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**Title:** New insights in the risk profile for arterial thrombosis: differences and similarities in risk factors between myocardial infarction and ischaemic stroke  
**Date:** 2017-11-21
Chapter 4

Hypercoagulability and the risk of myocardial infarction and ischaemic stroke in young women

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Journal of Thrombosis and Haemostasis, 2015
Abstract

**Background:** Myocardial infarction and ischaemic stroke are both acute forms of arterial thrombosis and share some but not all risk factors, indicating different pathophysiological mechanisms.

**Objective:** This study aims to determine if hypercoagulability has a differential effect on the risk of myocardial infarction and ischaemic stroke.

**Methods:** We reviewed the results from the Risk of Arterial Thrombosis in Relation to Oral Contraceptives study, a population based case control study involving young women (<50 years) with myocardial infarction, non-cardioembolic ischaemic stroke and healthy controls. From these data, relative odds ratios (OR\textsubscript{IS}/OR\textsubscript{MI}) and their corresponding confidence intervals for all prothrombotic factors that were studied in both subgroups were calculated.

**Results:** Twenty-nine prothrombotic risk factors were identified as measures of hypercoagulability. Twenty-two of these risk factors (22/29, 72%) had a relative odds ratios >1, for 12 (41%) it was >2, and for 5 (17%) it was >2.75. The five risk factors with the largest differences in associations were high levels of activated factor XI (FXI) and FXII, kallikrein, the presence of lupus anticoagulans, and a genetic variation in the factor XIII gene.

**Conclusion:** In young women, prothrombotic factors are more associated with risk of ischaemic stroke than myocardial infarction risk, suggesting a different role of hypercoagulability in the mechanism leading to these two diseases.
Introduction

Thrombotic diseases are among the leading causes of morbidity and mortality in the world. A large proportion of this burden can be ascribed to acute arterial thrombotic diseases, such as myocardial infarction and ischaemic stroke. The incidence of both these diseases rises sharply with age, largely due to age-related risk factors such as hypertension and atherosclerosis.¹

Both myocardial infarction and ischaemic stroke are multi-causal diseases, with a similar underlying mechanism of thrombus formation in the arteries supplying oxygen to either the heart or the brain.² Both the location and extent of the thrombus dictate the clinical presentation and consequence of the disease. Rudolph Virchow postulated in 1856 that the causes of thrombotic disorders, both venous and arterial, could be divided into three categories, which would now be called stasis, vessel wall damage and hypercoagulability.³

Hypercoagulability is the condition in which the coagulation system is out of balance and prone to thrombus formation. Such a hypercoagulable state can be the result of increased levels of coagulation factors, but also of a reduced fibrinolytic capacity. Hypercoagulability is a well-established risk factor for venous thrombosis, and, to a lesser extent, for arterial thrombosis.

However, although several markers of hypercoagulability have been linked to arterial disease, it is unknown whether their effect is similar for all forms of arterial thrombosis.⁴⁻⁹ There has never been a comprehensive assessment of hypercoagulability and its relation to different subtypes of arterial thrombosis.

Therefore, this paper aims to determine whether hypercoagulability and its specific constituents have a differential effect on the risk of myocardial infarction and ischaemic stroke using published data from the Dutch RATIO (Risk of Arterial Thrombosis in relation to Oral contraceptives) case-control study.
Methods

