New insights in the risk profile for arterial thrombosis : differences and similarities in risk factors between myocardial infarction and ischaemic stroke

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To Luisa who taught me the joy,
and to Pietro and Anna who fulfilled it.

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Chapter 1

General Introduction

Abstract

This thesis reports on risk factors involved in the aetiology and prognosis of the two main forms of arterial thrombosis, namely myocardial infarction and ischaemic stroke. The majority of risk factors under study are related to coagulation, and particularly to its pro-coagulant activity. There is also an underlying question in most of the chapters, which is whether the risk factors being investigated act differentially in myocardial infarction and ischaemic stroke. Finally, there is a discussion about the implications and interpretation of the findings from an aetiological point of view, and for the development of future research. As an introduction to this thesis, this chapter provides background information on myocardial infarction and ischaemic stroke. Moreover, it describes the study populations used to investigate the hypotheses along with an overview of the subsequent chapters.

Introduction

Myocardial infarction

Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide.[1] Myocardial infarction occurs when myocardial ischaemia, a diminished blood supply to the heart, exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis. Ischaemia at this critical level for an extended period results in irreversible myocardial cell damage and death. The myocardial ischaemia can be the result of an increased myocardial metabolic request, a decreased delivery of oxygen and nutrients to the myocardial tissue, or both. An interruption in blood flow occurs for example when a thrombus is superimposed over an ulcerated or unstable atherosclerotic plaque, resulting in coronary occlusion. A coronary artery stenosis caused by atherosclerosis or a dynamic stenosis associated with the vasospasm of the coronary artery can also limit the supply of oxygen and nutrients and precipitate a myocardial infarction.

Since 1998, the age-standardised incidence of myocardial infarction in the Netherlands decreased from 6.2 to 3.8 per 1000 person-year in 2007 in men and from 3.2 to 2.1 per 1000 person-year in 2007 in women.[2] In absolute terms, the decline in myocardial infarction incidence was smaller in the young (35-54 years: −3.8%) than in middle-aged individuals (55-84 years: −5.3%). Similarly, the annual percentage change in incidence was larger in men than women.[2] Incidences strongly differ per age category, and age is their strongest determinant. Myocardial infarction recurrence rates vary widely among studies and age populations, ranging at one year from 5.6% to 57% in autopsy-based population studies.[3,4] Out-of-hospital mortality decreased from 24.3% in 1998 to 20.6% in 2007 in men and from 33.0% to 28.9% in women. Hospitalised case-fatality declined from 2003 onwards. Similar pictures were found in other European countries and in the U.S.A.[5-7] In the latter, the annual incidences are slightly higher, with approximately 660 000 Americans having a new coronary attack each year.[8] Coronary heart disease alone caused approximately 1 out of 7 deaths in the U.S.A. in 2013. Even though incidences in developed countries are decreasing, a substantial

burden of morbidity persists: the overall estimated prevalence of coronary heart disease in the Netherlands is 3 % in women and 5 % in men.[9]

Seven primary risk factors for myocardial infarction have been identified so far, mostly associated with the development of atherosclerotic coronary artery disease: tobacco use, physical inactivity, obesity, family history of arterial disease, hyperlipidaemia, diabetes mellitus and hypertension.[1]

Ischaemic stroke

A stroke is defined as an acute loss of neurological function due to an abnormal perfusion of brain tissue. The majority of strokes are ischaemic (87%), and commonly result from an arterial obstruction by a thrombus or embolus, with the occurrence of cerebral infarction. The other strokes are haemorrhagic, either into the parenchyma or into the subarachnoid space. A transient ischaemic attack (TIA) is a brief episode of neurological dysfunction, with clinical symptoms typically lasting less than one hour, without evidence of acute infarction.[10] Ischaemic stroke has several aetiologies and classifications, of which the most adopted is the TOAST.[11,12] The TOAST classification has five categories: large-artery atherosclerosis, embolism, small-vessel disease, stroke of other determined aetiology, and stroke of undetermined aetiology. This classification reflects the mechanisms underlying the reduction in blood flow causing ischaemia. (i) Large-artery atherosclerosis accounts for a stenosis or occlusion of the major intra- and extra-cranial arteries due to the deposition of an atherosclerotic plaque, its rupture or a prolonged low-flow state due to relative hypotension. (ii) Embolism can be the consequence of thrombi resulting from turbulent or stagnant flow states in the heart. These thrombi can dislodge and occlude blood vessels in the intracranial circulation. The most common cause of cardioembolic stroke is atrial fibrillation, but emboli can also originate from cardiac valves or large arterial vessels. (iii) Changes in the arterial vasculature of small perforating arteries that result in the narrowing of the vessel lumen and the eventual occlusion may lead to lacunar infarcts. Chronic hypertension, as well as hyperlipidaemia, smoking, and diabetes are all causes of this kind of vessel damage. (iv) Vasculopathies, genetic disorders, and metabolic disorders are rare causes of other determined aetiologies. (v) Finally, in a significant number of cases (30-40%), no clear

explanation can be found for an ischaemic stroke event, despite an extensive diagnostic evaluation. These strokes are classified as strokes of undetermined aetiology, or cryptogenic strokes. The average age-adjusted incidence of ischaemic stroke in the Netherlands is 1.9 per 1000 person-years and as for myocardial infarction, the incidence rises with age.[13] Overall, the incidence of ischaemic stroke is slightly decreasing. From 2003 to 2013, the stroke death rates declined among the elderly from 5.3 to 2.4 per 1000 person-years and among the young from 0.05 to 0.02 per 1000 person-years.[1] Stroke recurrence is reported to be as high as 13% at one year.[14] Only about 10% of all strokes occur in people aged 18 to 50 years-old. However, in this age category the burden of the disease is the highest considering the long life expectancy.

Major risk factors for ischaemic stroke are the same as for myocardial infarction, as well as atrial fibrillation and carotid stenosis.

The RATIO case-control study

The RATIO (Risk of Arterial Thrombosis In relation to Oral contraceptives) is a multicentre, population-based, case-control study performed in the Netherlands between 1995 and 1998, and it serves as the basis for the questions posed in Chapters 3 and 4.[15,16] The coordinating centre is the department of Clinical Epidemiology at the Leiden University Medical Center. The RATIO study included young women (between 18 and 49 years old) with either myocardial infarction or ischaemic stroke. Exclusion criteria were TIA (defined as an event lasting <24 hours), haemorrhagic stroke, venous sinus thrombosis, carotid artery dissection, history of cardiovascular or cerebrovascular diseases, severe illness, aphasia, or cognitive impairment interfering with the questionnaire, and not speaking Dutch. The population-based group of control women was identified by random-digit dialing.[17] In this method, telephone numbers are randomly produced and dialled at any time during the day at least 7 times or until a successful connection is made. Eligible as controls were women who were aged 18 to 49 years and who did not meet the exclusion criteria that were used for selecting patients. The control group has been frequency matched with cases for age, area of residence, and calendar year. Finally, in recruitment of control subjects, women in the older age groups were over-sampled to minimise the age difference between the patients and control women.[16]

Therefore, adjustment for matching variables in the statistical models is always indicated. A structured and standardised questionnaire was used to collect information about classic cardiovascular risk factors, family history, oral contraceptive use as well as obstetrical history. The questions referred to the period before the index date (the date of myocardial infarction or ischaemic stroke for patients and mid-year of the same year for controls).

In total, 248 participants suffered from myocardial infarction, 203 from ischaemic stroke and 925 women served as healthy controls.[15,16] All qualifying events occurred between 1990 and 1997 and cases were included from 1995 to 1998. 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). Buccal swabs were collected if blood could not be sampled (13 participants with myocardial infarction, 15 with ischaemic stroke and 129 controls). To compensate for the loss of statistical power in the ischaemic stroke group, a further sample of 50 women who presented with an ischaemic stroke at the University Medical Center Utrecht were additionally recruited between 1996 and 2001. In total, blood samples were available from 205 cases with myocardial infarction, 175 cases with ischaemic stroke and 638 control subjects. Blood was collected after a median of 69 months (range 38 - 117) for myocardial infarction and 95 months for ischaemic stroke cases (range 23-146), thereby ensuring blood was sampled after the acute phase to minimise the risk of reverse causation. All participants gave informed consent and the study was approved by the ethics committees of the participating hospitals.[16]

Because the RATIO study focused on patients with a young age of onset, it has several advantages for the purpose of this thesis. Firstly, it is highly suitable to study non age-related risk factors, such as coagulation markers. Since age-related risk factors are prevalent in ageing patient groups, they may mask the effects of non-age-related risk factors. Secondly, because myocardial infarction and ischaemic stroke are so prevalent in ageing populations, research into the causes and consequences of these diseases often target these age categories.[18-20] The RATIO study helps to fill this gap in knowledge. Thirdly, studies into cardiovascular diseases are also disproportionally targeted at certain types of disease. The proportion of clinical research aimed at identifying new causes of myocardial infarction is larger than the studies that target ischaemic stroke, even when the difference in the incidence of these diseases is taken into account.[21] The RATIO study offers the unique opportunity to

study both forms of arterial thrombosis, myocardial infarction and ischaemic stroke, and to compare them within the same population. This makes the comparison easier and more reliable than when it is done between different studies with different designs and populations. Finally, falls in cardiovascular incidence in recent years have been reported to be greater in men and in the elderly than in women and the young. Therefore, the focus of the RATIO on women and on young patients made it particularly interesting in order to try to address those disparities.

The RATIO follow-up study

The RATIO follow-up study is a cohort study, described in detail in Chapter 7. It aims to provide answers to the need of further knowledge of secondary prevention strategies in young patients with myocardial infarction and ischaemic stroke.[22,23] Using data linkage between the original RATIO case-control study and existing national databases with follow-up data, provided by the Central Bureau of Statistics (CBS) in the Netherlands, the RATIO follow-up study was built. Not only does this study make it possible to determine the mortality and recurrence rate of myocardial infarction and ischaemic stroke, but also to investigate the coagulation risk factors related to prognosis and to compare these aspects between myocardial infarction and ischaemic stroke. The results from the RATIO follow-up project provide a direct insight of the consequences of cardiovascular diseases in young women.

Of the original study group, 226 women with myocardial infarction, 160 with ischaemic stroke, and 782 controls were linked to the national databases and included in the follow-up. With a previously reported recurrence rate and mortality in young patients with stroke of 0.5-3.6 per 100 person-year and 0.6-1.4 per 100 person-year respectively, and a follow up of 15 years, we expected a priori to find 15 to 84 recurrences of stroke and 17 to 38 deaths in the ischaemic stroke group.[18,19,24] In patients with young onset myocardial infarction, reported rates of recurrence and mortality are 1.8-2.2 and 1.2-2.0 per 100 person-year, leading to 59-70 expected recurrences and 40-64 expected deaths in the myocardial infarction group.[25]

The LILAC study

The LiLAC (Life Long After Cerebral Ischaemia) is a cohort study that serves as the basis for the investigation presented in Chapter 6, in order to answer the question about the role of concomitant headache in predicting vascular recurrences and death after a TIA or a minor stroke.

The LiLAC is based on the Dutch TIA Trial (DTT), a double blind, randomised study for the comparison of two doses of aspirin (30mg or 283 mg) in preventing vascular events in patients who had had a TIA or a minor stroke between 1986 and 1989. For logistical reasons, in the LiLAC study only patients from the 24 hospitals that had enrolled at least 50 patients in the DTT (2473 of the original 3150) were included. In order to be enrolled in the DTT patients must have had a TIA (symptoms lasting for less than 24 h) or an ischaemic stroke (symptoms persisting for more than 24 h), but non-disabling (modified Rankin grade < 3), within the past three months.[26] Patients with potential sources of embolism in the heart or conditions other than atherosclerosis that might have caused the cerebral ischaemia were excluded. In the Dutch TIA trial details of the history and the presence of vascular risk factors of each patient were recorded at baseline by a standardised questionnaire. The list also contained a number of detailed multiple-choice questions about the nature and time course of the symptoms. One question pertained to the presence and nature of headache. Follow-up in the DTT has been carried out until 1990. In the LiLAC study, follow-up was extended to the period between March, 2001, and December, 2003. Follow-up data were obtained from the clinicians involved in the DTT, the general practitioners and directly from patients, relatives or acquaintances. Twenty-six patients were completely lost to follow-up after close-out of the DTT. Seven patients were lost because they moved abroad and 19 for unknown reasons.

The LiLAC has several strengths that are useful for the purpose of this thesis. Firstly, it included a large sample of patients with a cerebrovascular accident, ensuring that patients with headache were a numerically sufficient group to allow enough statistical power. Secondly, the LiLAC is one of the longest follow-up studies available in the literature for patients with ischaemic stroke, thereby ensuring that outcomes of interest (vascular recurrences and death) were well represented. With a prevalence of headache at baseline of

17% among participants and an alpha error of 0.05, LiLAC has a power of 80% in detecting a relative risk of 0.86 or more extreme.

Outline of this thesis

In **Chapter 2** we present an individual patient data (IPD) meta-analysis and investigate the relationship between ADAMTS13, the von Willebrand Factor cleaving protease, and myocardial infarction. The IPD design was preferred over a meta-analysis of aggregate data, since we realised that only a few studies were available in the literature and we had the possibility to collect a large population sample, a goal hardly achievable with a single centre study.

A standard case-control study analysis, based on the RATIO study, is presented in **Chapter 3.** It investigates the relationship between pregnancy loss and both forms of arterial thrombosis. In this chapter we built a comprehensive statistical model in order to deal with all possible confounders and mediators that can affect the relationship of interest. **Chapters 4 and 5** share similarities. We used an unconventional approach in both chapters in order to compare the role of hypercoagulability in the aetiology of myocardial infarction and ischaemic stroke. In Chapter 4 we did this comparison within the frame of the RATIO case-control study, and we calculated relative odds ratios (ROR) for all the prothrombotic markers studied in the RATIO study to assess the difference in effects between the two diseases. We used a similar approach in Chapter 5, where the same question is investigated within the studies published in the literature, with the calculation of relative risk ratios (RRR). The direct comparison was maintained by selecting only those studies that have investigated the same marker in both diseases within the same study population. **Chapter 6** addresses the relationship between concomitant headache and vascular recurrences after TIA or minor strokes. It is based on the LiLAC cohort study. In **Chapter 7** the long term follow-up of the RATIO study is presented. Participants were followed until 2012. Results focus on mortality and on the relationship between hypercoagulability and fatal and non-fatal recurrences after myocardial infarction and ischaemic stroke. Finally, in **Chapter 8** we report a summary of the major findings and a general discussion on causality and prediction. Moreover, the comparison between myocardial infarction and ischaemic stroke and possible links with venous thrombosis are discussed in more detail.

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Chapter 2

Plasma ADAMTS13 levels and the risk of myocardial infarction: an individual patient data meta-analysis

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Abstract

Background: Low levels of ADAMTS13 have been repeatedly associated with an increased risk of ischaemic stroke but results for risk of myocardial infarction are inconclusive.

Objective: To perform an individual patient data meta-analysis from observational studies investigating the association between ADAMTS13 levels and myocardial infarction.

Methods: A one step meta-analytic approach with random treatment effects was used to estimate pooled odds ratios (OR) and corresponding 95% confidence intervals (CI) adjusted for confounding. Analyses were based on dichotomous exposures, with the 5th and 1st percentile of ADAMTS13 antigen levels as cut off values. Quartile analyses, with the highest quartile as a reference category, were used to assess a graded association between levels and risk ('dose' relationship). Additionally, we assessed the risk of the combined presence of low ADAMTS13 and high von Willebrand Factor (VWF) levels.

Results: Five studies were included, yielding individual data on 1501 cases and 2258 controls (mean age 49 years). Low levels of ADAMTS13 were associated with myocardial infarction risk, with an OR of 1.89 (95% CI 1.15-3.12) for values below the 5th percentile versus above, and an OR of 4.21 (95% CI 1.73-10.21) for values below the 1st percentile versus above. Risk appeared restricted to these extreme levels, as there was no graded association between levels of ADAMTS13 and myocardial infarction risk over quartiles. Finally, there was only a minor synergistic effect for the combination of low ADAMTS13 and high VWF levels.

Conclusion: Low levels of ADAMTS13 are associated with an increased risk of myocardial infarction.

Introduction

The thirteenth member of a disintegrin-like and metalloprotease with thrombospondin type 1 motif family (ADAMTS13) is a circulating plasma enzyme responsible for cleavage of the platelet-adhesive ultra-large forms of von Willebrand factor (VWF).[1] The cleavage of ultra-large VWF into smaller molecules by ADAMTS13 is an important regulatory mechanism in haemostasis since these smaller VWF molecules have a reduced platelet tethering capacity.[1,2] Severe deficiency of ADAMTS13 promotes VWF- induced platelet aggregation, and can result in thrombotic thrombocytopenic purpura (TTP).[2] Historically, after ADAMTS13 has been identified as the VWF-cleaving enzyme in 2001, research on this metalloprotease first focussed on the pathophysiology of TTP and the interactions of the enzyme with VWF.[3] In recent years, the focus shifted to the role of ADAMTS13 in the more common forms of thrombotic disease. This started with studies of VWF, the cleavage substrate of ADAMTS13, that have indicated an increase in risk of cardiovascular disease for high levels of VWF.[4] A similar association has been established for genetic factors influencing circulating VWF levels (most notably, ABO blood group).[5-8] These data suggested that low levels of ADAMTS13, which diminishes the VWF cleavage capacity and therefore increases the VWF activity, might also increase the risk of arterial thrombosis. Moreover, murine studies have shown that deletion of the murine ortholog of ADAMTS13 results in an increased predisposition to atherosclerosis and arterial thrombosis.[9-11]

With this background, a number of studies investigated the role of ADAMTS13 plasma levels in relation to the risk of arterial thrombosis, but with conflicting results for the two main forms of this disease.[12,13] Several studies reported that low levels of ADAMTS13 increased the risk of ischaemic stroke, but one study on myocardial infarction was negative.[14] A recent meta-analysis based on published results confirmed this association for ischaemic stroke (relative risk of 2.72, 95% confidence intervals 1.52 - 4.85, for low vs high levels of ADAMTS13), but failed to give a definitive answer for myocardial infarction. The pooled estimate for myocardial infarction was accompanied by wide confidence interval due to a lack of power (relative risk of 1.45, 95% CI 0.71-2.98).[15] The lack of precision of this meta-analytic approach, as well as the inability to discriminate specific patient subgroups, and uniform confounder adjustment, hampers the ability to determine the relevance of low ADAMTS13 levels in myocardial infarction.

