R: P2 L16: Use 'HRs' instead of 'HR'.
5) P4, L48: It would seem most important to state that control subjects had no prior MI or stroke.

A: the text has been modified as suggested.

6) P5, L 43: 'or to nobody at all' may need an explanation. How many were there of such instances?

A: 1376 women (248 myocardial infarction cases, 203 ischaemic stroke cases and 925 controls) participated in the RATIO case-control study. Of these women, 1168 (87%) were successfully linked to the registries (Figure). The follow-up therefore included 226 patients with myocardial infarction, 160 patients with ischaemic stroke and 782 control subjects.

We modified the text accordingly, adding this sentence (page 6 line 9):

“The original RATIO case control study included 1376 women (248 myocardial infarction cases, 203 ischaemic stroke cases and 925 controls). Of these women, 1168 (87%) were successfully linked to the registries.”

7) R: P6, L12: 'will invariably lead to hospitalization' should probably be 'will most likely lead to hospitalization'.

A: the text has been changed as the reviewer suggested (page 6 line 20).

“We chose to include these two acute cardiovascular diseases, as these will most likely lead to hospitalization and therefore be captured by the Dutch Hospital Data register.”

8) R: P7, L34-36: It may be relevant to shortly describe how some of these covariates were defined, e.g. alcohol consumption, diabetes, hypertension, hyperlipidaemia, and family history of CVD.

A: We added the following detailed description of covariates (page 4 line 22):

“All participants were asked to fill in the same structured questionnaire comprising questions on medical history of cardiovascular risk factors. Smoking was defined as having regularly smoked in the year before the index date. Alcohol consumption was defined as drinking at least one unit of alcohol per week. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m2). Women were classified as hypertensive, diabetic or hypercholesterolaemic when they reported a physician’s diagnosis or were taking medication for these conditions before
the index date. Family history of cardiovascular disease was defined as the presence of myocardial infarction, stroke or peripheral artery disease below 60 years of age in first degree relatives.”

9) R: References: Check journal's format - for example, annotations 'Br J Haematol' (ref #16) and 'British journal of haematology' (ref. #19) cannot both be correct.

A: all references were checked and corrected when needed.

10) R: Table 1: Add 'years' and 'kg/m2' to Age and BMI, use 'Medical history' instead of 'History of'.

A: The requested changes have been made.

Reviewer 2:

1) R: The authors have followed for a long time (18 years of median) a cohort of women from the RATIO study that have suffered an ischemic stroke or a myocardial infarction, to see if a series of coagulation parameters obtained at baseline were predictors of a second event. An important limitation is that information on medications during follow-up is not provided. Therefore, it is impossible to know the effect of drugs (ie antiplatelet and/or anticoagulants) in the positive association between hypercoagulability and stroke, as well as in the lack of association between hypercoagulability and myocardial infarction. Furthermore, probably almost all patients were on antiplatelet therapy after MI, but perhaps it was not the same after ischemic stroke.

A: We thank the reviewer for this comment, raised also by reviewer #1 (comment #3).

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2) R: Also, in Discussion: We think that other risk factors, apart from classical risk factors, could be important in young women, i.e hyperhomocysteinemia. Some discussion on this issue would be pertinent.

A: Due to the design of the analyses, in our prothrombotic score we only incorporated hypercoagulability markers measured in the RATIO study in both myocardial infarction and ischaemic stroke patients. Therefore, even though our prothrombotic score includes 18 protrombotic markers, some interesting factors including levels of homocysteine were unfortunately not included. Luckily, we were able to include the homozygous form of the MTHFR C677T SNP as a proxy marker for hyperhomocysteinemia.

We added this sentence in the discussion section (page 10 line 24):

“Even if our risk score does not include all markers of coagulation, we were still able to include 18 presumed markers of hypercoagulability as they were measured in both case groups of the RATIO study.”