Study design

We used published data from the RATIO study which was originally set up to determine the risk of myocardial infarction and ischaemic stroke in relation to oral contraceptive use, and was described in detail in previously.\textsuperscript{10-12} In this study, cases where women who were diagnosed with a myocardial infarction, non-cardio embolic ischaemic stroke or peripheral arterial disease in one of the sixteen Dutch participating hospitals. Women free from arterial disease were approached by random digit dialling to participate in the shared control group which was frequency matched on age, year of event and area of residence of all case groups (Figure 1). Informed consent was obtained from all participants, and the study was approved by the medical ethics committees of the participating hospitals. Initially, women were asked to provide data on risk factors (oral contraceptive use, presence of a previous diagnosis of, or treatment for hypertension, hypercholesterolemia, and diabetes mellitus) in the year prior to the event (cases) or index date (controls) through a detailed questionnaire. Blood and DNA samples were collected in a later phase in order to investigate prothrombotic factors as a measure of hypercoagulability and their role in the aetiologic mechanism of myocardial infarction and ischaemic stroke. Blood samples were drawn post acute phase (>23 months). These samples were in an earlier phase used to measure several prothrombotic factors, in order to study their role in the aetiology of myocardial infarction, ischaemic stroke or both, depending on the research question. Since all these different research questions have different analytical approaches the current analysis relies on the odds ratios as previously published.\textsuperscript{10,13-24} This way, a direct comparison of the effects can be obtained while preserving any choices made during the original analyses. Since this analysis compares the effects on myocardial infarction and ischaemic stroke risk, only the prothrombotic factors that have been measured in both subgroups were eligible for the current analysis.

Statistical analyses

As said, the current analyses includes published data from women who suffered from myocardial infarction (N=205) or ischaemic stroke (N=175) and control women (N=638, Figure 1). In these women, several prothrombotic factors, all reflective of a hypercoagulable state, were measured and these were published previously. The individual analyses differ
from each other due to differences in selection of participants, available data, cut off
determination and adjustment for confounding. In general all factors were analysed with
unconditional logistic regression models, with stratification variables (i.e. age, area of
residence and year of event) to obtain odds ratios for myocardial infarction (OR\textsubscript{MI}) and
ischaemic stroke (OR\textsubscript{IS}) as measures of rate ratios. These odds ratios, as well the choice for
sources of confounding, were collected from the published results. These previous
publication distinguished four adjustment models: Model 1 indicates adjustment for
stratification variables age, area of residence and year of event. Model 2 additionally includes
hypertension, diabetes and hypercholesterolaemia. Model 3 includes variables from model 2
with the addition of smoking. Model 4 includes the same variables from model 3 with the
addition of body mass index (BMI). Exposures that were originally analysed with model 2
(exposures number 1, 10, 21, 22 and 28) were additionally reanalysed according to model 3
to account for potential residual confounding.

A direct comparison of these effect estimates was obtained with the relative odds ratio (ROR)
which is calculated as

$$ROR = \left( \frac{OR\textsubscript{IS}}{OR\textsubscript{MI}} \right)$$

If the ROR >1 the effect on risk is larger for ischaemic stroke, and conversely, if the ROR <
1 the effect is larger for myocardial infarction. When the ROR = 1 there is no difference in
effect size. The corresponding 95% confidence interval was obtained from the variance of
the natural logarithm of the ROR, which was calculated as the sum of the variances of the
natural logarithm of the OR\textsubscript{MI} and OR\textsubscript{IS}. This method yields a conservative estimate of the
variance, because the shared control group is not taken into account.\textsuperscript{25}

To assess to what extent the burden of the two diseases can be attributed to a hypercoagulable
state, a population attributable fraction (PAF, also known as population attributable risk, or
PAR) was estimated for both myocardial infarction and ischaemic stroke. It is based both on
the magnitude of the effect as well as on the prevalence of the risk factor of interest. The PAF
was calculated by the formula

$$\text{population attributable fraction} = p_{\text{cases}} \left( \frac{OR - 1}{OR} \right)$$
where $P_{\text{cases}}$ represents the proportion of exposed cases (of all cases) and OR represents the odds ratio of the risk factor of interest for either myocardial infarction or ischaemic stroke.26-28

Figure 1. Flowchart of the RATIO study.

Flowchart of the study population. RATIO, Risk of Arterial Thrombosis in Relation to Oral Contraceptives.
Results

The baseline characteristics of the RATIO participants who provided citrate plasma samples are presented in Table 1. As expected, classic cardiovascular risk factors were more prevalent in the case groups than in the controls. The number of women who were active smokers at the time of the event was much higher among women with a myocardial infarction (82%) than in women suffering from ischaemic stroke (58%).

Table 1. Characteristics of the women who participated in RATIO.