A more powerful and less biased approach collects individual patient data (IPD) directly from the researchers responsible for each study. Use of IPD has several advantages over the aggregate data approach, including standardization of statistical analyses, assessment of potential causes of heterogeneity, adjustment for confounding on individual information and the investigation of interactions and non-linear effects.[16]

Methods

Search strategy and selection of studies

This systematic review and IPD meta-analysis of studies investigating ADAMTS13 levels in myocardial infarction were conducted according to the principles of the PRISMA statement.[17] We searched for all publications reporting the association between ADAMTS13 and myocardial infarction up to February 2014. Publications were identified with a systematic search in PubMed (1950-2014). The search strategy was composed by the following Boolean combination of search terms: ADAMTS13 "AND" myocardial infarction "OR" heart disease "OR" coronary disease. Publications were selected independently by two authors (AM, LAL) from the resulting listing.

Publications were included when: (1) they reported original data about the association between ADAMTS13 levels in humans (i.e., plasma antigen or activity) and incident myocardial infarction; (2) the outcome was myocardial infarction as an acute vascular event rather than surrogate (e.g., studies reporting only coronary artery plaque were excluded). Studies that combined several forms of arterial thrombosis (e.g. myocardial infarction and transient ischaemic attack, ischaemic stroke, peripheral artery disease) were included as long as, at the patient level data, it was possible to select myocardial infarction cases from the combined endpoint. No restriction was applied on study design, except they were controlled.

The reference list of the included studies was checked for relevant publications that were not identified by the literature search. For practical reasons, and in order to minimize the impact of publication bias, studies were eligible only when the sample size was reasonably large (predefined cut off of more than 50 cases).

Study design

Corresponding authors of the selected publications were contacted and asked to provide information on an individual level regarding clinical and demographic characteristics of cases and controls. Requested variables were: age, sex, height, weight, and known clinical cardiovascular risk factors. These included history of hypertension, diabetes, hypercholesterolemia (defined as total cholesterol greater than 200 mg/dl) and smoking

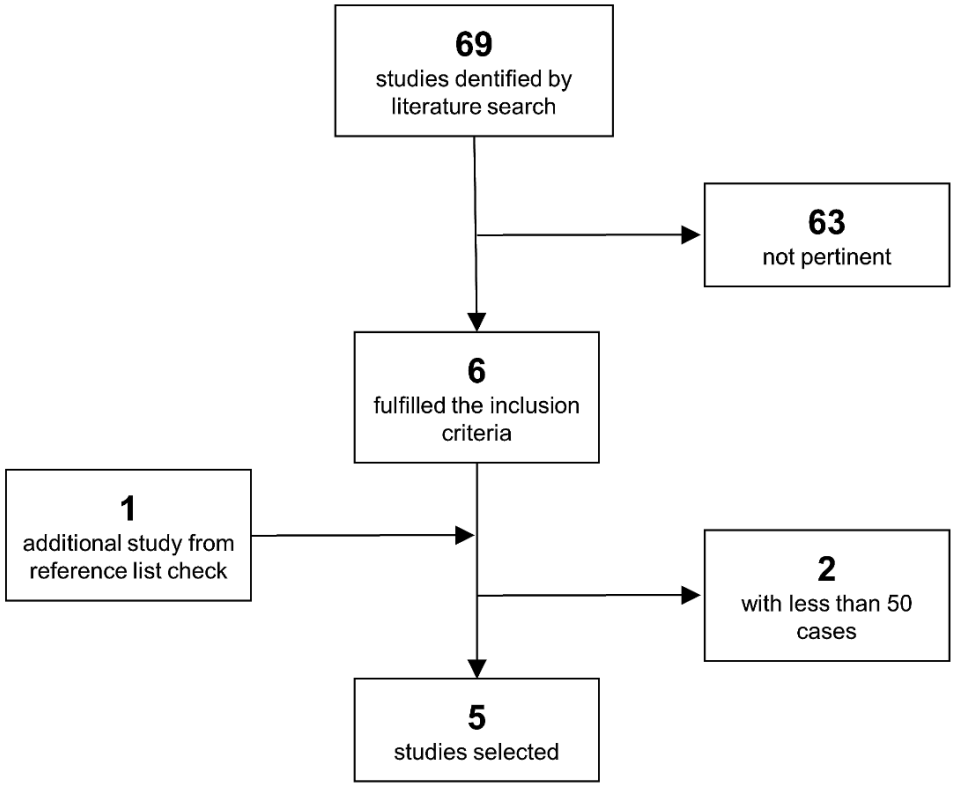
habits. Moreover, the authors were asked to provide the following laboratory variables: time from the event to the blood collection, ADAMTS13 level (plasma antigen levels) and VWF level (plasma antigen levels).

Statistical analysis

ADAMTS13 and VWF levels from each study were standardized by dividing each single value by the mean value of the corresponding control group and expressed as percentage. The overall association between ADAMTS13 and myocardial infarction was determined using a one-step meta-analytic approach on individual patient data. This was applied by mixed logistic regression model with random treatment effects to obtain odds ratios (OR) and corresponding 95% confidence intervals (95% CI) as measures of relative risk.[18] All models included the variables age and sex. Additional adjustment for potential confounders (e.g., hypercholesterolemia, hypertension, diabetes mellitus, body mass index and smoking) was done in separate models. The main analyses were based on a dichotomous exposures, with a-priori cut-off values set at the 5th and 1st percentile of the ADAMTS13 distribution of the pooled control group. Dummy variables with predefined cut off points were created to assess the combined effect of high levels of VWF (above the 90th percentile, already showed to be associated with myocardial infarction)[12] and low levels of ADAMTS13 (below the 5th percentile). Quartile analyses, with the highest quartile as reference category, were used to determine the association over the full range of ADAMTS13 values. The presence of a non-linear effect of the ADAMTS13 levels distribution on the risk of myocardial infarction (expressed as log odds) was also evaluated using a restricted cubic spline function with 3 knots, which was the one that maximized the Akaike's information criterion [(model likelihood ratio $\chi 2$ -2p), with p equal to the number of parameters in the model aside from the intercept (i.e., the number of knots - 1)].[19] Predefined subgroups analyses were based on sex (male vs female) and age (below vs above 45 years old at the time of the event). Because of the reduction of the number of studies available for each subgroup, a fixed effect model was used, which included the variables age, sex, hypercholesterolemia, hypertension, diabetes mellitus, body mass index, smoking, study indicator (i.e., the original study population to which each patient belongs) and interaction terms between exposure and study indicator. Finally, in order to minimize the effect of an acute phase reaction, we restricted the

main analysis to those studies in which the blood draw was performed at least one month from the event. Statistical analyses were performed using STATA version 13.0, SPSS version 20.0 and software R version 3.0.2.

Fig. 1. Flow chart of the step of studies selection.



The figure shows the three steps in studies selection: identification of studies which reported the association between ADAMTS13 and incident myocardial infarction as acute vascular event and with an adequate sample size of more than 50 myocardial infarction cases.

Table 1. Main characteristics of the studies included in the IPD meta-analysis.

Study	Reference	Original study size (cases / controls)	Age	Sex	Recruitment period	Blood draw (months)	Nation	Case-control matching variables
SMILE	Chion et al. 2007 [14]	560 / 646	>18	male	1994-1997	>3	The Netherlands	age
GLAMIS	Crawley et al. 2008 [20]	466 / 484	>18	both	1994-1995	>3	UK	sex and age
ATTAC	Bongers et al. 2009 [21]	169 / 332	<55 men <40 women	both	before 2005	>1	The Netherlands	age
Milan	Peyvandi et al. 2010 [13]	138 / 199	18-45	female	1998-2001	<1	Italy	age and geographic origin
RATIO	Andersson et al. 2012 [12]	202 / 626	<50	female	1990-1995	>38	The Netherlands	age, area of residence and index year

Results

A total of 69 publications were identified by the search strategy. Of these, six studies fulfilled the inclusion criteria and one additional study was included after checking the reference lists of the included publications (Fig. 1). The studies that were identified were conducted with a case-control design and had various conclusions regarding the association of low levels of ADAMTS13 with myocardial infarction, encompassing a protective effect (Chion et al. [14]), no effect (Horii et al. [22] and Peyvandi et al. [13]) and a risk-increasing effect (Kaikita et al.[23], Matsukawa et al. [24], Crawley et al. [20], Bongers et al. [21] and Andersson et al. [12]). Ultimately, five studies fulfilled the criterion of an adequate sample size, and therefore were included in the analysis. The main characteristics of all study populations, such as study design and moment of blood draw, are summarised in Table 1. In all these studies investigators measured ADAMTS13 antigen levels by an enzyme-linked immunosorbent assay (ELISA), and in only one study (ATTAC study) also the activity of ADAMTS13 was measured.

All corresponding authors from the selected studies proved willing to provide the requested information, yielding a total of 1501 myocardial infarction cases and of 2258 controls. All cases were recruited after their first event. Demographic and clinical characteristics of cases and controls are shown in Table 2. Overall, participants were young, with similar mean ages for cases and controls (51 years vs 47 years), whereas there was a preponderance of men within cases (66% vs 49%). As expected, cardiovascular risk factors were more prevalent in cases than in controls.

Table 2. Demographic and clinical characteristics for cases and controls included in the IPD meta-analysis.

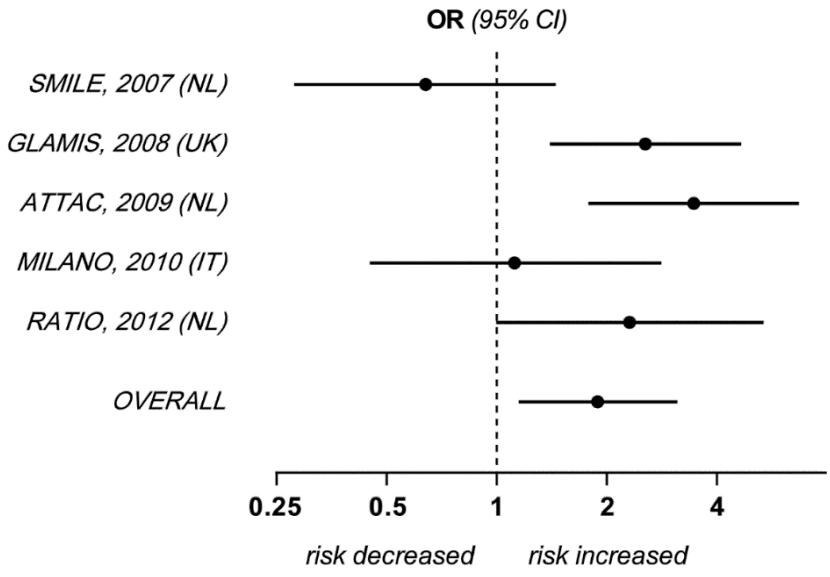
	SMILE		GLAMIS		ATTAC		Milan		RATIO		Overall	
	Cases (551)	Controls (635)	Cases (447)	Controls (472)	Cases (165)	Controls (329)	Cases (136)	Controls (196)	Cases (202)	Controls (628)	Cases (1501)	Controls (2258)
Age, mean (SD)	56.2 (9.1)	57.4 (10.8)	54.8 (7.5)	55.1 (7.5)	42.8 (5.6)	38.4 (7.9)	39.3 (5.6)	39.4 (5.2)	42.2 (6.1)	38.4 (7.9)	51.0 (10.1)	47.4 (12.3)
Sex, n (%)												
female	-	-	116 (26)	126 (27)	63 (38)	207 (63)	136 (100)	196 (100)	202 (100)	628 (100)	517 (34)	1155 (51)
male	551 (100)	635 (100)	331 (74)	346 (73)	102 (62)	122 (37)	-	-	-	-	984 (66)	1103 (49)
BMI (SD)	27.1 (3.4)	26.9 (3.5)	28.0 (4.8)	26.9 (4.6)	26.9 (4.8)	25.1 (4.3)	24.1 (4.3)	23.1 (4.7)	27 (5.2)	24.4 (4.1)	27.1 (4.4)	25.6 (4.3)
Months from MI, mean (min-max)	38 (3-72)	-	6 (3-9)*	-	2 (1-7)	-	<1 (0-1)	-	70 (38-112)	-	24 (0-112)	-
History of (%):												
Hypertension	154 (28)	118 (18)	176 (39)	83 (18)	36 (24)	22 (8)	34 (26)	18 (9)	74 (37)	39 (6)	474 (32)	274 (12)
Diabetes	26 (5)	21 (3)	45 (10)	10 (2)	15 (9)	5 (2)	7 (5)	2 (1)	10 (5)	9 (1)	103 (7)	47 (2)
Smoking	345 (63)	208 (33)	197 (44)	131 (28)	145 (88)	166 (51)	95 (70)	92 (47)	167 (83)	264 (43)	949 (63)	864 (38)
Hypercholesterolemia	164 (30)	10 (2)	142 (32)	153 (33)	81 (60)	133 (48)	52 (39)	73 (40)	20 (10)	19 (3)	458 (31)	388 (18)

MI, myocardial infarction; BMI, body mass index; SD, standard deviation;

**Details for single participants are not available, samples were drown between 3 and 9 months from the event*

Fig. 2 shows the ORs for the association between ADAMTS13 levels and the risk of myocardial infarction based on the 5th percentile comparison in each single study.

Fig. 2. Forest plot for the association between low levels of ADAMTS13, which is below the 5th percentile, and the risk of myocardial infarction by studies.



Dots indicate odds ratios (ORs) and solid bars indicate 95% confidence intervals. The scale is logarithmic. ORs were calculated for each study population by multivariable logistic regression model and were adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. The overall OR was calculated by one step individual patient data meta-analytic approach by mixed logistic regression with random treatment effects. NL, The Netherlands; UK, United Kingdom; IT, Italy.

When all the studies were pooled together, low levels of ADAMTS13 (i.e., below the 5th percentile vs above the 5th percentile) were associated with almost a twofold increase in risk of myocardial infarction (fully adjusted OR 1.89, 95% CI 1.15 - 3.12). This association became stronger with a more extreme cut-off (fully adjusted OR 4.09, 95% CI 1.73 - 10.21 for levels below vs above the 1st percentile), as shown in Table 3. Additional adjustment for VWF levels did not affect the estimates (OR 1.79, 95% CI 1.05 - 3.06 for the 5th percentile cut-off and OR 4.16, 95% CI 1.74 - 9.98 for the 1st percentile cut-off). When the comparison was made by quartiles of ADAMTS13 distribution, moderately low levels of ADAMTS13 were not associated with a substantial increase in risk of myocardial infarction (lowest quartile vs highest quartile, fully adjusted OR 1.28, 95% CI 0.68 - 2.45), and no trend was seen for intermediate quartiles, indicating a threshold rather than a 'dose' relationship (Table 3).

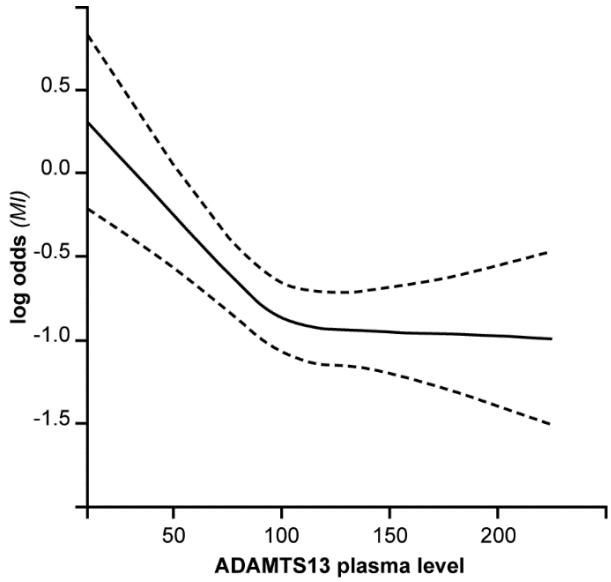
Table 3. Risk of myocardial infarction in relation to ADAMTS13 levels.

Standardized levels ADAMTS13	**Cases 1501**	**Controls 2258**	**OR (95% CI)**	**OR_1 (95% CI)**
<=5th percentile (<=64%)	130	112	1.75 (0.98-3.12)	1.89 (1.15-3.12)
>5th percentile (>64%)	1371	2146	ref	ref
<=1st percentile (<=52%)	67	22	4.09 (1.41-11.83)	4.21 (1.73-10.21)
>1st percentile (>52%)	1437	2236	ref	ref
Q1 (<83%)	420	564	1.38 (0.69-2.78)	1.28 (0.68-2.45)
Q2 (from 83% to 97%)	379	565	1.23 (0.76-2.01)	1.25 (0.78-1.97)
Q3 (from 97% to 112%)	361	565	1.12 (0.83-1.52)	1.08 (0.81-1.46)
Q4 (>112%)	341	564	ref	ref

ORs, as measure of relative risk, are calculated by mixed logistic regression model with random treatment effects and are all adjusted for age and sex. OR_1 values are also adjusted for body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. Q indicates quartile; and ref, reference.

The form of the relationship between levels of ADAMTS13 and the risk of myocardial infarction is depicted in Fig. 3. There is appreciably a non-linear component of this association (i.e., a slightly more rapid increase in the risk of myocardial infarction) for levels below 80%.

Fig. 3. Restricted cubic spline curve showing the model-predicted probability of myocardial infarction against plasma levels of ADAMTS13.



The solid line represents the model predicted probability of myocardial infarction [log odds (myocardial infarction)] for each level of ADAMTS13, adjusted for age, sex, body mass index, study indicator and history of smoking, hypercholesterolemia, hypertension, diabetes. Dashed lines represent 95% confidence intervals. Splines are the result of a curve smoothing method based on a regression model which shapes a function of the log odds of myocardial infarction associated with each value of ADAMTS13. It does so by allowing a different fitted curve for different ranges of the exposure of interest, with the constraint that the result can be plotted as a continuous curve.