<table>
<thead>
<tr>
<th></th>
<th>Myocardial infarction</th>
<th>Ischaemic stroke</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=205</td>
<td>N=175</td>
<td>N=638</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>43</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>195 (95%)</td>
<td>167 (97%)</td>
<td>602 (94%)</td>
</tr>
<tr>
<td>History of *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>53 (26%)</td>
<td>50 (29%)</td>
<td>40 (6%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (5%)</td>
<td>7 (4%)</td>
<td>10 (2%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>21 (10%)</td>
<td>14 (8%)</td>
<td>19 (3%)</td>
</tr>
<tr>
<td>Oral contraceptives use *</td>
<td>81 (40%)</td>
<td>92 (53%)</td>
<td>213 (33%)</td>
</tr>
<tr>
<td>Smoking *</td>
<td>169 (82%)</td>
<td>101 (58%)</td>
<td>270 (42%)</td>
</tr>
</tbody>
</table>

*All data are pertinent to the year of event (cases) or index date (controls)*
Table 2. Prothrombotic risk factors in the RATIO study, the effect on myocardial infarction and ischaemic stroke sorted to their ascending RORs.

<table>
<thead>
<tr>
<th>#</th>
<th>Prothrombotic factor</th>
<th>ref</th>
<th>OR MI</th>
<th>OR IS</th>
<th>ROR</th>
<th>95%CI</th>
<th>model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anticardiolipin antibodies, p95</td>
<td>14</td>
<td>1.80</td>
<td>0.90</td>
<td>0.50</td>
<td>0.17 - 1.45</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>hypofibrinolysis vs. normofibrinolysis*</td>
<td>20</td>
<td>2.82</td>
<td>1.50</td>
<td>0.53</td>
<td>0.22 - 1.27</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Prekallikrein:ag p90</td>
<td>30</td>
<td>1.54</td>
<td>0.90</td>
<td>0.58</td>
<td>0.23 - 1.52</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>F13A1 Pro564Leu, dominant</td>
<td>13,15</td>
<td>1.40</td>
<td>0.89</td>
<td>0.64</td>
<td>0.39 - 1.05</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>F13A1 Val34Leu, dominant</td>
<td>13,15</td>
<td>1.07</td>
<td>0.77</td>
<td>0.72</td>
<td>0.44 - 1.17</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Factor XII:ag, p90</td>
<td>30</td>
<td>1.18</td>
<td>1.03</td>
<td>0.87</td>
<td>0.34 - 2.23</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Factor XII:ag, p10</td>
<td>30</td>
<td>1.54</td>
<td>1.36</td>
<td>0.88</td>
<td>0.39 - 2.00</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>Prothrombin G20210A, dominant</td>
<td>16,17</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.22 - 4.54</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>High molecular weight kininogen:ag, p10</td>
<td>31</td>
<td>1.39</td>
<td>1.49</td>
<td>1.07</td>
<td>0.43 - 2.67</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>Oral contraceptive use vs. non use</td>
<td>10,12</td>
<td>2.00</td>
<td>2.30</td>
<td>1.15</td>
<td>0.69 - 1.91</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>MTHFR TT snp, recessive</td>
<td>16,24</td>
<td>1.30</td>
<td>1.50</td>
<td>1.15</td>
<td>0.57 - 2.33</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>VWF:ag q4 vs q1</td>
<td>22</td>
<td>4.20</td>
<td>6.70</td>
<td>1.60</td>
<td>0.60 - 4.26</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>Factor V Leiden, dominant</td>
<td>16,17</td>
<td>1.10</td>
<td>1.80</td>
<td>1.64</td>
<td>0.65 - 4.11</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Factor XI:ag, p90</td>
<td>30</td>
<td>1.61</td>
<td>2.65</td>
<td>1.65</td>
<td>0.79 - 3.44</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>High molecular weight kininogen:ag, p90</td>
<td>31</td>
<td>1.05</td>
<td>1.82</td>
<td>1.73</td>
<td>0.74 - 4.08</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>FGB -455 G/A, dominant</td>
<td>19</td>
<td>0.98</td>
<td>1.76</td>
<td>1.80</td>
<td>0.53 - 6.08</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>FGA 312Ala, dominant</td>
<td>19</td>
<td>1.22</td>
<td>2.33</td>
<td>1.90</td>
<td>0.79 - 4.61</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>F13B His95Arg, dominant</td>
<td>13,15</td>
<td>0.79</td>
<td>1.70</td>
<td>2.15</td>
<td>1.14 - 4.05</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>ADAMTS13:ag, q1 vs q4</td>
<td>22</td>
<td>1.40</td>
<td>3.10</td>
<td>2.21</td>
<td>0.93 - 5.27</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>prekallikrein:ag, p10</td>
<td>30</td>
<td>0.60</td>
<td>1.33</td>
<td>2.22</td>
<td>0.79 - 6.24</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>anti prothrombin antibodies, p95</td>
<td>14</td>
<td>0.80</td>
<td>1.80</td>
<td>2.25</td>
<td>0.63 - 8.03</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>anti-β2-glycoprotein antibodies, p95</td>
<td>14</td>
<td>1.20</td>
<td>2.80</td>
<td>2.33</td>
<td>0.92 - 5.93</td>
<td>2</td>
</tr>
<tr>
<td>#</td>
<td>Factor</td>
<td>Value</td>
<td>OR</td>
<td>95% CI</td>
<td>p90</td>
<td>Reference</td>
<td></td>
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<tr>
<td>----</td>
<td>---------------------------------------------</td>
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<td>------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Factor XIa AT-INH, p90</td>
<td>0.94</td>
<td>2.33</td>
<td>2.48</td>
<td>1.13</td>
<td>- 5.41</td>
<td>3</td>
</tr>
<tr>
<td>24</td>
<td>Hyperfibrinolysis vs. normofibrinolysis</td>
<td>1.60</td>
<td>4.07</td>
<td>2.54</td>
<td>1.03</td>
<td>- 6.27</td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>Factor XIIa C1-INH, p90</td>
<td>0.82</td>
<td>2.26</td>
<td>2.76</td>
<td>1.27</td>
<td>- 5.99</td>
<td>3</td>
</tr>
<tr>
<td>26</td>
<td>Kallikrein C1-INH, p90</td>
<td>1.50</td>
<td>4.34</td>
<td>2.89</td>
<td>1.42</td>
<td>- 5.89</td>
<td>3</td>
</tr>
<tr>
<td>27</td>
<td>Factor XIa C1-INH, p90</td>
<td>0.96</td>
<td>2.76</td>
<td>2.89</td>
<td>1.31</td>
<td>- 6.34</td>
<td>3</td>
</tr>
<tr>
<td>28</td>
<td>Lupus anticoagulant, ≥1.15</td>
<td>5.30</td>
<td>43.1</td>
<td>8.13</td>
<td>1.30</td>
<td>- 50.9</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>F13A1 Tyr204phe, dominant</td>
<td>0.82</td>
<td>9.10</td>
<td>11.1</td>
<td>4.52</td>
<td>- 27.2</td>
<td>1</td>
</tr>
</tbody>
</table>

# = number, ref = reference, OR_MI = odds ratio from myocardial infarction analyses, OR_IS = odds ratio from ischaemic stroke analyses, ROR = relative odds ratio (OR_IS/OR_MI), 95%CI = 95% confidence interval, :ag = antigen levels, C1-INH = C1-inhibitor levels, AT-INH = antitrypsin-inhibitor levels, dominant = analyses based on dominant inheritance pattern, p90 = 90th percentile. * categorization of fibrinolysis is based on a tertile analyses, where the lowest tertile of clot lysis time in the control group is regarded as hypofibrinolysis, middle tertile as normofibrinolysis and the highest tertile as hyperfibrinolysis. Model 1 indicates adjust for stratification variables age, area of residence and year of event. Model 2 additionally includes hypertension, diabetes and hypercholesterolaemia. Model 3 includes variables from model 2 with the addition of smoking. Model 4 includes the same variables from model 3 with the addition of BMI. Exposures that were originally analyzed with model 2 (exposures number 1, 10, 21, 22 and 28) were additionally evaluated according to model 3, showing similar results.
A direct comparison of the effect estimates between myocardial infarction and ischaemic stroke was possible for a total of 29 prothrombotic factors. All factors, the corresponding OR\textsubscript{MI}, OR\textsubscript{IS} and ROR are listed in Table 2. The majority of risk factors had a stronger association with the ischaemic stroke risk than the myocardial infarction risk: twenty-two of these risk factors (22/29, 72%) had a relative odds ratios >1, 12 (41%) >2, and 5 (17%) > 2.75. Additional adjustments did not materially affect these results (supplementary Table 1).