Table 4 shows the combined effect of ADAMTS13 and VWF levels on the risk of myocardial infarction. VWF levels above the 90th percentile compared with levels below the 90th percentile were associated with an increase in the risk of myocardial infarction (OR 1.72, 95% CI 1.22 - 2.42). When the analysis was restricted to levels of VWF below the 90th percentile, the risk associated with low ADAMTS13 levels was similar to that of the main analysis (OR 1.77, 95% CI 1.00 - 3.25 for ADAMTS13 levels below the 5th percentile vs above the 5th percentile). The risk of myocardial infarction conferred by a combination of low levels of ADAMTS13 and high levels of VWF was only slightly higher than could be expected by the separate effect, without showing evidence of a strong interaction (expected OR 1+0.77+0.72=2.49; calculated OR 3.17, 95% CI 1.18 - 8.63).

Table 4. Risk of myocardial infarction in relation to the combination of low ADAMTS13 and high VWF plasma levels.

High VWF (> 90th percentile)	**Low ADAMTS13 (< 5th percentile)**	**Cases, n (%)**	**Controls, n (%)**	**OR (95 % CI)**
-	-	1137 (76)	1872 (86)	ref
+	-	225 (15)	201 (9)	1.72 (1.22 - 2.42)
-	+	94 (6)	92 (4)	1.77 (1.00 - 3.25)
+	+	35 (2)	17 (1)	3.17 (1.18 - 8.63)

ORs, as measure of relative risk, are calculated by mixed logistic regression model with random treatment effects and are adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes. Relative risks are calculated for four strata, high plasma levels of VWF, i.e. above the 90th percentile, (+/-), low plasma levels of ADAMTS13, i.e. below the 5th percentile, (-/+), or both (+/+) with the -/- category as reference.

Data on VWF was available for 1491 cases (99% of total) and 2182 controls (97% of total). Ref indicates reference.

The results from subgroup analyses are shown in Table 5. There was a similar relative effect of ADAMTS13 on the risk of myocardial infarction for subject below and above 45 years old, whereas there was a considerable difference between women and men. Women with low levels of ADAMTS13 (i.e. levels below the 5th percentile compared with levels above the 5th

percentile) had an almost 3 fold increased risk of myocardial infarction (OR 2.78, 95% CI 1.61 - 4.88), while in men a 1.7 fold increase was observed (OR 1.66, 95% CI 1.08 - 2.56). When, to remove centre effects in this comparison, the analysis was restricted to studies including both sexes (i.e. GLAMIS and ATTAC), this difference in relative rates persisted: for ADAMTS13 levels below vs above the 5th percentile OR in women 4.00, 95% CI 1.63 - 9.72; OR in men 2.50, 95% CI 1.48 - 4.15.

Table 5. Risk of myocardial infarction in relation on levels of ADAMTS13 for age and sex subgroups.

	ADAMTS13 <5th percentile		**ADAMTS13 >5th percentile**		
	Cases	**Controls**	**Cases**	**Controls**	**OR (95% CI)**
Age at the event					
below 45 years	57 (12%)	59 (6)	428 (88%)	966 (94%)	1.99 (0.93-4.26)
above 45 years	73 (7%)	53 (4)	943 (93%)	1180 (96%)	2.41 (0.74-7.86)
Sex					
female	59 (11%)	62 (5%)	458 (89%)	1093 (95%)	2.78 (1.61-4.88)
male	71 (7%)	50 (6%)	913 (93%)	1053 (94%)	1.66 (1.08-2.56)

ORs are calculated by multivariable logistic regression model with fixed effect and adjusted for age, sex, body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes.

Finally, when the analysis was restricted to the studies in which the blood samples were collected after the acute phase (i.e. >1 month from the event), the results did not differ much from the main analysis (ADAMTS13 levels below vs above the 5th percentile, fully adjusted OR 1.96, 95% CI 1.08 - 3.55).

Discussion

Our analysis of 1501 cases and 2258 controls from 5 case-control studies indicates that only low levels of ADAMTS13 (i.e., standardized antigen levels below 64%, a cut-off that corresponds to values varying from 61% [ATTAC] to 78% [Milan] antigen levels relative to normal pooled plasma among the original studies) are associated with a moderate increase in risk of myocardial infarction. This association is not mediated by levels of VWF antigen.

The relationship between ADAMTS13 and myocardial infarction has been previously studied by Sonneveld and colleagues.[15] They have summarized the same five studies in a meta-analysis of aggregate data. However, they have pooled ORs measured with different cut-off levels of ADAMTS13 (tertiles and quartiles) and with different adjustments, and were unable to give a definitive answer. By performing a meta-analysis based on IPD we obtained a less biased estimate, we determined the dose response relationship, and we investigated the association in specific subgroups of patients.

Subgroups analysis showed that the association between ADAMTS13 and the risk of myocardial infarction was similar between ages, but, while present in both sexes, seemed to be more pronounced in women. Partly, this difference in relative risk could have resulted from the division of the studies in the two subgroups (i.e. SMILE, GLAMIS and ATTAC for men and GLAMIS, RATIO, Milan, ATTAC for women), but also because the incidence of myocardial infarction is higher in men than in women, and therefore a similar absolute risk difference between sexes for low vs high levels of ADAMTS13 will lead to a higher relative risk in women than in men.[25] However, our data do not allow to further investigate if it reflects a true sex-specific effect, chance, or a difference in the presence of unmeasured confounding between men and women (for example alcohol consumption or physical activity).

The relationship between ADAMTS13 and other types of arterial thrombosis such as ischaemic stroke has been investigated in some studies.[12,21,26,27] Their results suggest that even moderately reduced levels of ADAMTS13 increased the risk of ischaemic stroke. A meta-analysis of aggregate data, pooling these publications, showed a strong association between ADAMTS13 and ischaemic stroke for the lowest quartile of the ADAMTS13 distribution (pooled OR for low vs high ADAMTS13 levels, 2.72, 95% CI 1.52 - 4.86).[15] For

myocardial infarction our IPD meta-analysis showed an association for the lowest levels of ADAMTS13 (OR for the lowest quartile vs the highest quartile 1.28, 95% CI 0.68 - 2.45, whereas OR for levels below vs above the 1st percentile 4.21, 95% CI 1.73 - 10.21). This indicates that, although ADAMTS13 levels are associated with both forms of arterial thrombosis, the effects are different, which may reflect pathophysiological differences between the two disorders.

The pathophysiological mechanisms that underlie the association of low ADAMTS13 levels with myocardial infarction are not fully understood. We believe that this mechanism may be due to one, or a combination of, the following: 1) an effect of ADAMTS13 concentration upon the initiation and progression of the atherosclerotic plaque itself;[9] 2) the influence of ADAMTS13 concentration upon acute thrombus formation;[10] 3) the influence of ADAMTS13 upon amplification of the thrombus and deleterious post-thrombotic inflammation.[11]

Murine models have revealed that complete ADAMTS13 deficiency augments the development of atherosclerotic lesions in a manner that is dependent on VWF.[9,28,29] Although not formally examined in these studies, it is likely that complete (rather than heterozygous) deficiency is necessary for this enhanced lesion development. This may argue against a role for more subtle variation in ADAMTS13 concentration upon plaque development. In humans, severe ADAMTS13 deficiency (<5% activity) is a cause for widespread microvascular thrombosis due to the lack of cleavage of the hyperactive ultra-large von Willebrand factor (ULVWF) multimers. This can also be associated with myocardial infarction in some patients with TTP.[30] However, it appears in both mice and humans that ADAMTS13 levels >50% are not associated with any detectable difference in plasma VWF multimer distribution.[31]

In our analysis, we did not find that the association of ADAMTS13 with the risk of myocardial infarction was mediated by levels of VWF (i.e., adjustment for VWF levels did not materially affect the results). Moreover, we found only a minor synergistic effects between low levels of ADAMTS13 and high levels of VWF. Therefore, it is possible that plasma levels of ADAMTS13 slightly below the lower bound of the normal range (i.e. ~60%) may influence the kinetics of the thrombus growth, in a manner that is independent of circulating VWF levels. In physiologic conditions, the proteolysis of circulating VWF is

determined primarily by the unfolding of ULVWF multimers rather than ADAMTS13 plasma levels. Conversely, at sites of injury or plaque rupture, where the thrombus is consolidated by platelet binding through fibrinogen and fibrin and active VWF can become protected against proteolysis, changes in concentration of ADAMTS13 may be far more important in controlling the thrombus formation.[32] As such, low ADAMTS13 might leads to the formation of a more extensive platelet plug that is further stabilized/consolidated by additional prothrombotic mechanisms.

Our study has some limitations. To increase the number of studies that could provide data and the homogeneity of the information, we intentionally limited the number of variables we used for our analyses. This, however, could have led to residual confounding and have limited the number of additional analysis (e.g. stratification for other blood parameters). We also excluded studies with less than 50 cases. This might have led to a small reduction in power, but reduced the impact of publication bias, to which small studies are more prone than large studies. Given the considerable study sample size we have reached, it is unlikely that the inclusion of these studies would have altered our findings. Finally, because of the case-control design of the studies included, blood was collected after the event in the case groups. This might lead to reverse causation, which is when the consequence of an event is mistaken for the cause. Several factors could have influenced ADAMTS13 levels. First, the acute phase response to the arterial thrombotic event. We were not able to perform subgroups analysis based on time from the event to the blood sampling due to the little overlap of these time periods between the five studies. However, since only in one study blood was collected in the acute phase of myocardial infarction (i.e. the Milan study), and we did not find an effect on timing of the blood draw in the individual patient data analysis, the results are unlikely to be explained by the transient effects of the acute phase. Second, it has to be considered whether ADAMTS13 levels were influenced by the presence of chronic heart failure (CHF) in patients with myocardial infarction. There is no information in the literature on ADAMTS13 levels in CHF patients as compared with healthy subjects, but one study found that low ADAMTS13 activity levels were predictors of adverse functional outcome in CHF patients.[33] Therefore, we cannot exclude that ADAMTS13 levels are decreased in CHF. However, given that ADAMTS13 is mainly produced in the liver and therefore not or hardly affected by endothelial dysfunction, it seems most likely that that relationship was

ADAMTS13 causing worsening of CHF rather than the other way round.[1] Moreover, participants in our analysis were relatively young (mean age 51 years for cases), in which age category CHF and other age-related risk factors such as endothelial lesions and dysfunction through atherosclerosis are uncommon. Third, some medications, such as the thienopyridines (e.g., ticlopidine, clopidogrel, and prasugrel) which are often prescribed after myocardial infarction, have been associated with acute TTP episodes.[34] This occurrence, however, is extremely rare, and the underlying mechanisms seems to be an acute induced autoimmune reactions with a dramatic clearance of ADAMTS13, rather than a moderate decrease of its plasma concentration. It is therefore unlikely that post-hoc use of thienopyridines offers an alternative explanation of our findings. Because only one of the five included studies investigated the activity of ADAMTS13 and VWF (ATTAC study), we choose to focus on antigen levels, which not always are a good representation of the molecule's activity. However, plasma antigen levels of ADAMTS13 correlate well with its activity.[35] Only in rare cases, when inhibitor antibodies against ADAMTS13 are present, antigen levels do not reflect activity. Therefore, we do not believe that this could have influenced our main results.

In conclusion, with an IPD meta-analytic approach we demonstrated that low levels of ADAMTS13 increased the risk of myocardial infarction. This association is valid only for low levels of ADAMTS13, and therefore differs from its relation with ischaemic stroke.

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Chapter 3

Pregnancy loss and risk of ischaemic stroke and myocardial infarction

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Abstract

Background and Aims: To investigate whether pregnancy loss increases the risk of arterial thrombosis in young women, and risk factors involved in this association.

Methods: Women (age 18 to 50 years) with ischaemic stroke or myocardial infarction and at least one pregnancy were contrasted for pregnancy loss with a control group. Odds ratios (OR) with 95% confidence intervals (CI), adjusted for matching variables, cardiovascular risk factors (smoking, hypertension, alcohol consumption, diabetes, hyperlipidemia, body mass index), cardiovascular family history and the presence of antiphospholipid antibodies, were calculated for the number of pregnancy losses as well as the type of unsuccessful pregnancy (early miscarriage, late miscarriage and stillbirth).

Results: 165 ischaemic stroke cases, 218 myocardial infarction cases and 743 controls, with an average of 2.7, 2.5 and 2.5 pregnancies per woman, were included. Women with multiple (≥3) pregnancy loss had a doubled risk of arterial thrombosis (OR 2.37, 95% CI 0.99-5.70) compared with women without pregnancy loss, similarly to women who experienced stillbirth (OR 1.68, 95% CI 0.79-3.55). Both relative risks were higher for ischaemic stroke (OR 3.51, 95% CI 1.08-11.35 and 2.06, 95% CI 0.81-5.23, respectively) than for myocardial infarction (OR 2.04, 95% CI 0.71-5.86 and 1.04, 95% CI 0.39-2.79). Adjustment for antiphospholipid antibodies did not affect the estimates.

Conclusion: Women with multiple pregnancy loss and those with at least one stillbirth had an increased risk of ischaemic stroke and, to a lesser extent, of myocardial infarction, even when other cardiovascular risk factors and antiphospholipid antibodies were accounted for.

Introduction

Arterial thrombotic diseases, such as myocardial infarction and ischaemic stroke, are the leading causes of morbidity and mortality in western countries.[1, 2] The young and women have a low cardiovascular risk, and therefore research into myocardial infarction and ischaemic stroke has traditionally been focused on the elderly and men, leaving women and the young underrepresented in cardiovascular research.[3, 4] This could be one of the reasons why in the last decades morbidity and mortality related to cardiovascular diseases improved more for men than for women.[5]

Nonetheless, the few studies carried out in women suggest some sex-specific risk factors for arterial thrombosis, such as adverse pregnancy outcome.[6-8] Endothelial dysfunction has been linked with preeclampsia and recurrent miscarriages and has been suggested to be a marker of increased future cardiovascular risk.[9, 10] Moreover, hypercoagulability due to the presence of antiphospholipid antibodies, known to be associated with both recurrent miscarriages and cardiovascular events, could play a role in this association.[11]

However, studies that linked pregnancy losses with myocardial infarction and ischaemic stroke later in life yielded contrasting results. A large prospective cohort study found that women with stillbirth or recurrent miscarriage had an approximately 2-fold increased risk of subsequent myocardial infarction and ischaemic stroke at older ages than women with only successful pregnancies.[12] Other studies reported similar findings for myocardial infarction, but not for ischaemic stroke.[13, 14] Variables acting as potential confounders or mediators, such as classic cardiovascular risk factors and the presence of antiphospholipid antibodies, have been rarely (cardiovascular risk factors) or even never (antiphospholipid antibodies) investigated for this association.

We aimed to study whether adverse pregnancy outcome increases the risk of arterial thrombosis in women at young age when residual confounding, family history of cardiovascular disease and the presence of antiphospholipid antibodies are taken into account. Additionally, we investigated whether this risk differed between myocardial infarction and ischaemic stroke.

Methods

Patients

Women who participated in the Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) case-control study were eligible for this analysis. Cases selection within the RATIO case-control study has been described in detail previously.[7, 15, 16] In short, all women 18 to 50 years of age who presented with a first event of myocardial infarction or ischaemic stroke to one of the 16 participating hospitals in the Netherlands between 1990 and 1995 were eligible and approached for study participation. Diagnosis was made on the basis of clinical symptoms and confirmed by appropriate tests. Myocardial infarction was diagnosed by the presence of clinical symptoms, elevated cardiac enzyme levels, and corresponding electrocardiographic changes. Clinical symptoms of ischaemic stroke were confirmed by either computed tomography or magnetic resonance imaging. Ischaemic stroke of cardioembolic origin was excluded by the presence of atrial fibrillation or suggestive cardiac ultrasound imaging. All cases were included from 1995 to 1998.

Women were approached to participate as a control subject by random digit dialing and were matched with cases according to age (in 5-year categories), area of residence, and year of event. To be eligible as control women should not have a history of coronary heart disease, cerebrovascular event, or peripheral vascular disease.

In this analysis we included women who experienced at least one pregnancy before the event (cases) or the matched index year (controls). All participants gave informed consent and the study was approved by the ethics committees of the participating hospitals.

Data collection

The study consisted of two phases. In the first phase patients and controls were asked to fill in the same structured questionnaire comprising questions on demographic characteristics, medical history of cardiovascular risk factors, obstetrical history and a family history of vascular disease. These questions were targeted to the year before diagnosis (cases) or the matched index year (controls). The questionnaire included detailed information about previous pregnancies and their outcomes. Information collected included various aspects of

obstetrical history, pregnancies, parity, gestation, and terminations. Miscarriage was defined as the naturally-occurring expulsion of the product of conception before the 22th week of gestation, and stillbirth as fetal death at the 22th week of gestation or later or at a birthweight of at least 500g.[17] Smoking was defined as having regularly smoked in the year before the index date. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Women were classified as hypertensive, diabetic or hypercholesterolemic 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 under 60 years of age in first degree relatives.

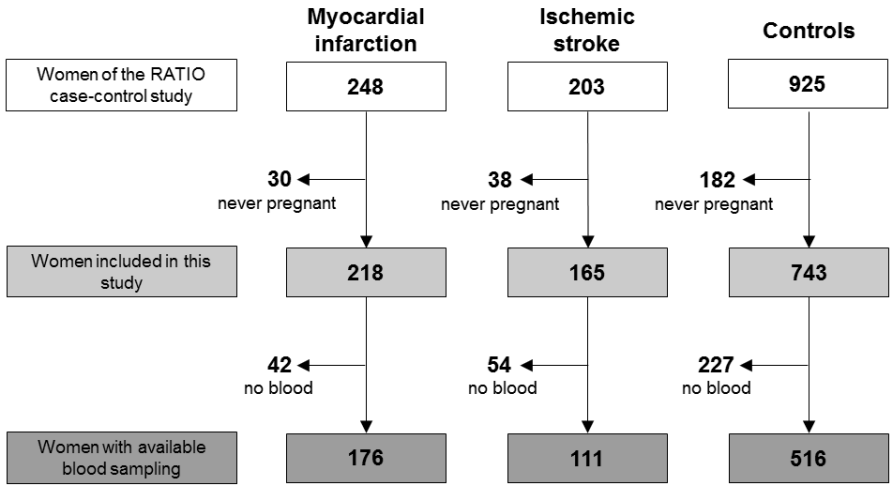
In the second phase of the study all participants were re-approached to donate blood. Blood samples were collected after a median of 69 months (range 38 to 117 months) for myocardial infarction cases and 95 months (range 23 to 146 months) for ischaemic stroke cases. Blood draw procedures and measurements are described in detail in previous publications.[6] The presence of lupus anticoagulant was detected with dilute Russell's viper venom time (dRVVT) reagents (LA-screen and LA-confirm; Gradipore, Australia). In the event of a prolonged coagulation time (LA-screen time >99th percentile of time recorded for 40 healthy volunteers), LA-confirm assays were done. Normalized ratios for LA-screen and LA-confirm coagulation times (ratios/c) were calculated and samples were deemed positive for lupus anticoagulant when the ratios/c was 1.15 or higher, on the basis of the 99th percentile of the value recorded for 40 healthy volunteers. This measurement is in accordance with current recommendations for testing lupus anticoagulant.[18] IgG anti-β2-glycoprotein 1 antibodies were measured as described previously and reported as percentage of a positive control.[6] IgG anticardiolipin antibody concentrations (GPL) were measured in plasma samples with a commercially available kit (Corgenix, Broomfi eld, CO, USA) in accordance with the instructions of the manufacturer. For both antibodies cut-off was set at the 90th percentile of the concentration recorded for the control group.