**Figure 2. Population attributable fractions and corresponding confidence intervals.**

Population attributable fractions from the myocardial infarction analyses are represented by blue squares where the ischaemic stroke results are denoted by red dots. The corresponding lines represent the corresponding 95% confidence intervals. Exposure # refers to the exposure number as denoted in Table 2 in which the exposures are ranked according to PAF\textsubscript{IS} in ascending order.
The PAFs of the 29 prothrombotic factors showed a similar picture: the PAFs for ischaemic stroke were generally higher than for myocardial infarction (Figure 2). Only 4 prothrombotic factors (4/29, 14%) yielded a PAF >0.1 in the myocardial infarction analyses, indicating that 10% of the myocardial infarction cases in women in this age group could be attributed to each one of these 4 prothrombotic factors. The impact of prothrombotic factors on ischaemic stroke incidence was much higher: fourteen (14/29, 48%) prothrombotic factors yielded a PAF>0.1 in the ischaemic stroke analyses.
Figure 3. Prothrombotic risk factors in the RATIO study and their effect on myocardial infarction and ischaemic stroke.

Each point depicts the log odds ratio as a measure of effect (left panel) or the population attributable fraction (right panel) of a particular risk factor on the risk of myocardial infarction (x-axis) as well as the effect on the risk of ischaemic stroke (y-axis). The red dashed lines indicate the null effect for either myocardial infarction (vertical line) or ischaemic stroke (horizontal line). The blue diagonal line represents the theoretical line along which all points would cluster when the role of thrombotic factors is similar in the aetiology of myocardial infarction and ischaemic stroke. MI = myocardial infarction, IS = ischaemic stroke, Log OR = natural logarithm of the odds ratio, PAF = population attributable fraction.

Figure 3 depicts the OR (left) and the PAF (right) of each risk factor for both the myocardial infarction (plotted on the x-axis) and ischaemic stroke analyses (y-axis). In the left panel, the distance from any point perpendicular to the blue diagonal line is reflective of the ROR. The overall picture that arises from Figure 3 is that the relative risk associated with several measures of hypercoagulability is different for myocardial infarction and ischaemic stroke.
Discussion

Our results suggest that in young women, the increase in ischaemic stroke risk conveyed by prothrombotic factors is overall higher than that in myocardial infarction. When considering the population attributable fractions, the impact of hypercoagulability on the incidence of myocardial infarction is minimal, whereas up to 20-30% of the ischaemic stroke incidence may be attributed to the studied prothrombotic factors.

The largest difference in effect was observed for a genetic variant of coagulation factor XIII (ROR 11.1), a protein which crosslinks fibrin monomers and thereby affects the clot structure. High levels of activated factor XII (ROR 2.8), kallikrein (ROR 2.9) and factor XI (ROR 2.9) point towards a specific role of the intrinsic coagulation system in ischaemic stroke. FXI can be activated by FXII, but also independent of FXII by thrombin in a positive feedback mechanism. Lupus anticoagulant (ROR 8.1) is a marker for the antiphospholipid syndrome. Some have proposed a link between the antiphospholipid syndrome and the intrinsic coagulation system, whereby anti-β2-glycoprotein antibodies might play a role in disrupting the activation of FXII and FXI. Therefore the intrinsic coagulation system might be the driving force in the observed difference between myocardial infarction and ischaemic stroke. This is interesting because the proteins from this system are not only directly involved in thrombus propagation, but also linked to processes such as fibrinolysis, inflammation and neutrophilic-extracellular-trap mediated coagulation.