Statistical analysis

We use means or proportions to describe obstetrical variables and cardiovascular risk factors. We calculated odds ratios (OR) as estimate of relative risks for any arterial thrombosis and for myocardial infarction and ischaemic stroke separately. We contrasted women with at least one pregnancy loss (either miscarriage or stillbirth) with women without pregnancy loss. Odds ratios and 95% confidence intervals (CI) were obtained by multivariable logistic regression adjusted for matching variables (age, area of residence, year of index event). Additional adjustment for common cardiovascular risk factors (i.e., body mass index, history of smoking, hypercholesterolemia, hypertension, diabetes and alcohol consumption) and family history of a cardiovascular event was done in separate models.

A "dose response relationship" between pregnancy loss and arterial thrombosis was investigated by the use of exposure categories, based on the crude number of pregnancy losses (none, one, two or more), and the type of pregnancy loss (early miscarriage before 13 weeks of gestation, late miscarriage from 13 weeks to 22 weeks, and stillbirth). Finally, the analyses were restricted to those women for whom blood samples were available in order to adjust the estimates for antiphospholipid antibodies (presence of lupus anticoagulant, anti-β2-glycoprotein 1 IgG, anticardiolipin IgG). For all analyses, results were presented for the two case groups pooled together, and for each case group separately (myocardial infarction and ischaemic stroke).

Figure 1. Women included in the analysis.



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

Results

The study included 218 women with myocardial infarction, 165 with ischaemic stroke and 743 controls who experienced at least one pregnancy (Figure 1). Table 1 summarizes demographic and clinical characteristics of cases and controls. Mean age was 42.1 for cases and 39.2 for controls. As expected, cardiovascular risk factors were more prevalent in cases than in controls.

Table 1. Baseline demographic and clinical characteristics for cases and controls.

	Any arterial thrombosis n=383	**Myocardial infarction n=218**	**Ischaemic stroke n=165**	**Controls n=743**
Age at the event, mean (SD)	42.1 (6.8)	43.2 (5.8)	40.6 (7.6)	39.2 (7.7)
BMI, mean (SD)	26.5 (5.6)	27.0 (5.4)	25.8 (5.8)	24.4 (4)
Risk factors, n (%)				
Diabetes	22 (6%)	13 (6%)	9 (6%)	11 (2%)
Hypertension	89 (23%)	47 (22%)	42 (26%)	47 (6%)
Hyperlipidaemia	35 (9%)	26 (12%)	9 (6%)	20 (3%)
Alcohol consumption	229 (60%)	129 (59%)	100 (61%)	488 (66%)
Smoking	281 (73%)	180 (83%)	101 (61%)	312 (42%)
Cardiovascular family history*	219 (57%)	139 (67%)	80 (53%)	261 (37%)
Antiphospholipid antibodies#				
Lupus anticoagulant †	25 (9%)	5 (3%)	20 (18%)	4 (1%)
Anti-β2-glycoprotein I IgG >27.8% ‡	37 (13%)	15 (9%)	22 (20%)	51 (10%)
Anticardiolipin IgG >14.5 ‡	31 (11%)	22 (13%)	9 (8%)	51 (10%)

BMI, body mass index and SD, standard deviation. * Data on cardiovascular family history are available for 96% of the subjects in the myocardial infarction group, 91% of the subjects in the ischaemic stroke group, and 95% of the subjects in the control group. # Blood samples were available for 81% of the subjects in the myocardial infarction group, 67% of the subjects in the ischaemic stroke group, and 69% of the subjects in the control group. † Normalized ratios for LA-screen and LA-confirm coagulation times ($ratio_{s/c}$) higher than 1.15. ‡ 90^{th} percentile of controls.

There were 995 pregnancies amongst the cases (either myocardial infarction or ischaemic stroke), of which 170 (17%) ended in a pregnancy loss. The women in the control group reported 1861 pregnancies, of which 326 (18%) ended in a pregnancy loss (Table 2).

Among cases, 122 (32%) women experienced at least one pregnancy loss [63 (29%) with myocardial infarction and 59 (36%) with ischaemic stroke]. In the control group, 234 (32%) women experienced at least one pregnancy loss, leading to an OR for any arterial thrombosis of 1.05 (95% CI 0.79-1.40) (Table 3). This estimate did not change substantially when common cardiovascular risk factors and cardiovascular family history were taken into account (fully adjusted OR 1.01, 95% CI 0.71-1.41). Fully adjusted odds ratios were slightly higher for women with ischaemic stroke (OR 1.17, 95% CI 0.74-1.87) than myocardial infarction (OR 0.83, 95% CI 0.54-1.27).

Table 2. Obstetrical history for cases and controls.

	Any arterial thrombosis n=383	**Myocardial infarction n=218**	**Ischaemic stroke n=165**	**Controls n=743**
Total pregnancies	995	544	451	1861
Total pregnancy losses (%)	170 (17)	91 (17)	79 (18)	326 (18)
Mean pregnancies per woman	2.6	2.5	2.7	2.5
Women with at least one pregnancy loss (%)	122 (32)	63 (29)	59 (36)	234 (32)
Women by number of pregnancy loss (%)				
one	89 (23)	45 (21)	44 (27)	160 (22)
two	18 (5)	8 (4)	10 (6)	56 (8)
three or more	15 (4)	10 (5)	5 (3)	18 (2)

Table 3. Risk of any arterial thrombosis, myocardial infarction and ischaemic stroke by the occurrence of at least one pregnancy loss.

	Pregnancy loss (%)	No pregnancy loss (%)	OR (95% CI)	OR_1 (95%CI)	OR_2 (95%CI)
Control (n=743)	234 (32)	509 (68)	ref	ref	ref
Any arterial thrombosis (n=383)	122 (32)	261 (68)	1.05 (0.79-1.40)	0.89 (0.64-1.23)	1.01 (0.71-1.41)
Myocardial infarction (n=218)	63 (29)	155 (71)	0.95 (0.67-1.35)	0.70 (0.46-1.06)	0.83 (0.54-1.27)
Ischaemic stroke (n=165)	59 (36)	106 (64)	1.23 (0.82-1.83)	1.03 (0.66-1.59)	1.17 (0.74-1.87)

ORs are calculated by logistic regression and are adjusted for age, area of residence, year of index event. OR_1 are also adjusted for body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes and alcohol consumption. OR_2 are additionally adjusted for cardiovascular family history. Ref, reference.

When the analyses were based on the number of pregnancy losses, we found that women who experienced three or more pregnancy losses had a double risk of any arterial thrombosis (OR 1.95, 95% CI 0.92-4.14, fully adjusted OR 2.37, 95% CI 0.99-5.70), compared with women without pregnancy loss (Table 4). Women who had a stillbirth had a similar increased risk of any arterial thrombosis (OR 2.14, 95% CI 1.15-4.00), but after adjustment for common cardiovascular risk factors and cardiovascular family history the OR decreased to 1.68 (95% CI 0.79-3.55).

When the two case groups were analyzed separately, recurrent pregnancy losses and stillbirth had a more pronounced effect on the risk of ischaemic stroke than on that of myocardial infarction (≥ 3 pregnancy losses OR 2.00, 95% CI 0.86-4.69 for myocardial infarction and OR 2.38, 95% CI 0.80-7.08 for ischaemic stroke; stillbirth OR 2.01, 95% CI 0.95-4.23 for myocardial infarction and OR 2.54, 95% CI 1.12-5.72 for ischaemic stroke), especially when cardiovascular risk factors and cardiovascular family history were taken into account (≥ 3 pregnancy losses OR 2.04, 95% CI 0.71-5.86 for myocardial infarction and OR 3.51, 95%

CI 1.08-11.35 for ischaemic stroke; stillbirth OR 1.04, 95% CI 0.39-2.79 for myocardial infarction and OR 2.06, 95% CI 0.81-5.23 for ischaemic stroke).

Table 4. Risk of arterial thrombosis in relation on the number and type of pregnancy losses.

	Cases n (%)	Controls n (%)	OR (95% CI)	OR_1 (95% CI)	OR_2 (95% CI)
Any arterial thrombosis					
Number of pregnancy losses					
none	261 (68)	509 (69)	ref	ref	ref
one	89 (23)	160 (22)	1.09 (0.79 - 1.51)	0.91 (0.63 - 1.32)	0.97 (0.66 - 1.42)
two	18 (5)	56 (8)	0.64 (0.35 - 1.18)	0.51 (0.25 - 1.02)	0.56 (0.27 - 1.23)
three or more	15 (4)	18 (2)	1.95 (0.92 - 4.14)	1.92 (0.83 - 4.46)	2.37 (0.99 - 5.70)
Type of pregnancy loss					
early miscarriage	76 (20)	163 (22)	0.96 (0.69 - 1.34)	0.83 (0.56 - 1.21)	0.94 (0.63 - 1.40)
late miscarriage	13 (3)	28 (4)	0.88 (0.43 - 1.83)	0.89 (0.40 - 1.97)	0.96 (0.43 - 2.17)
stillbirth	26 (7)	24 (3)	2.14 (1.15 - 4.00)	1.53 (0.73 - 3.20)	1.68 (0.79 - 3.55)
Myocardial Infarction					
Number of pregnancy losses					
none	155 (71)	509 (69)	ref	ref	ref
one	45 (21)	160 (22)	0.98 (0.66 - 1.46)	0.69 (0.43 - 1.11)	0.74 (0.45 - 1.21)
two	8 (4)	56 (8)	0.52 (0.23 - 1.15)	0.39 (0.15 - 1.00)	0.55 (0.21 - 1.46)
three or more	10 (5)	18 (2)	2.00 (0.86 - 4.69)	1.73 (0.63 - 4.71)	2.04 (0.71 - 5.86)
Type of pregnancy loss					
early miscarriage	42 (20)	163 (22)	0.92 (0.62 - 1.38)	0.70 (0.44 - 1.14)	0.85 (0.52 - 1.39)
late miscarriage	5 (2)	28 (4)	0.58 (0.21 - 1.60)	0.61 (0.21 - 1.83)	0.69 (0.23 - 2.07)
stillbirth	13 (6)	24 (3)	2.01 (0.95 - 4.23)	1.00 (0.39 - 2.50)	1.04 (0.39 - 2.79)

Ischaemic stroke

Number of pregnancy losses					
none	106 (64)	509 (69)	ref	ref	ref
one	44 (27)	160 (22)	1.25 (0.80 - 1.97)	1.06 (0.65 - 1.72)	1.18 (0.71 - 1.98)
two	10 (6)	56 (8)	0.85 (0.38 - 1.93)	0.62 (0.25 - 1.55)	0.64 (0.23 - 1.76)
three or more	5 (3)	18 (2)	2.38 (0.80 - 7.08)	2.35 (0.73 - 7.52)	3.51 (1.08 - 11.35)
Type of pregnancy loss					
early miscarriage	34 (21)	163 (22)	1.03 (0.64 - 1.66)	0.89 (0.52 - 1.49)	1.00 (0.58 - 1.73)
late miscarriage	8 (5)	28 (4)	1.33 (0.52 - 3.39)	1.29 (0.48 - 3.44)	1.48 (0.54 - 4.07)
stillbirth	13 (8)	24 (3)	2.54 (1.12 - 5.72)	1.69 (0.68 - 4.22)	2.06 (0.81 - 5.23)

ORs are calculated by logistic regression and are adjusted for age, area of residence and year of index event. OR_1 are also adjusted for body mass index and history of smoking, hypercholesterolemia, hypertension, diabetes and alcohol consumption. OR_2 are additionally adjusted for cardiovascular family history. Women who never experienced pregnancy loss are the reference category for all comparisons. Gestational age at the time of the pregnancy loss was available in 724 controls (97%) and in 376 cases (98%). Gestational age categories are based on the most late pregnancy loss. Ref, reference.

Finally, when the analyses were restricted to women for whom blood samples were available (72% of the overall participants, Figure 1), the models yielded similar, although slightly attenuated, results (for women with ≥3 pregnancy losses fully adjusted OR for any arterial thrombosis 1.38, 95% CI 0.57-3.33; for women with at least one stillbirth fully adjusted OR for any arterial thrombosis 1.51, 95% CI 0.69-3.32). In these models, the additional adjustment for the presence of antiphospholipid antibodies did not affect the estimates (OR 1.42, 95% CI 0.56-3.51 for women with ≥ 3 pregnancy losses; OR 1.44, 95% CI 0.64-3.26 for women with at least one stillbirth). Similar results were found when the two diseases were analyzed separately (data not shown).

Discussion

Historically, after the evidence emerged that postmenopausal women experience more cardiovascular accidents than premenopausal women, research into cardiovascular diseases focused on the influence of reproductive factors on the occurrence of arterial thrombosis in older ages.[19] Of those factors, only pregnancy loss emerged as a possible predictor of cardiovascular risk, but with contrasting results.[8, 12, 14, 20, 21] A study on a German cohort found an association between stillbirth (hazard ratio, HR 3.40, 95%1.60-7.20) and multiple pregnancy losses (HR 5.06, 95% CI 1.30-20.30) with myocardial infarction, but not with ischaemic stroke.[14] This study had the limitation of a small sample size (82 cases of myocardial infarction and 112 of ischaemic stroke). A larger study on a Danish cohort found that women who experienced at least one miscarriage had a mild increased risk of both myocardial infarction (incidence rate ratio, IRR 1.13, 95% CI 1.03-1.21) and ischaemic stroke (IRR 1.16, 95% CI 1.07-1.25).[12] However, the risk was doubled for women who experienced three or more miscarriages or at least one stillbirth. Because this study was based on data from national registries, information about potential confounders, such as common cardiovascular risk factors, were obtained indirectly, only for a subgroup of women and included diabetes and smoking only.

Our study included women with arterial thrombotic events at a young age (mean age 42 years) and results were adjusted for several common cardiovascular risk factors, cardiovascular familial history and the presence of antiphospholipid antibodies. Similar to the Danish registry study, our analysis showed that women with at least one pregnancy loss did not have an increased risk of arterial thrombosis (OR 1.05, 95% CI 0.79 - 1.40), but that this risk was increased in women with three or more pregnancy losses (OR 1.95, 95% CI 0.92 - 4.14) or stillbirths (OR 2.14, 95% CI 1.15 - 4.00). Our analyses allowed to take other cardiovascular risk factors, cardiovascular familial history and the presence of antiphospholipid antibodies into account. After adjustments for these risk factors, the associations were more pronounced for ischaemic stroke than for myocardial infarction.

Because the risk factor profiles are different between young and old populations, this difference could be partly related to the different age category between our study and the previous ones.[22] Moreover, stroke of cardioembolic origin has been excluded from our study.

Cardioembolic stroke is often related to atrial fibrillation, a condition unlikely to be related with pregnancy loss. Therefore the presence of this subtype of stroke in previous studies could have diluted the relative risk of ischaemic stroke compared with that of myocardial infarction. Notably, the differences between myocardial infarction and ischaemic stroke are more appreciable after the adjustment for cardiovascular risk factors (especially for stillbirth, for which the association with myocardial infarction disappeared after adjustment). This suggests that the mechanisms underlying the association between pregnancy loss and arterial thrombosis may differ between the two diseases.

Several mechanisms have been proposed to explain the association between pregnancy loss and arterial thrombosis. First, the immune system and inflammatory mechanisms play a role in pregnancy loss, especially in recurrent miscarriages. Therefore, alloimmune and autoimmune factors have been linked with both pregnancy loss and arterial thrombosis, including the presence of antiphospholipid antibodies.[23, 24] In our study we did not observe that the effect of multiple pregnancy losses or stillbirth on the risk of arterial thrombosis was mediated by the presence of antiphospholipid antibodies. However, blood was available only for a subgroup of patients and, moreover, we cannot exclude that other immunological factors have played a role.

Genetic components have been also proposed to link miscarriage and atherosclerotic disease. A previous study reported an association between a parental history of ischaemic heart disease and recurrent miscarriage in daughters.[13] Moreover, in the study on the Danish cohort, the strongest association between miscarriages and arterial thrombosis was found in the youngest women.[12] These findings, together with the fact that some familial forms of myocardial infarction and ischaemic stroke are well known to present at younger ages than non-familial forms, corroborate the hypothesis of a common underlying genetic background of the two diseases. Previous studies did not examined these associations, specifically among young women. In our study, we adjusted the analysis for the presence of a positive cardiovascular familial history, but the risk estimates did not change, meaning that, despite the young age of our cases, a genetic background is not likely to play a role in the association between pregnancy loss and arterial thrombosis.