Ischaemic stroke is a heterogeneous disease, in which several different causal mechanisms can be discerned, as is done in the TOAST classification. There are five TOAST categories, each with their own causes and consequences: cardioembolism, large-artery atherosclerosis, small-vessel occlusion, stroke of other determined aetiology and stroke of undetermined aetiology. Interestingly, ‘stroke of undetermined origin’ comprises about one third of all strokes, a proportion that might be higher in the young. Although women with ischaemic stroke with an overt cardiac-embolic-stroke were excluded from the RATIO study, all other subtypes are combined as data needed for classification are not available. Therefore, new studies are needed to further elucidate the role of hypercoagulability in subtypes of ischaemic stroke. An important factor to consider is the concept of ‘paradoxical embolism’ where an ischaemic stroke is caused through the embolization of thrombus which passes a patent
foramen ovale. If all our cases where of this origin, our main result is not noteworthy since we would in fact be comparing the presence of markers of hypercoagulability in deep venous thrombosis patients to their presence in myocardial infarction patients. However, data from other studies suggest that a patent foramen ovale is present in about 40-50% of patients with cryptogenic stroke, making this a paradoxical embolization only a possible option in about 15-20% of these patients.\textsuperscript{46} This number, together with other explanations doubting the clinical relevance of the detection of patent foramen ovale in young stroke victims, indicate that paradoxical embolism is not likely to fully explain our results.\textsuperscript{47}

Several aspects are of importance in the consideration of our results: first, the original goal of the study implicated that the participants of the RATIO were women between the ages of 18 and 50. This dictated a case-control design with the added benefit that the young age of our cases and controls harbors a reduced atherosclerotic burden which could mask the effect of hypercoagulability. The role of other age-related cardiovascular risk factors will also be minimized, reducing the problem of confounding. The incidence of myocardial infarction and ischaemic stroke in women in the Netherlands in this age category is low and comparable (i.e. \(~12-14\) per 100 000 person years) which makes a direct comparison of relative odds ratios possible without scaling effects.\textsuperscript{48,49} Second, the design of the RATIO study also dictates the use of a single control group for both OR\textsubscript{MI} and OR\textsubscript{IS}, leading to an overestimation of the standard error or the ROR and thereby yielding conservative estimates of the precision of our analyses. Third, this direct comparison within the same study increases the comparability because there is no difference in blood sampling, handling and measurement, case ascertainment procedures, administered questionnaires etcetera. With these similarities, bias might have a similar impact on both the myocardial infarction and ischaemic stroke analyses.

Fourth, it is possible that some of our prothrombotic factors might display a change in risk for either myocardial infarction or ischaemic stroke merely by chance. Also, these has to be emphasized that the coagulation factors used in this analyses as a proxy for a hypercoagulable state might be non-dependent. However, it is unlikely that chance or inter variable dependency will fully explain the overall picture.
Hypercoagulability and arterial thrombosis

This study also has several limitations. First, our results are applicable to young women, and it is unclear to what extent these results can be generalized to different patient populations. An important aspect to consider during replication of our results is the differences in the distribution of stroke subtypes amongst different age groups.\(^5\) Second, case control studies inherently harbor the possibility of the reverse causation, which occurs when an effect of the disease is mistaken for its cause. Although the risk of reverse causation was reduced by the blood draw after the acute phase of the diseases, long-term effects of the disease or related treatments can be responsible for part of our results. Third, residual confounding might still distort our results. For example, some possible sources of confounding were not taken into account in the original analyses. Adjustment for a more inclusive selection of potential sources of confounding (i.e. model 3) did change the point estimates marginally, but never the direction or order of magnitude of the relative odds ratios. Also, the data used to reduce confounding are mostly self-reported, which harbors the possibility of residual confounding. If this residual confounding differs in strength for the two diseases, our direct comparison is biased. However, we do not believe that that this bias is the sole explanation of our main finding, i.e. that the association between markers of hypercoagulability and myocardial infarction / ischaemic stroke risk is differential.