Finally, it has been demonstrated that endothelial dysfunction, a possible cause of placentation-related defects, persists after the complicated pregnancy. Patients with recurrent

pregnancy loss have been found to have lower endothelium-independent vasodilatation compared with women with successful pregnancies.[10] Endothelial dysfunction could be a plausible underlying mechanism common to pregnancy loss (stillbirth and recurrent miscarriage in particular), and arterial thrombosis. Under this hypothesis, the stronger overall associations observed for stillbirth and multiple miscarriages could be explained by the fact that placental problems related to endothelial dysfunction are much more prevalent in stillbirth and multiple pregnancy loss than in sporadic miscarriages, that are more often due to fetal genetic abnormalities or infections.[25] Preeclampsia, closely linked to endothelial dysfunction and known to be associated with both myocardial infarction and ischaemic stroke, could have contributed to some of the stillbirths, however we were unable to adjust for the presence of such condition due to the lack of data. Whether the mechanisms underlying the associations observed for multiple pregnancy loss and stillbirth overlap or whether separate processes are involved is unclear.

Some limitations apply to this study. First, because of the relatively limited number of cases, our risk estimates are imprecise and accompanied by wide confidence intervals. However, women who participated in our study were well characterized, and the diagnosis of myocardial infarction and ischaemic stroke was confirmed objectively, at variance with previous studies. Moreover, women were young, and therefore with a low prevalence of atherosclerotic burden and age-related risk factors, that could potentially obscure the effects of non-age-related risk factors. This made our study more suitable to study risk factors such as pregnancy outcomes. Second, given the case-control design, exposures have been obtained after the event. However, since the purpose of the original RATIO study was to investigate the use of oral contraceptive and the risk of arterial thrombosis, a particular attention was placed upon history of pregnancy and pregnancy outcomes. Nonetheless, even if unlikely, recall bias might have played a role in the overall analyses, but cannot explain the different results of myocardial infarction and ischaemic stroke analyses. Finally, due to the rarity of lupus anticoagulant and the fact that not all patients were willing to donate blood, the statistical power of our secondary analysis was limited and this affects the strength of our conclusion about the role of antiphospholipid antibodies.

In conclusion, we found that multiple pregnancy loss and stillbirth are associated with an increased risk of arterial thrombosis at young age. This increase in risk was more pronounced

for ischaemic stroke than for myocardial infarction, especially when common cardiovascular risk factors and cardiovascular family history were taken into account. The presence of antiphospholipid antibodies is not likely to have mediated this association.

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Chapter 4

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

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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_{IS}/OR_{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.[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.[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_{MI}) and ischaemic stroke (OR_{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 idex (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

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

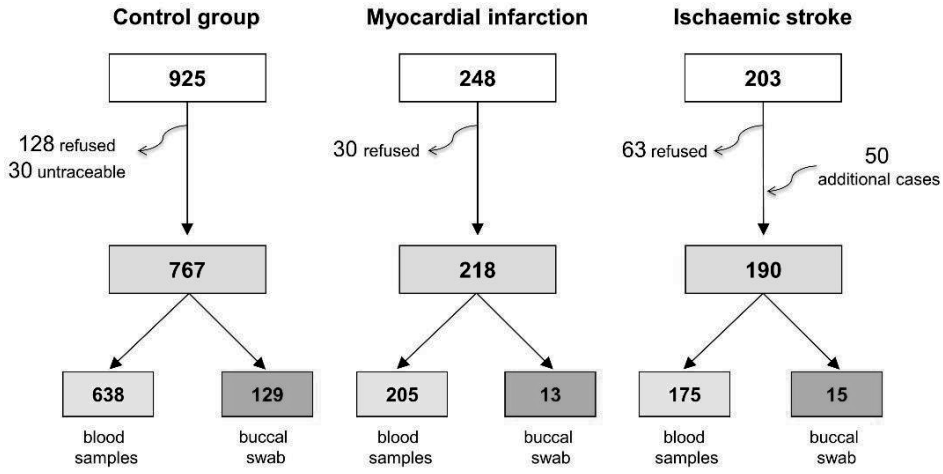
If the ROR >1 the effect on risk is larger for ischaemic stroke, and conversely, if the ROR < 1 the effect is larger for myocardial infarction. When the ROR = 1 there is no difference in effect size. The corresponding 95% confidence interval was obtained from the variance of the natural logarithm of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

$$population\ attributable\ fraction = P_{cases}\left(\frac{OR - 1}{OR}\right)$$

, where P_{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.

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

** 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.

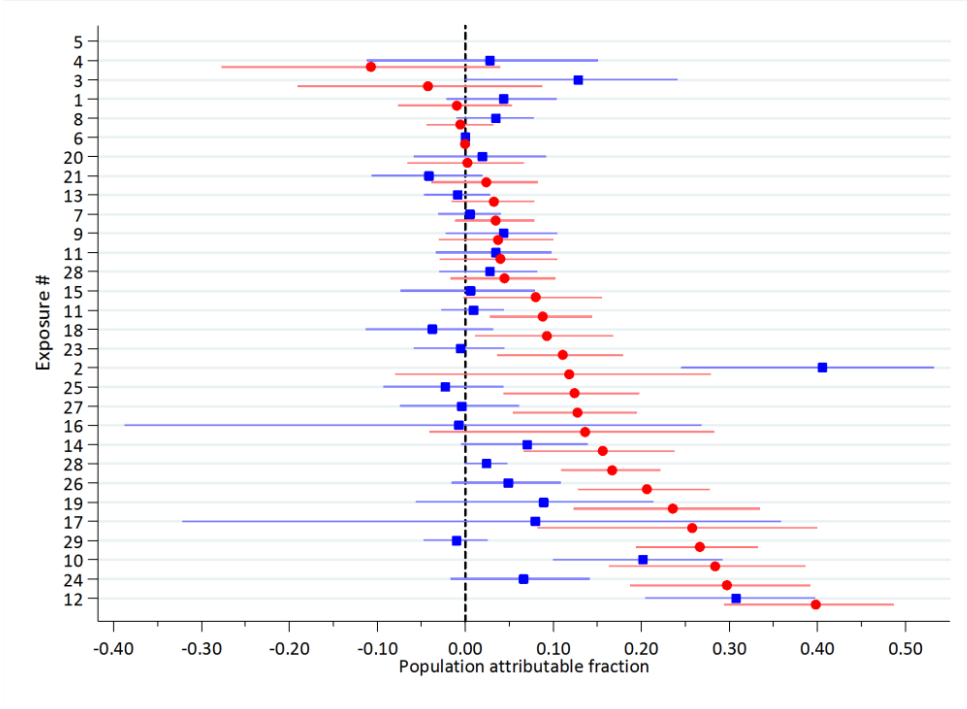
#	Prothrombotic factor	ref	OR MI	OR IS	ROR	95%CI	model
1	Anticardiolipin antibodies, p95	[14]	1.80	0.90	0.50	0.17 - 1.45	2
2	hypofibrinolysis vs. normofibrinolysis*	[20]	2.82	1.50	0.53	0.22 - 1.27	4
3	Prekallikrein:ag p90	[30]	1.54	0.90	0.58	0.23 - 1.52	3
4	F13A1 Pro564Leu, dominant	[13,15]	1.40	0.89	0.64	0.39 - 1.05	1
5	F13A1 Val34Leu, dominant	[13,15]	1.07	0.77	0.72	0.44 - 1.17	1
6	Factor XII:ag, p90	[30]	1.18	1.03	0.87	0.34 - 2.23	3
7	Factor XII:ag ,p10	[30]	1.54	1.36	0.88	0.39 - 2.00	3
8	Prothrombin G20210A, dominant	[16,17]	1.00	1.00	1.00	0.22 - 4.54	1
9	High molecular weight kininogen:ag, p10	[31]	1.39	1.49	1.07	0.43 - 2.67	2
10	Oral contraceptive use vs. non use	[10,12]	2.00	2.30	1.15	0.69 - 1.91	1
11	MTHFR TT snp, recessive	[16,24]	1.30	1.50	1.15	0.57 - 2.33	1
12	VWF:ag q4 vs q1	[22]	4.20	6.70	1.60	0.60 - 4.26	3
13	Factor V Leiden, dominant	[16,17]	1.10	1.80	1.64	0.65 - 4.11	1
14	Factor XI:ag, p90	[30]	1.61	2.65	1.65	0.79 - 3.44	3
15	High molecular weight kininogen:ag, p90	[31]	1.05	1.82	1.73	0.74 - 4.08	2
16	FGB -455 G/A, dominant	[19]	0.98	1.76	1.80	0.53 - 6.08	1
17	FGA 312Ala, dominant	[19]	1.22	2.33	1.90	0.79 - 4.61	1
18	F13B His95Arg, dominant	[13,15]	0.79	1.70	2.15	1.14 - 4.05	1
19	ADAMTS13:ag, q1 vs q4	[22]	1.40	3.10	2.21	0.93 - 5.27	3
20	prekallikrein:ag, p10	[30]	0.60	1.33	2.22	0.79 - 6.24	3
21	anti prothrombin antibodies, p95	[14]	0.80	1.80	2.25	0.63 - 8.03	2
22	anti-β2-glycoprotein antibodies, p95	[14]	1.20	2.80	2.33	0.92 - 5.93	2

23	Factor XIa AT-INH, p90	[29]	0.94	2.33	2.48	1.13	- 5.41	3
24	Hyperfibrinolysis vs. normofibrinolysis8	[20]	1.60	4.07	2.54	1.03	- 6.27	4
25	Factor XIIa C1-INH, p90	[29]	0.82	2.26	2.76	1.27	- 5.99	3
26	Kallikrein C1-INH, p90	[29]	1.50	4.34	2.89	1.42	- 5.89	3
27	Factor XIa C1-INH, p90	[29]	0.96	2.76	2.89	1.31	- 6.34	3
28	Lupus anticoagulant, ≥1.15	[14]	5.30	43.1	8.13	1.30	- 50.9	2
29	F13A1 Tyr204phe, dominant	[13,15]	0.82	9.10	11.1	4.52	- 27.2	1

*# = 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 fibrinolysation 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.[10,12-17,19,20,22,24,29-31] All factors, the corresponding OR_{MI}, OR_{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).

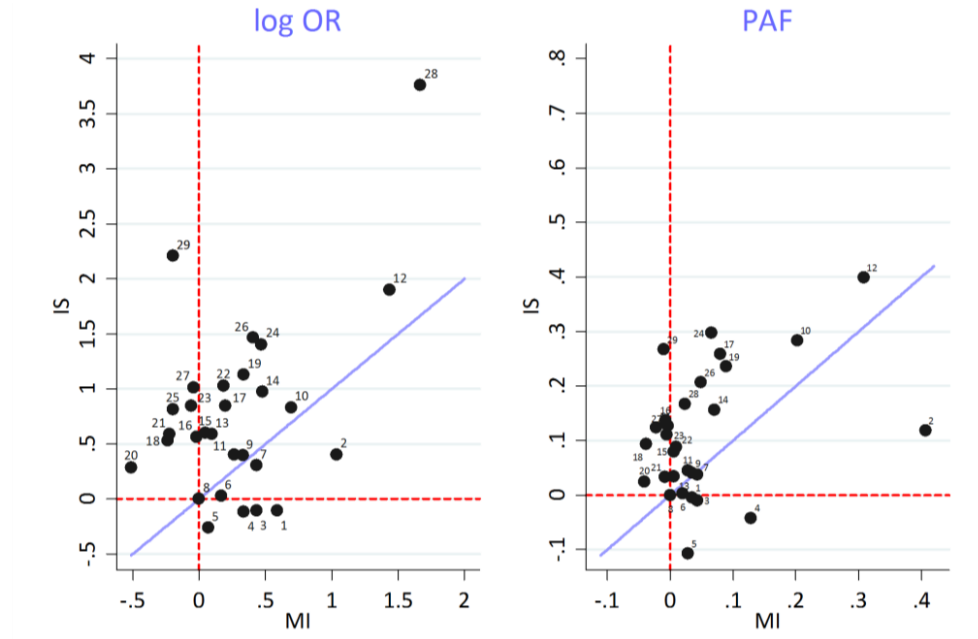
Figure 2. Population attributable fractions and corresponding confidence intervals.



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_{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.[32,33] 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.[34-37] 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.[37-39]

Ischaemic stroke is a heterogeneous disease, in which several different causal mechanisms can be discerned, as is done in the TOAST classification.[40] 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.[41-44] 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.[45] 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.[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.[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.[48,49] Second, the design of the RATIO study also dictates the use of a single control group for both OR_{MI} and OR_{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.

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.[50] 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.

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

 # = 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.

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Chapter 5

Hypercoagulability is a stronger risk factor for ischaemic stroke than for myocardial infarction: a systematic review

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Abstract

Background and Purpose: Hypercoagulability increases the risk of arterial thrombosis, however, this effect may differ between various manifestations of arterial disease.

Methods: In this study, we compared the effect of coagulation factors as measures of hypercoagulability on the risk of ischaemic stroke and myocardial infarction by performing a systematic review of the literature. The effect of a risk factor on ischaemic stroke (relative risk for ischaemic stroke, RR_{IS}) was compared with the effect on myocardial infarction (RR_{MI}) by calculating their ratio ($RRR=RR_{IS}/RR_{MI}$). A relevant differential effect was considered when RRR was >1+ its own standard error (SE) or <1-SE.

Results: We identified 70 publications, describing results from 31 study populations, accounting for 351 markers of hypercoagulability. The majority (203/351, 58%) had an RRR greater than 1. A larger effect on ischaemic stroke risk than myocardial infarction risk (RRE>1+1SE) was found in 49/343 (14%) markers. Of these, 18/49 (37%) had an RRR greater than 1+2SE. On the opposite side, a larger effect on myocardial infarction risk (RRR<1-1SE) was found in only 17/343 (5%) markers.

Conclusions: These results suggest that hypercoagulability has a more pronounced effect on the risk of ischaemic stroke than that of myocardial infarction.

Introduction

Myocardial infarction (MI) and ischaemic stroke (IS), the main manifestations of arterial thrombosis, are the most common causes of morbidity and mortality globally.[1, 2] Many risk factors are shared by both diseases because the pathophysiologic mechanism is similar: the formation of a thrombus in the arteries supplying oxygen to either the heart or the brain.[3] Platelets play a pivotal role in the formation and propagation of the thrombus, and therefore are the primary targets of antithrombotic therapy in arterial disease.[4] However, arterial thrombus formation is also determined by the activation of the coagulation cascade.[5-7] These two mechanisms work intertwined and independently: thrombin transforms fibrinogen into fibrin, but also activates platelets, both important factors in thrombus growth and stability. As a consequence, drugs that target thrombin generation (vitamin K antagonists and FXa inhibitors), thrombin's catalytic function (direct thrombin inhibitors) or thrombin's activation of platelets (PAR1 antagonists) all have the potential to inhibit arterial thrombosis.[8]

On the contrary, hypercoagulability, a condition in which the haemostatic balance is tilted towards thrombus formation, increases the risk of arterial thrombosis.[9] An increased risk of both MI and IS has been reported for high levels of FVIII, fibrinogen, plasminogen, VWF, FX and FXIII.[5] It has also been observed that some factors associated with hypercoagulability, for example elevated FXI and FXII levels, increase the risk of IS, but not that of MI.[10, 11] Recently we showed in a study of women under 50 years of age that hypercoagulability increases the risk of IS, whereas the risk of MI is only affected marginally.[12] However, it is unclear to which extent these findings truly reflect a different role of hypercoagulability in these two diseases, or whether a difference is only present in this specific patient group, for the differential effect may be limited to specific age and sex categories.[10, 11]

Differences in causal mechanisms, overall and in subgroups, are not easily recognizable because most studies only investigated one or a combination of arterial thrombosis manifestations. Some studies differentiated between MI and IS, but the results were often fragmented into several publications in different specialty journals.

If true, the hypothesis that MI and IS behave differently from a prothrombotic perspective is a strong stimulus for researches into the role of coagulation on the aetiology of IS, a field in which data are lacking compared with the equivalent of MI.[13]

Therefore, we set out to identify studies that investigated markers of hypercoagulability in association with the risk of both IS and MI, in order to compare these effects directly and to test the hypothesis that hypercoagulability has a differential effect on these two main forms of arterial thrombosis.

Methods

Literature search and study selection

We used a systematic approach to identify study populations (with both cohort and case-control study design) in which the effect of a prothrombotic factor was studied on both MI and IS. We included in our analysis only direct comparisons within the same study population to reduce bias due to differences in study design, data acquisition, data analyses and underlying research questions. The data needed for this direct comparison were obtained by a systematic and comprehensive three-stage approach.

1. Identification and selection of the publications

We searched for all publications reporting the association between a measure of coagulation and MI or IS up to July 2012 (Fig. 1; step 1). Publications were identified with a systematic search in four different search engines, PubMed (1950-2012), EMBASE (1980-2012), the Science Citation Index through Web of Science (1945-2012) and the Cochrane Library (1898-2012). The search strategy applied in each database was composed by the combination of four concepts: presence of *ischaemic stroke* or *myocardial infarction* (combined with either "AND" and "OR") *"*AND" *coagulation "*AND" *risk "*AND" *cohort* OR *case control*. These concepts were extensively searched either by the use of subject headings or free text words (S1 File). From the resulting list, publications were selected independently by two authors (AM, BS).

Publications were included when: (1) they reported original data about the association between a measure of coagulation (either coagulation factor plasma levels, activity, genetic mutation or aggregated measures) and MI or IS separately; (2) the outcomes were the clinical endpoints MI or IS as acute vascular events rather than surrogates (e.g., studies reporting only on carotid intima-media thickness or coronary artery plaque were excluded); (3) the magnitude of the association was reported as a point estimate such as odds ratio (OR), risk ratio (RR) or hazard ratio (HR), or these could be inferred from the raw data. Studies that combined several forms of stroke (e.g. transient ischaemic attack, haemorrhagic stroke, sinus thrombosis) were included as long as IS was at least part of the combined endpoint used. Review articles or previous systematic reviews were excluded but used to check for relevant publications that were not identified by the literature search.

2. Identification and selection of the studies

The selected publications were then used to identify unique study populations (Fig. 1; step 2). Publications were considered to be pertain of the same study population when it was clearly stated as such in the text (e.g., by study name) or when the publication shared the same group of participants, based on method description, inclusion procedures, number and baseline characteristics. Studies that included only MI or IS and therefore could not be used for a direct comparison were excluded.

3. Comparison of the publications from the same study and data extraction

We selected publications from the same study populations in which the risk of a prothrombotic factor could be directly compared for MI and IS (Fig. 1; step 3). This was possible when the same factor was measured in MI cases and IS cases in the same publication, or in two publications on the same study population (one on MI and one on IS) and similar analytical approaches were used for both diseases. Additionally, all reference lists were scanned to identify publications that were missed during the previous two steps. Also, key authors of each selected publication were entered in individual Pubmed searches to further identify missed publications.

Information was extracted from each selected article with a standardized form. Extracted data were: 1) study outcome (i.e. stroke of any origin, ischaemic stroke, acute myocardial infarction, angina); 2) characteristics of the marker of hypercoagulability (i.e. name, type of assay (phenotype or genotype) and the effect estimator used in the analysis); 3) study type (case-control or follow-up study); 4) magnitude of the association as a relative effect estimate (adjusted) with corresponding confidence intervals; 5) study population characteristics (i.e. number of participants, age, sex and baseline risk profile). Finally we performed a study quality assessment to assess the presence of bias that could substantially influence the results. We considered the results possibly biased if: 1) blood samples were taken in the acute phase (first month after the event), which could lead to reverse causation; 2) lack of adjustment for age and sex (for non- genetic exposures); 3) high probability of selection bias; 4) different follow-up duration between the study groups (>2 years). Studies with these characteristics were analysed separately.

Markers of hypercoagulability were categorized in markers of pro-coagulant activity, markers of anti-coagulant activity, markers of fibrinolysis and markers of platelet function and other pathways (including ADAMTS13 and von Willebrand factor).

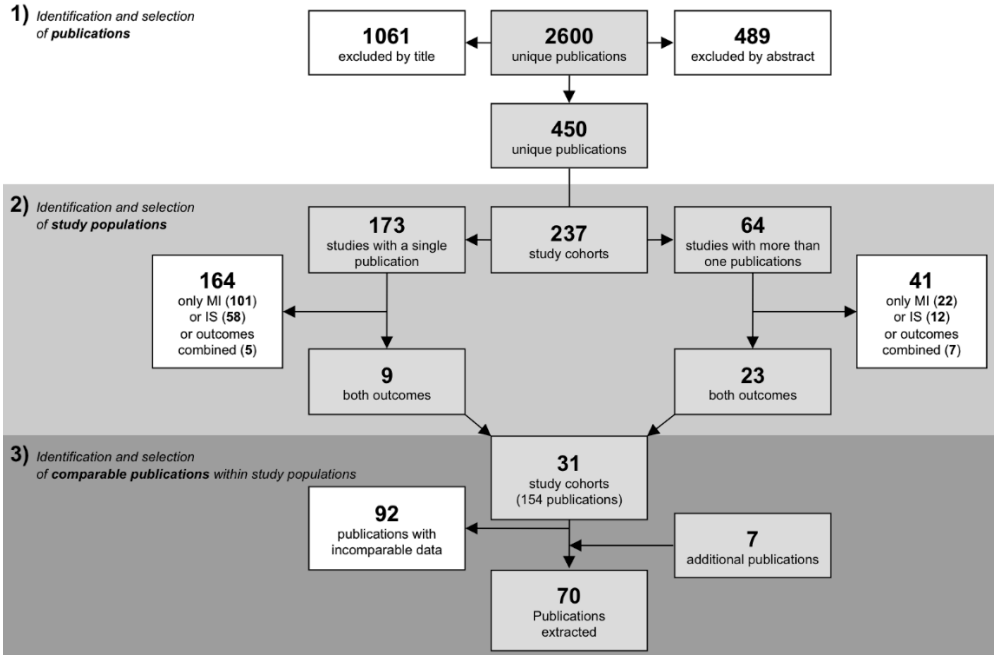
Statistical analysis

The relative risks for IS (RR_{IS}) were compared with the relative risks for MI (RR_{MI}) by calculating their ratio ($RRR=RR_{IS}/RR_{MI}$) per study with a corresponding 95% confidence intervals (CI), where the variance was based on the sum of the variances of RR_{MI} and RR_{IS}. When a risk factor has a similar effect on MI and IS (either increasing the risk, decreasing the risk or no effect), the RRR equals 1, whereas an RRR>1 indicates a greater effect on IS risk than on MI, and vice versa. Each RRR refers to one single marker and, when two different studies investigated the same coagulation marker, their RRR_S are presented separately. To filter out small variations due to chance we used the standard error of the RRR: markers with RRRs within 1+ its own standard error (SE) and 1-SE were considered to only marginally differ and to affect the risk of IS and MI equally, whereas markers with RRRs<1-SE or RRRs>1+SE were considered to have a substantially different effect.

Subgroups were based on age (younger or older than 50 years old for women and 55 for men), sex, stroke type (only ischaemic or haemorrhagic also included), baseline risk of study population (general population or patients affected by one or more diseases with a high impact on cardiovascular risk, such as atrial fibrillation, end stage renal disease or previous cardiovascular events), type of the investigated marker (phenotypic or genotypic), study design of the original publication (case-control or follow-up), and probability of bias (high or low).

Design and results of this systematic review are reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement).[14]

Fig. 1. Flow chart of the steps of data collection.



The figure shows the three steps in the data collection: (1) identification of publications which report on the effect of measures of hypercoagulability and the risk of myocardial infarction (MI) or ischaemic stroke (IS) (2) identification of study populations (3) identification of publications with comparable data. Comparable data can be found in the same publication or in two different publications.

Results

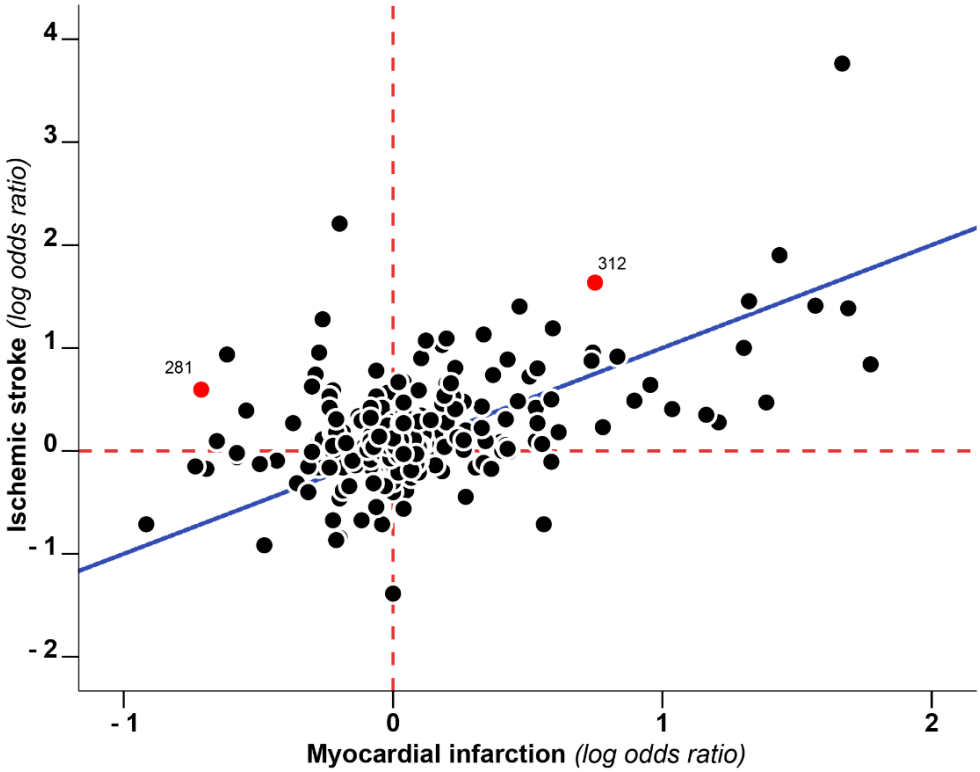
Literature search and studies selection

The first step of the search procedure yielded a total of 2600 publications (Fig. 1). Review of titles and abstracts identified 450 potentially relevant publications. With full text reading, publications from the same study group were clustered in 237 study populations (data from 64 study populations were used in more than one publication and 173 were single publication). Thirty-one of these study populations, accounting for 154 publications, included reports on effect sizes on both MI and IS separately. Supplemental Table 1 (S1 Table) shows the characteristics of these 31 study populations. Finally, 70 publications from these study populations reported comparable measures, and were eligible for data extraction.

Markers of hypercoagulability

A total of 351 markers of hypercoagulability were extracted. 203 (203/351, 58%) of those had an RRR>1, 140 (140/351, 40%) <1 and 8 (8/351, 2%) =1 (Fig. 2, S1 Fig. and S1-4 Tables for the detailed list). 205 of these markers involved pro-coagulant factors, 46 anti-coagulant factors, 63 markers of fibrinolysis and 37 markers of platelet function and other pathways. For 8 markers SE was not calculable due to lack of data. Of the remaining 343 markers, 277 (81%) had an RRR between 1-1SE and 1+1SE, indicating no large difference in the effect on the risk of IS and MI. Half of these markers (150/277, 54% for MI and 126/277, 46% for IS) did not show an effect on risk of either of the outcomes ($0.9>RR_{MI}>1.1$ and $0.9>RR_{IS}>1.1$, S1 Fig.). Of the 66 markers that were associated with either MI or IS, 49 (14% of all, 74% of those with an effect) had an RRR greater than 1+1SE, indicating a larger effect on IS risk than MI risk. Of these, 18/49 (37%) had an RRR greater than 1+2SE. The RRR of 17 (5% of all 343, 26% of those with an effect) markers of hypercoagulability was <1-1SE. There were no markers with an RRR<1-2SE (Table 1 and Fig. 3).

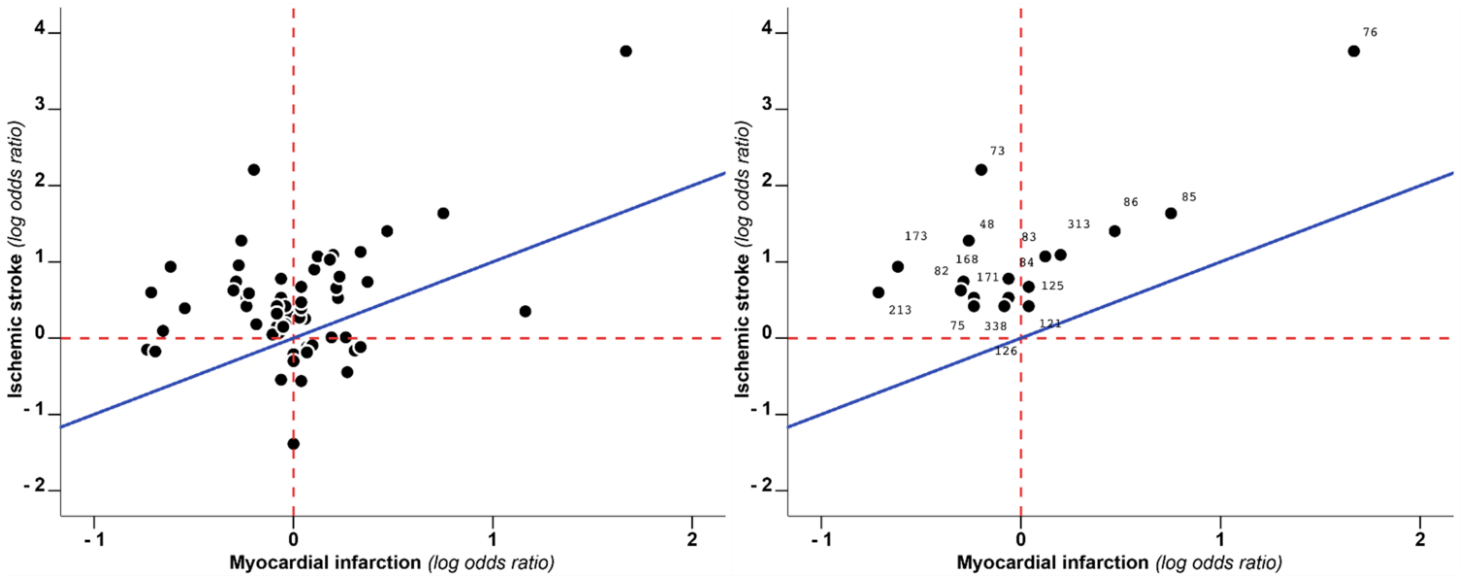
Fig. 2. Prothrombotic risk factors and their effect on myocardial infarction and ischaemic stroke.



Each point depicts the log odds ratio as a measure of effect 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.

As an explicative example red dots represent: #312: KAL-C1-INH (RR_{IS} 5.14 e RR_{MI} 2.12). #281: FXIIIA SNP rs3024462 allele (RR_{IS} 1.82 e RR_{MI} 0.49).

Fig. 3. Prothrombotic risk factors with RRR either >1+SE and <1-SE (left) and either > 1+SE and <1-SE (right).



Each point depicts the log odds ratio as a measure of effect 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. On the left are depicted RRR>1+SE and RRR<1-SE. on the right RRR>1+2SE. No factors had RRR<1-2SE. Numbers represent the ID of the corresponding marker in Table 1 and S2-4 Tables.

Pro-coagulant factors contributed for the greatest part to the difference between RRRs (pro-coagulant factors with RRR>1+1SE 30/199 (15%) and with RRR<1-1SE 6/199 (3%); anti-coagulant factors with RRR>1+1SE 4/45 (9%) and with RRR<1-1SE 4/45 (9%); factors involved in fibrinolysis with RRR>1+1SE 7/63 (11%) and with RRR<1-1SE 4/63 (6%); others factors with RRR>1+1SE 8/36 (22%) and with RRR<1-1SE 3/36 (8%)). Within pro-coagulant factors the largest RRRs, indicative of a larger effect on IS than on MI, were observed for FV Leiden mutation (RRR 3.42, 95% CI 0.11-104), a genetic variant in the gene coding for coagulation factor VIII (F8 rs6655259, RRR 4.72, 95% CI 0.62-35.73), presence of lupus anti-coagulant (RRR 8.13, 95% CI 0.61-108.76) and three variants of FXIII (F13A1 V34L, RRR 4.66, 95% CI 0.44-49.10; F13A1 T204P, RRR 11.1, 95% CI 5.64-21.82 and F13A1 rs3024462, RRR 3.71, 95% CI 0.62-22.35)

Table 1. Factors that showed a predominant association with ischaemic stroke or myocardial infarction (RRR>1+SE and RRR<1-SE).

RRR>1+SE			RRR<1-SE		
ID[1]	**Coagulation factor (contrast)**	**RRR (95% CI)**	**ID**	**Coagulation factor (contrast)**	**RRR (95% CI)**
	Pro-coagulant				
317	FXIIIA SNP Tyr204phe (dominant) *	11.1 (5.64 - 21.82)	53	fibrinopeptide A (T3 vs T1)	0.63 (0.31 - 1.26)
282	FVIII SNP 165293 rs6655259 (allele) *	4.72 (0.62 - 35.73)	301	FXIIIA SNP Pro564Leu (dominant)	0.64 (0.31 - 1.29)
331	FXIIIA SNP Val34Leu (L/L vs V/V) *	4.66 (0.44 - 49.1)	121	FGA 3807 (allele)	0.77 (0.52 - 1.12)
281	FXIIIA SNP 177424 (allele) *	3.71 (0.62 - 22.35)	123	FX SNP 9501 (allele)	0.77 (0.51 - 1.17)
294	FV Leiden (dominant)	3.42 (0.11 - 104)	284	fibrinogen (SD)	0.78 (0.61 - 0.98)
280	FVIII SNP 25167 (allele) *	2.8 (0.7 - 11.2)	128	FXI SNP 3450 (allele)	0.81 (0.59 - 1.11)
315	FXIa-C1-INH (>90 percentile)*	2.58 (0.77 - 8.72)			
313	FXIIa-C1-INH (>90 percentile) *	2.53 (0.74 - 8.6)			
105	FV Leiden (allele) *	2.44 (0.6 - 10)			
310	FXIa-AT-INH (>90 percentile) *	2.32 (0.68 - 7.95)			
104	FV SNP Rs7542281 (allele)	2.22 (0.65 - 7.56)			
307	FXIIIB SNP His95Arg (dominant) *	2.15 (0.88 - 5.25)			
64	d-dimer (SD (log scale)) *	1.88 (0.81 - 4.4)			
278	FVIII SNP 95826 (allele) *	1.81 (1.02 - 3.2)			
102	FV SNP Rs6035 (allele)	1.74 (0.56 - 5.4)			
277	FXI SNP 4197 (allele)	1.58 (0.61 - 4.1)			
276	FVIII SNP 55941 (allele)	1.5 (0.9 - 2.5)			
275	FV SNP upper 38592 (allele)	1.49 (0.69 - 3.21)			
63	fibrinogen (SD (log scale)) *	1.46 (0.96 - 2.22)			
274	FVIII SNP 139972 (allele)	1.45 (0.68 - 3.08)			
37	d-dimer (T3 vs T1)	1.44 (0.63 - 3.32)			
100	FV SNP Rs3753305 (allele)	1.39 (0.67 - 2.86)			
347	trombin generation (PEAK) (SD)	1.27 (0.83 - 1.95)			

RRR>1+SE			RRR<1-SE		
ID[1]	**Coagulation factor (contrast)**	**RRR (95% CI)**	**ID[1]**	**Coagulation factor (contrast)**	**RRR (95% CI)**
269	FXIIIA SNP 4377 (allele)	1.26 (0.93 - 1.71)			
268	FX SNP 4544 (allele)	1.25 (0.83 - 1.87)			
267	FGA 5498 (allele)	1.25 (0.84 - 1.86)			
265	FXI SNP 10942 (allele)	1.22 (0.83 - 1.78)			
264	FV SNP lower 29565 (allele)	1.21 (0.87 - 1.69)			
262	TFPI SNP 2418 (allele)	1.17 (0.9 - 1.51)			
260	FGA 251 (allele)	1.15 (0.87 - 1.52)			
	Anticoagulant				
103	prot C SNP Rs2069920 (allele)	1.92 (0.93 - 3.96)	71	thombomodulin SNP Rs3176123 (allele)	0.62 (0.3 - 1.28)
13	prot C (Q1 vs Q5) *	1.65 (1.05 - 2.6)	118	prot C receptor SNP 837 (allele)	0.74 (0.46 - 1.2)
101	prot C SNP Rs1401296 (allele) *	1.42 (0.67 - 3.03)	127	thombomodulin SNP 6235 (allele)	0.81 (0.58 - 1.13)
266	prot C SNP 11310 (allele)	1.22 (0.92 - 1.62)	130	prot C SNP 4515 (allele)	0.83 (0.63 - 1.1)
	Fibrinolysis				
314	CLT (hypo vs. normofibrinolysis) *	2.54 (0.71 - 9.09)	350	t-PA (Q4 vs Q1)	0.44 (0.17 - 1.15)
329	PAI-1 SNP 4G/5G (4G/5G vs 4G/4G)	2.12 (0.51 - 8.69)	125	t-PA SNP 30619 (allele)	0.78 (0.51 - 1.18)
20	TAFI SNP 1040C/T (CC vs TT)	1.79 (0.45 - 7.11)	129	plasminogen SNP 18114 (allele)	0.83 (0.63 - 1.11)
326	PAI-1 SNP 4G/5G (allele)	1.68 (0.45 - 6.24)	131	TAFI SNP 54691 (allele)	0.83 (0.61 - 1.12)
341	t-PA (SD)	1.54 (0.55 - 4.34)			
22	t-PA (T3 vs T1)	1.5 (0.69 - 3.27)			
62	t-PA (SD (log scale))	1.35 (0.81 - 2.25)			
	Other				
316	lupus anticoagulant (ratio >=1.15)[1] *	8.13 (0.61 - 108)	24	whole blood aggregation (Q5 vs Q1)	0.25 (0.07 - 0.93)
330	GPIb SNP thr/Met (recessive)	2.55 (0.48 - 13.69)	25	PLT aggregation (first) (Q5 vs Q1)	0.49 (0.18 - 1.31)
312	KAL-C1-INH (>90 percentile) *	2.42 (0.77 - 7.64)	70	ICAM1 SNP Rs3093030 (allele)	0.55 (0.26 - 1.17)
311	anti-beta2GP (>95 percentile)	2.33 (0.63 - 8.71)			
309	anti-prothrombin IgG (>95 percentile)	2.25 (0.38 - 13.5)			
308	ADAMTS-13 (Q1 vs Q4)	2.21 (0.65 - 7.51)			

327	GPIa SNP C807T (recessive)	1.78 (0.45 - 7.04)
23	VWF (T3 vs T1)	1.56 (0.72 - 3.34)

([1]) Normalised ratios for LA-screen and LA-confirm coagulation times. The positivity for lupus anticoagulant was considered when the ratio was 1.15 or higher, on the basis of the 99th percentile of the value recorded for 40 healthy volunteers. More details can be found in the original publication. (*) Prothrombotic factors with an RRR greater than 1+2SE. No prothrombotic factor had an RRR less than 1-2SE.