Our findings could be of importance to the treatment and secondary prevention of both diseases. Current treatments that target the haemostatic system reduce the coagulation capacity and therefore can induce major bleeding episodes. Currently, upstream coagulation factors such as FXI and FXII are promising targets for treatments that theoretically could lower thrombosis risk without increase bleeding risk.\(^{39,51}\) Our results suggest that such new therapies might be most effective in the treatment and prevention of ischaemic stroke. However, despite our results, the role of the intrinsic coagulation system in cardiovascular disease is still far from clear and needs to be studied in future, perhaps in a prospective study.\(^9,37,52\)

Myocardial infarction and ischaemic stroke are both acute forms of arterial thrombosis and are as such unequivocally linked to coagulation and thrombus formation. However, the role of hypercoagulability in this causal mechanism seems to mainly affect the risk of ischaemic stroke. We demonstrated this in a single study on 29 prothrombotic factors, which reduces the external validity of our results, but strengthens the internal validity because the possibility
of bias is minimized. Future studies must be undertaken to determine whether the role of hypercoagulability in the subtypes of ischaemic stroke is also differential.
Supplementary Table 1. Non-genetic exposures which initially were analysed according to model 2, were additionally analysed according to model 3. This table provides a direct comparison of these results.

<table>
<thead>
<tr>
<th>#</th>
<th>Prothrombotic factor</th>
<th>ref</th>
<th>OR MI</th>
<th>OR IS</th>
<th>ROR</th>
<th>95%CI</th>
<th>model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anticardiolipin antibodies, p95</td>
<td>[14]</td>
<td>1.80</td>
<td>0.90</td>
<td>0.50</td>
<td>0.17 - 1.45</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>Anticardiolipin antibodies, p95</td>
<td>[14]</td>
<td>1.27</td>
<td>1.00</td>
<td>0.79</td>
<td>0.17 - 3.65</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>Oral contraceptive use vs. non use</td>
<td>[10,12]</td>
<td>2.00</td>
<td>2.30</td>
<td>1.15</td>
<td>0.69 - 1.91</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Oral contraceptive use vs. non use</td>
<td>[10,12]</td>
<td>1.88</td>
<td>2.53</td>
<td>1.35</td>
<td>0.57 - 3.15</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>anti prothrombin antibodies, p95</td>
<td>[14]</td>
<td>0.80</td>
<td>1.80</td>
<td>2.25</td>
<td>0.63 - 8.03</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>anti prothrombin antibodies, p95</td>
<td>[14]</td>
<td>0.88</td>
<td>1.73</td>
<td>1.97</td>
<td>0.33 - 11.9</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>anti-β2-glycoprotein antibodies, p95</td>
<td>[14]</td>
<td>1.20</td>
<td>2.80</td>
<td>2.33</td>
<td>0.92 - 5.93</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>anti-β2-glycoprotein antibodies, p95</td>
<td>[14]</td>
<td>1.30</td>
<td>2.90</td>
<td>2.22</td>
<td>0.46 - 10.7</td>
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<tr>
<td>28</td>
<td>Lupus anticoagulant, ≥1.15</td>
<td>[14]</td>
<td>5.30</td>
<td>43.1</td>
<td>8.13</td>
<td>1.30 - 50.9</td>
<td>1</td>
</tr>
<tr>
<td>28</td>
<td>Lupus anticoagulant, ≥1.15</td>
<td>[14]</td>
<td>4.28</td>
<td>56.7</td>
<td>13.3</td>
<td>0.78 - 224</td>
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</tbody>
</table>

# = number, ref = reference, OR MI = odds ratio from myocardial infarction analyses, OR IS = odds ratio from ischaemic stroke analyses, ROR = relative odds ratio (OR IS / OR MI), 95%CI = 95% confidence interval, p95 = 95th percentile. Model 1 indicates adjust for stratification variables age, area of residence and year of event. Model 2 additionally includes hypertension, diabetes and hypercholesterolemia. Model 3 includes variables from model 2 with the addition of smoking.


use in a multicenter clinical trial. TOAST.