Subgroups

Table 2 shows the results of the subgroup analyses. The largest RRRs were found in young individuals (RRR>1+1SE: 17/43 (40%); RRR>1+2SE: 9/17 (53%); 5 studies). Of those RRRs, 33/43 (77%) belonged to populations of only women. However, 20 RRRs out of 43 (46%) come from a single study population (the RATIO study). After the exclusion of this study, the RRRs greater than 1+1SE became 6 (6/23, 26%) (data not shown). A substantial part of the studies (15 studies, 216 factors) combined haemorrhagic and ischaemic stroke as a single outcome. When restricted to those that excluded haemorrhagic stroke, 26 out of 130 markers (20%) had an RRR >1+1SE (RRR>1+2SE 33/84 (39%)) and 6 (6/130, 4%) an RRR <1-1SE. Larger RRRs were also found in populations at a relatively low risk of arterial thrombosis (RRR>1+1SE 29/157, 20%), and for phenotypic measurements (RRR>1+1SE 18/85, 21%), whereas only 15 RRRs were potentially affected by high risk of bias and their exclusion did not change the results. A graphical representation of relevant RRRs by subgroups is shown in the supplementary (S2 Fig.).

Table 2. Distribution of RRRs greater than 1+1SE and smaller than 1-1SE for different subgroups of population.

Subgroups	Prothrombotic markers	>1+1SE	<1-1SE
		N (%)	N (%)
Sex			
Male	32	3 (10)	2 (6)
Female	38	16 (42)	2 (5)
No distinction	273	30 (11)	13 (5)
Age at onset[1]			
Young	43	17 (40)	1 (2)
Old	300	32 (11)	16 (5)
Ischaemic stroke type			
Only ischaemic	130	26 (20)	6 (5)
Ischaemic and haemorrhagic	213	23 (11)	11 (5)
Cardiovascular risk[2]			
High	186	20 (11)	9 (5)
Low	157	29 (19)	8 (5)
Bias risk			
Low	328	46 (14)	17 (5)
High	15	2 (13)	0 (0)
Type of marker			
Phenotypic	85	18 (21)	5 (6)
Genotypic	258	31 (12)	12 (5)
Study design			
Case-control	222	34 (15)	10 (5)
Follow-up	121	15 (12)	7 (6)

([1]) Young age at onset is defined as younger than 50 years old for women and 55 for men. ([2]) Low risk for arterial thrombosis is defined as a risk comparable with the general population. High risk for arterial thrombosis is given to populations affected by one or more diseases with a high impact on cardiovascular risk (such as atrial fibrillation, end stage renal disease, previous cardiovascular event).

Discussion

We investigated whether markers of hypercoagulability have a differential role on the risk of MI and IS. This systematic review indicates that overall hypercoagulability has a larger effect on the risk of IS than on the risk of MI (14% of the 343 markers studied had an RRR>1+1SE compared with 5% of markers with RRR<1-1SE). The majority of the markers included in this study were pro-coagulant factors, in which the difference between RRRs was remarkable (15% of these factors had an RRR>1+1SE compared with only 3% with an RRR<1-1SE), whereas no difference was found for anti-coagulant factors and a small difference for factors involved in the fibrinolytic system. The differential role was more pronounced in young patients (40% of factors with RRR>1+1SE) and after the exclusion of studies that used haemorrhagic stroke and IS as a combined endpoint (20% of factors with RRR>1+1SE). Studies with young populations were only 5 and one of those (the RATIO study) accounted for half of the RRRs. However, after the exclusion of the RATIO study from the analysis, the percentage of large RRRs remained higher than that in the other subgroups (26% of factors with RRR>1+1SE). Our study is the first that has systematically summarized the data available on the relationship between hypercoagulability and the two main manifestations of arterial thrombosis. These data support the hypothesis that hypercoagulability increases the risk of IS more than that of MI.

Ischaemic stroke is a heterogeneous disease in which several causal mechanisms play a role. According to the TOAST classification, subtypes of IS can be divided in five main categories, i.e., stroke from cardioembolic origin, large vessel atherosclerosis, small vessel occlusion, stroke of other determined origin and stroke of undetermined origin.[15] All these categories have specific risk factors, such as for example atrial fibrillation for cardioembolic stroke, whereas stroke of undetermined origin has none. Stroke of undetermined origin includes a third of all strokes and half of the strokes in the young.[16-18] When the analyses were restricted to the young, the difference between MI and IS was more marked (40% of the factors had RRR greater than 1+1SE). Unfortunately, no data on TOAST classification were available in the included studies; however, we can hypothesize that the larger difference found in the young is associated with the higher incidence of stroke of undetermined origin in these patients. Notably, increasing evidence suggests that most strokes of undetermined origin are caused by covert thromboembolic events.[19] In our study we found that, whereas factors

associated with platelet activation are similarly involved in the two diseases, many markers of abnormal secondary haemostasis, such as for example FV Leiden or the presence of Lupus anticoagulant, have a greater role in the risk of IS than that of MI. These markers are known risk factors for venous thrombosis, and this supports the hypothesis that, although paroxysmal atrial fibrillation and subsequent embolization of a thrombus is undoubtedly responsible for a fraction of strokes of undetermined origin, hypercoagulability in itself should also be considered in cryptogenic strokes. Unfortunately, the lack of a disease classification in the available literature prevents the possibility to further investigate this hypothesis, and underlines the need of new studies in etiologic research, particularly for IS.[13, 20]

Some methodological issues should be considered. First, due to our within study population approach we could only include a small part of the available data on measures of coagulation and the risk of arterial thrombosis. This approach reduced the power of the study, but it is unlikely that this selection can explain our findings. Even more, our method increased the reliability of the results since it removes publication bias and bias introduced by differences in data collection, laboratory analysis, quality of data and statistical analysis between different study populations. Moreover, even if a bias was present in an original study, it is likely to have had a similar effect on the MI and IS analyses, leaving the RRR estimates unaffected (except for the null effects). A subgroup analysis restricted to studies with low evidence of bias yielded results similar to the overall findings, thereby indicating that there is a high level of similarity between the RR_{MI} and RR_{IS} when assessing the RRR. Finally, when the analysis was restricted to follow-up studies, results did not change substantially, indicating that reverse causation cannot explain the observed difference between MI and IS. A second limitation is the lack of a standard measure of precision of the RRR estimates. As a measure of variance, i.e. of standard error, we adopted the sum of the variance of the original IS estimate plus the MI estimate. This method is probably an overestimation of the true variance, because the two risk ratios included in the RRR share, at least partly, the same population, and therefore our approach may be considered conservative. This yielded confidence intervals of RRRs larger than the true ones, hence we arbitrarily predefined that RRRs 1±1SE reflected a true difference in the effect on MI and IS risk.

Third, MI and IS have approximately the same incidence in the general population; however, this is not the case in specific subgroups, for example, in patients with atrial fibrillation.[2] Unfortunately we were unable to adjust our estimates for the presence of such factors. However, when we limited our analyses to studies that included only low risk study populations, we found that the difference between risk factors for the two diseases was even more pronounced (19% of the RRR greater than 1+1SE, 157 factors, 25 study populations).

To identify new treatments to prevent arterial thrombosis, it is important that reliable data on potential risk factors are available. In literature, data on risk factors for IS are lacking compared with the equivalent data for MI, and there are very few data on specific subtypes of IS.[13] Our findings, supporting the hypothesis that IS and MI behave differently from a prothrombotic perspective, are a warning for the need of new investigations on the role of coagulation in subtypes of IS, especially in the young. The presence of coagulation markers that are risk factors specifically for IS and not for MI can have implications in the medical treatment of IS, especially in the era of the new direct oral anti-coagulants, drugs that are specific for a single coagulation factor (thrombin for dabigatran, activated FX for apixaban, edoxaban and rivaroxaban, and other direct inhibitors against FXI and FXII are in pre-clinical phase studies).[21, 22] Because of differences in the role of hypercoagulability in MI and IS, their efficacy in the prevention of the two main forms of arterial thrombosis might differ.

Supplementary

S1 File. Search strategies

PubMed

(Medical Subject Headings), Major (Major Medical Subject Headings).

((((stroke OR strokes) NOT "stroke volume") OR "Stroke"[Mesh] OR "Cerebral Stroke" OR "Cerebral Strokes" OR "Brain Vascular Accident" OR "Brain Vascular Accidents" OR "Cerebrovascular Apoplexy" OR "Cerebrovascular Stroke" OR "Cerebrovascular Strokes" OR CVA[tw] OR CVAs[tw] OR Apoplexy[tw] OR "Cerebrovascular Accident" OR "Cerebrovascular Accidents" OR "Acute Stroke" OR "Acute Strokes") AND ("Myocardial Infarction"[Mesh] OR "myocardial infarction" OR "Myocardial Infarctions" OR "Myocardial Infarct" OR "Myocardial Infarcts" OR "Myocardial Ischemia"[mesh] OR "Cardiovascular Diseases"[Mesh:NoExp] OR "coronary heart disease" OR "coronary disease") AND (coagulation OR "Blood Coagulation"[Mesh] OR "Hemostasis"[Mesh:NoExp] OR "blood clotting" OR "Blood Coagulation Factors"[Mesh] OR Blood Coagulation Tests OR Coagulants OR Hemostatics OR Partial Thromboplastin Time OR Prothrombin Time OR Thrombin Time OR Whole Blood Coagulation Time OR coagulant OR fibrinogen OR fibrinogens OR fibrinolysis) AND (risk OR risks OR "Risk factors"[mesh] OR "Risk"[mesh]) AND ("Cohort Studies"[mesh] OR cohort OR cohorts OR followup OR "follow up" OR "Epidemiologic Studies"[mesh] OR "Case-Control Studies"[mesh] OR casecontrol OR "case-control" OR "case-controlled" OR "Retrospective Studies"[mesh] OR "Longitudinal Studies"[mesh] OR "Cross-Sectional Studies"[mesh] OR "Prospective studies"[mesh] OR "Retrospective Studies" OR "Longitudinal Studies" OR "Cross-Sectional Studies" OR "Prospective studies" OR "Retrospective Study" OR "Longitudinal Study" OR "Cross-Sectional Study" OR "Prospective study" OR "Comparative Study"[Publication Type])) OR (((((stroke[ti] OR strokes[ti]) NOT "stroke volume") OR "Stroke"[Majr] OR "Cerebral Stroke"[ti] OR "Cerebral Strokes"[ti] OR "Cerebrovascular Apoplexy"[ti] OR "Cerebrovascular Stroke"[ti] OR "Cerebrovascular Strokes"[ti] OR CVA[ti] OR CVAs[ti] OR Apoplexy[ti] OR "Cerebrovascular Accident"[ti] OR "Cerebrovascular Accidents"[ti] OR "Acute Stroke"[ti] OR "Acute Strokes"[ti]) OR ("Myocardial Infarction"[Majr] OR "myocardial infarction"[ti] OR "Myocardial Infarctions"[ti] OR "Myocardial Infarct"[ti] OR "Myocardial Infarcts"[ti] OR "Myocardial Ischemia"[majr] OR "Cardiovascular Diseases"[Majr:NoExp])) AND (coagulation[ti] OR "Blood Coagulation"[Majr] OR "Hemostasis"[Majr:NoExp] OR "blood clotting"[ti] OR "Blood Coagulation Factors"[Majr] OR Coagulants[ti] OR Hemostatics[ti] OR Hemostatic[ti] OR Haemostatics[ti] OR Haemostatic[ti] OR Haemostasis[ti] OR Hemostasis[ti] OR Partial Thromboplastin Time[ti] OR Prothrombin Time[ti] OR Thrombin Time[ti] OR coagulant[ti] OR fibrinogen[ti] OR fibrinogens[ti] OR fibrinolysis[ti]) AND (risk OR risks OR "Risk factors"[mesh] OR "Risk"[mesh]) AND ("Cohort Studies"[mesh] OR cohort OR cohorts OR followup OR "follow up" OR "Epidemiologic Studies"[mesh] OR "Case-Control Studies"[mesh] OR casecontrol OR "case-control" OR "case-controlled" OR "Retrospective Studies"[mesh] OR "Longitudinal Studies"[mesh] OR "Cross-Sectional Studies"[mesh] OR "Prospective studies"[mesh] OR "Retrospective Studies" OR "Longitudinal Studies" OR "Cross-Sectional Studies" OR

"Prospective studies" OR "Retrospective Study" OR "Longitudinal Study" OR "Cross-Sectional Study" OR "Prospective study" OR "Comparative Study"[Publication Type])) **AND english[la]**

EMBASE

((*Stroke/ OR *Stroke patient/ OR Stroke.ti OR "Cerebral Strokes".ti OR "Cerebrovascular Apoplexy".ti OR "Cerebrovascular Stroke".ti OR "Cerebrovascular Strokes".ti OR CVA.ti OR CVAs.ti OR Apoplexy.ti OR "Cerebrovascular Accident".ti OR "Cerebrovascular Accidents".ti OR "Acute Stroke".ti OR "Acute Strokes".ti) OR (exp *Heart Infarction/ OR *Ischemic Heart Disease/ OR *Heart Disease/ OR "myocardial infarction".ti OR "Myocardial Infarctions".ti OR "Myocardial Infarct".ti OR "Myocardial Infarcts".ti)) AND (exp *Blood clotting/ OR exp *blood clotting factor/ OR *Hemostasis/ OR coagulation.ti OR "blood clotting".ti OR Coagulants.ti OR Hemostatics.ti OR Hemostatic.ti OR Haemostatics.ti OR Haemostatic.ti OR Haemostasis.ti OR Hemostasis.ti OR Partial Thromboplastin Time.ti OR Prothrombin Time.ti OR Thrombin Time.ti OR coagulant.ti OR fibrinogen.ti OR fibrinogens.ti OR fibrinolysis.ti) AND (exp risk factor/ OR exp risk/ OR risk*.mp) AND (cohort analysis/ OR cohort*.mp OR exp follow up/ OR followup.mp OR "follow up".mp OR Case Control Study/ OR casecontrol*.mp OR "case-control*".mp OR Retrospective Study/ OR Longitudinal Study/ OR Cross-Sectional Study/ OR Prospective study/ OR ("Retrospective Studies" OR "Longitudinal Studies" OR "Cross-Sectional Studies" OR "Prospective studies" OR "Retrospective Study" OR "Longitudinal Study" OR "Cross-Sectional Study" OR "Prospective study" OR "Comparative Study" OR "Comparative studies").mp OR Comparative Study/)

Web of Science

TI=((Stroke OR "Cerebral Strokes" OR "Cerebrovascular Apoplexy" OR "Cerebrovascular Stroke" OR "Cerebrovascular Strokes" OR CVA OR CVAs OR Apoplexy OR "Cerebrovascular Accident" OR "Cerebrovascular Accidents" OR "Acute Stroke" OR "Acute Strokes") OR ("Heart Infarction" OR "Ischemic Heart Disease*" OR "Heart Disease*" OR "myocardial infarction" OR "Myocardial Infarctions" OR "Myocardial Infarct" OR "Myocardial Infarcts")) AND TI=("Blood clotting" OR Hemostasis OR coagulation OR "blood clotting" OR Coagulants OR Hemostatics OR Hemostatic OR Haemostatics OR Haemostatic OR Haemostasis OR Hemostasis OR Partial Thromboplastin Time OR Prothrombin Time OR Thrombin Time OR coagulant OR fibrinogen OR fibrinogens OR fibrinolysis) AND TS=risk* AND TS=("cohort analysis" OR "cohort stud*" OR "follow up stud*" OR "followup stud*" OR "Case Control Stud*" OR casecontrol* OR "case-control*" OR "Retrospective Stud*" OR "Longitudinal Stud*" OR "Cross-Sectional Stud*" OR "Prospective stud*" OR "Retrospective Studies" OR "Longitudinal Studies" OR "Cross-Sectional Studies" OR "Prospective studies" OR "Retrospective Study" OR "Longitudinal Study" OR "Cross-Sectional Study" OR "Prospective study" OR "Comparative Study" OR "Comparative studies" OR "Comparative Stud*")

Cochrane

ti/ab/kw

((Stroke OR "Cerebral Strokes" OR "Cerebrovascular Apoplexy" OR "Cerebrovascular Stroke" OR "Cerebrovascular Strokes" OR CVA OR CVAs OR Apoplexy OR "Cerebrovascular Accident" OR "Cerebrovascular Accidents" OR "Acute Stroke" OR "Acute Strokes") OR ("Heart Infarction" OR "Ischemic Heart Disease*" OR "myocardial infarction" OR "Myocardial Infarctions" OR "Myocardial Infarct" OR "Myocardial Infarcts"))

all text

("Blood clotting" OR Hemostasis OR coagulation OR "blood clotting" OR Coagulants OR Hemostatics OR Hemostatic OR Haemostatics OR Haemostatic OR Haemostasis OR Hemostasis OR Partial Thromboplastin Time OR Prothrombin Time OR Thrombin Time OR coagulant OR fibrinogen OR fibrinogens OR fibrinolysis) AND risk* AND ("cohort analysis" OR "cohort stud*" OR "follow up stud*" OR "followup stud*" OR "Case Control Stud*" OR casecontrol* OR "case-control*" OR "Retrospective Stud*" OR "Longitudinal Stud*" OR "Cross-Sectional Stud*" OR "Prospective stud*" OR "Retrospective Studies" OR "Longitudinal Studies" OR "Cross-Sectional Studies" OR "Prospective studies" OR "Retrospective Study" OR "Longitudinal Study" OR "Cross-Sectional Study" OR "Prospective study" OR "Comparative Study" OR "Comparative studies" OR "Comparative Study*")

S1 Table. Characteristics of the 31 studies selected.

Name of the study[1]	Publications (N and references)	Year[2]	Study design[3]	Age[4]	Sex[5] (male%)	MI cases[6] (min-max)	IS cases[6] (min-max)	Prothrombotic markers[7]
ARIC	4 [23-25]	1987-1989	F-UP	45-64	42	368-1257	89-613	*1-14*
ATTAC	3 [26, 27]	2008	CC	18-55	37	198-271	103-150	*15-20*
British Regional Heart	2 [28, 29]	1980-1998	F-UP	40-50	100	198	187	*21-23*
Caerphilly	3 [30-32]	1979-1983	F-UP	45-59	100	54-353	26-156	*24-39*
Cardiovascular Health	4 [33-36]	1989	F-UP	> 65	39	158-494	115-442	*40-45*
Copenhagen City Heart	2 [37, 38]	1976-1978	F-UP	20-95	43	469-720	410-614	*46-51*
Dai K et al	1 [39]	2000	CC	54	50	49-103	107-150	*52*
Edinburg Artery	2 [40, 41]	1987	F-UP	55-74	52	166-248	45-168	*53-64*
Engstrom G et al	1 [42]	1973-1974	F-UP	47	100	611	238	*65*
Finrisk '92	2 [43, 44]	1992	F-UP	45-64	48	133	75	*66-69*
Finrisk '92-'97	1 [45]	1992-1997	F-UP	57	72	401	149	*70-105*
Framingham	1 [46]	1948	F-UP	47-79	44	214	92	*106,107*
Gonzalez-Conejero R et al	3 [47-49]	1998	CC	18-90	62	101	104	*108-111*
Group Health Cooperative	1 [50]	1995-2002	CC	30-79	39	856	368	*112-282*
Health ABC	1 [51]	1997-1998	F-UP	70-79	49	177	104	*283*
Health Survey for England	1 [52]	1994-2004	F-UP	55	45	102	126	*284*
Karakus Z et al	1 [53]	2005	CC	<50	60	63	22	*285, 286*

Northern Sweden Health and Disease	2 [54, 55]	1985-1999	F-UP	30-60	62	78	108	*287, 288*
Northwick park Heart II	1 [11]	1989	F-UP	50-60	100	231	56	*289-292*
Onohara T et al	1 [56]	1985-1995	F-UP	69	81	36	32	*293*
Pestana CI et al	1 [57]	2005-2007	CC	58	51	175	54	*294*
Physicians' Health	3 [58-60]	1984	F-UP	40-84	100	374-404	209-259	*295-297*
RATIO	9 [61-68]	1990-1995	CC	18-49	0	248	203	*298-317*
Roosendaal FR et al	8 [69-74]	1991-1995	CC	18-44	0	79-84	104-107	*318-331*
Rotterdam	4 [75-78]	1990	F-UP	>55	40	115-473	112-290	*332-335*
Ruan C et al	1 [79]	2003	CC	na	na	103	150	*336*
Santamaria A et al	2 [80]	1998-2003	CC	21-80	57	174	205	*337*
Smith FB et al	1 [81]	1989-1990	F-UP	65	64	160	79	*338-341*
Three-City	2 [82, 83]	1999-2001	F-UP	>65	39	88-94	87-90	*342-347*
TPT	1 [84]	1983-1992	F-UP	45-69	100	1515	391	*348, 349*
Women's Health Initiative	2 [85, 86]	1992	F-UP	50-79	0	304	972	*350, 351*

MI: myocardial infarction; IS: ischaemic stroke; na: information not available. ([1]) Name of the study; when this was not available, the name of the first author of the first publication is displayed. ([2]) Inclusion period, when this was not available, the year of the first publication is displayed. ([3]) F-UP: follow-up study cohort; CC: case-control study. ([4]) Age at inclusion; range, or cut off. If criteria were not available, the mean baseline age as displayed in the earliest publication. ([5]) Percentages of males reported in the first publication. In follow-up studies we report the percentage of male in the entire cohort; in case-control studies that in the control group. ([6]) Numbers show the minimum and maximum sample size of cases included in each study, and may vary over time or analyses. ([7]) Numbers show the ID of the marker. For the entire list of markers of prothrombotic state, refer to S2-4 Tables.

S2 Table. Phenotypic measurements sorted alphabetically.

ID	Factor (contrast)	RR MI	RR IS	RRR (95% CI)
308	ADAMTS13 (Q1 vs Q4)	1.40	3.10	2.21 (0.65 - 7.51)
24	aggregation (whole blood) (Q5 vs Q1)	1.00	0.25	0.25 (0.07 - 0.93)
14	alpha2 antiplasmin (Q4 vs Q1)	0.81	1.36	1.68 (0.39 - 7.21)
311	anti-beta2GP (>95 percentile)	1.20	2.80	2.33 (0.63 - 8.71)
298	anti-cardiolipin IgG (>95 percentile)	1.80	0.90	0.5 (0.11 - 2.25)
309	anti-prothrombin IgG (>95 percentile)	0.80	1.80	2.25 (0.38 - 13.5)
30	APC ratio (T3 vs T1)	0.70	0.73	1.04 (0.42 - 2.59)
38	aPTT (T3 vs T1)	0.76	1.11	1.46 (0.59 - 3.62)
335	aPTT (for Protein C) (Q1 vs Q5)	1.53	2.43	1.59 (0.35 - 7.26)
39	bleeding time (T1 vs T3)	0.90	1.42	1.58 (0.19 - 12.93)
16	CLT (fibrinolytic potential) (>90 percentile)	2.60	1.90	0.73 (0.24 - 2.26)
300	CLT (hyperfibrinolysis vs. normofibrinolysis) (T3 vs T2)	2.82	1.50	0.53 (0.16 - 1.8)
314	CLT (hypofibrinolysis vs. normofibrinolysis) (T1 vs T2)	1.60	4.07	2.54 (0.71 - 9.09)
344	d-dimer (Q5 vs Q1)	1.49	1.09	0.73 (0.12 - 4.38)
351	d-dimer (Q4 vs Q1)	1.70	1.52	0.89 (0.39 - 2.06)
57	d-dimer (T3 vs T1)	1.59	1.62	1.02 (0.43 - 2.41)
21	d-dimer (T3 vs T1)	1.39	1.56	1.12 (0.52 - 2.42)
333	d-dimer (high vs low)	2.10	2.60	1.24 (0.27 - 5.63)
340	d-dimer (SD)	1.02	1.27	1.25 (0.72 - 2.16)
37	d-dimer (T3 vs T1)	1.45	2.09	1.44 (0.63 - 3.32)
64	d-dimer (SD (log scale))	1.04	1.96	1.88 (0.81 - 4.4)
28	F1+2 fragment (T3 vs T1)	1.03	0.96	0.93 (0.35 - 2.46)
58	F1+2 fragment (T3 vs T1)	0.92	1.00	1.09 (0.45 - 2.64)
1	fibrinogen (Q4 vs Q1)	2.18	1.26	0.58
106	fibrinogen (T1 vs T3)	0.62	0.40	0.65
342	fibrinogen (Q5 vs Q1)	2.45	1.63	0.67 (0.14 - 3.25)
293	fibrinogen (>300 mg/dl)	3.68	2.72	0.74 (0.11 - 5.05)
284	fibrinogen (SD)	1.30	1.01	0.78 (0.61 - 0.98)
348	fibrinogen (SD (log scale))	1.52	1.36	0.89 (0.65 - 1.24)
339	fibrinogen (SD)	1.13	1.14	1.01 (0.68 - 1.51)
69	fibrinogen (SD)	1.02	1.05	1.03 (0.69 - 1.53)
65	fibrinogen (Q4 vs Q1)	2.30	2.50	1.09 (0.61 - 1.93)
31	fibrinogen (T3 vs T1)	1.26	1.51	1.2 (0.56 - 2.55)
61	fibrinogen (T3 vs T1)	1.66	2.06	1.24 (0.56 - 2.75)
63	fibrinogen (SD (log scale))	1.04	1.52	1.46 (0.96 - 2.22)
107	fibrinogen (T1 vs T3)	0.56	0.94	1.68
53	fibrinopeptide A (T3 vs T1)	1.36	0.85	0.63 (0.31 - 1.26)
11	FII (Q4 vs Q1)	0.89	1.34	1.51 (0.32 - 7.04)
4	FIX (Q4 vs Q1)	0.93	0.96	1.03 (0.27 - 3.92)
6	FV (Q4 vs Q1)	1.22	1.59	1.3 (0.34 - 5.02)

55	FVII (T3 vs T1)	1.12	0.89	0.79 (0.31 - 2.05)
59	FVII (SD (log scale))	0.98	1.08	1.1 (0.63 - 1.93)
67	FVII:ag (SD)	1.11	1.01	0.91 (0.57 - 1.44)
68	FVII:c (SD)	1.01	0.93	0.92 (0.58 - 1.47)
3	FVII:c (Q4 vs Q1)	1.00	1.00	1.00
36	FVII:c (T3 vs T1)	0.65	0.91	1.4 (0.54 - 3.6)
349	FVII:c (unit log) (SD (log scale))	0.98	1.07	1.09 (0.74 - 1.6)
41	FVIII:c (SD)	1.20	1.16	0.97 (0.74 - 1.26)
42	FVIII:c (SD)	1.13	1.15	1.02 (0.78 - 1.32)
12	FVIII:c (Q4 vs Q1)	1.22	1.93	1.58
29	FVIII:c (T3 vs T1)	1.12	1.10	0.98 (0.4 - 2.41)
8	FX (Q4 vs Q1)	0.61	0.88	1.44 (0.37 - 5.66)
5	FXI (Q4 vs Q1)	1.27	1.62	1.28 (0.33 - 4.86)
290	FXIa-AT-INH (T3 vs T1)	1.31	1.22	0.93 (0.23 - 3.82)
310	FXIa-AT-INH (>90 percentile)	0.94	2.18	2.32 (0.68 - 7.95)
292	FXIa-C1-INH (T3 vs T1)	1.05	1.51	1.44 (0.41 - 5.02)
315	FXIa-C1-INH (>90 percentile)	1.13	2.92	2.58 (0.77 - 8.72)
2	FXII (Q4 vs Q1)	1.17	1.16	0.99 (0.27 - 3.7)
291	FXIIa-C1-INH (T3 vs T1)	0.73	0.86	1.18 (0.37 - 3.77)
313	FXIIa-C1-INH (>90 percentile)	0.74	1.87	2.53 (0.74 - 8.6)
289	KAL-C1-INH (T3 vs T1)	0.73	0.67	0.92 (0.28 - 2.99)
312	KAL-C1-INH (>90 percentile)	2.12	5.14	2.42 (0.77 - 7.64)
316	lupus anticoagulant (ratio >=1.15)	5.30	43.10	8.13 (0.61 - 108.76)
288	PAI-1 (Q4 vs Q1)	3.35	1.32	0.39 (0.08 - 1.88)
34	PAI-1 (T3 vs T1)	1.30	1.61	1.24 (0.49 - 3.13)
66	plasminogen (SD)	1.41	1.10	0.78 (0.42 - 1.44)
9	plasminogen (Q4 vs Q1)	0.81	1.20	1.48 (0.34 - 6.4)
25	PLT aggregation (first) (Q5 vs Q1)	1.31	0.64	0.49 (0.18 - 1.31)
33	PLT aggregation (irreversible) (high vs low)	1.04	1.26	1.21 (0.47 - 3.15)
35	PLT retention (Q5 vs Q1)	0.80	1.05	1.31 (0.39 - 4.43)
13	prot C (Q1 vs Q5)	0.92	1.52	1.65 (1.05 - 2.6)
10	prot C (high) (Q4 vs Q1)	1.03	1.54	1.50
26	TAT (T3 vs T1)	0.97	0.71	0.73 (0.27 - 1.95)
287	t-PA (Q4 vs Q1)	5.89	2.32	0.39 (0.07 - 2.07)
350	t-PA (Q4 vs Q1)	3.20	1.42	0.44 (0.17 - 1.15)
56	t-PA (T3 vs T1)	1.80	1.65	0.92 (0.38 - 2.22)
32	t-PA (T3 vs T1)	1.10	1.33	1.21 (0.54 - 2.73)
62	t-PA (SD (log scale))	1.25	1.69	1.35 (0.81 - 2.25)
22	t-PA (T3 vs T1)	0.92	1.38	1.5 (0.69 - 3.27)
341	t-PA (SD)	1.04	1.60	1.54 (0.55 - 4.34)
345	trombin generation (PEAK) (SD)	1.71	1.31	0.77 (0.38 - 1.53)
346	trombin generation (PEAK) (SD)	1.04	1.31	1.26 (0.76 - 2.1)
347	trombin generation (PEAK) (SD)	1.03	1.31	1.27 (0.83 - 1.95)
54	VWF (T3 vs T1)	1.53	1.02	0.67 (0.31 - 1.44)
343	VWF (Q5 vs Q1)	1.52	1.06	0.7 (0.16 - 3.06)

27	VWF (T3 vs T1)	1.09	0.97	0.89 (0.43 - 1.86)
332	VWF (Q4 vs Q1)	1.39	1.25	0.9 (0.41 - 1.97)
338	VWF (SD)	1.04	0.97	0.93 (0.65 - 1.34)
60	VWF (SD (log scale))	0.95	1.15	1.21 (0.76 - 1.92)
7	VWF (Q4 vs Q1)	1.21	1.71	1.41
23	VWF (T3 vs T1)	1.24	1.93	1.56 (0.72 - 3.34)
304	VWF (Q4 vs Q1)	4.20	6.70	1.6 (0.4 - 6.38)

ID, identification number; RR IS, relative risk for ischaemic stroke; RR MI, relative risk for myocardial infarction; RRR relative risk ratio.

S3 Table. Genotypic measurements sorted alphabetically.

ID	Factor(contrast)	RR MI	RR IS	RRR (95% CI)
184	antithrombin SNP 1734 (allele)	1.02	1.01	0.99 (0.64 - 1.54)
244	antithrombin SNP 2415 (allele)	1.00	1.11	1.11 (0.75 - 1.65)
261	antithrombin SNP 5403 (allele)	0.91	1.06	1.16 (0.54 - 2.51)
173	antithrombin SNP 7199 (allele)	1.05	1.01	0.96 (0.62 - 1.49)
226	antithrombin SNP 9089 (allele)	1.01	1.08	1.07 (0.81 - 1.41)
260	FGA 251 (allele)	0.93	1.07	1.15 (0.87 - 1.52)
121	FGA 3807 (allele)	1.07	0.82	0.77 (0.52 - 1.12)
267	FGA 5498 (allele)	0.92	1.15	1.25 (0.84 - 1.86)
194	FGA 6534 (allele)	0.96	0.97	1.01 (0.77 - 1.33)
162	FGA 9205 (allele)	0.99	0.94	0.95 (0.66 - 1.37)
299	FGA SNP Thr312Ala rs6050 (allele)	0.82	0.43	0.52 (0.15 - 1.8)
212	FGB 1083 (allele)	0.97	1.01	1.04 (0.71 - 1.52)
177	FGB 11079 (allele)	1.02	0.99	0.97 (0.73 - 1.28)
139	FGB 1643 (allele)	1.02	0.91	0.89 (0.64 - 1.24)
134	FGB 9487 (allele)	1.00	0.87	0.87 (0.62 - 1.23)
306	FGB SNP 455G/A rs1800790 (allele)	0.98	1.76	1.8 (0.32 - 10.06)
183	FGG 129 (allele)	0.94	0.93	0.99 (0.73 - 1.34)
255	FGG 5836 (allele)	1.13	1.28	1.13 (0.57 - 2.24)
148	FGG 902 (allele)	1.10	1.02	0.93 (0.7 - 1.22)
234	FGG 9340 (allele)	0.94	1.02	1.09 (0.81 - 1.46)
296	FII SNP C148T (or G455A) (allele)	1.10	0.93	0.85 (0.45 - 1.6)
227	FII SNP 21239 (allele)	1.00	1.07	1.07 (0.82 - 1.4)
253	FII SNP 280 (allele)	0.94	1.06	1.13 (0.7 - 1.82)
259	FII SNP 3696 (allele)	1.03	1.18	1.15 (0.51 - 2.58)
252	FII SNP 4992 (allele)	1.03	1.16	1.13 (0.83 - 1.52)
257	FII SNP 5389 (allele)	1.01	1.15	1.14 (0.86 - 1.51)
215	FII SNP 5467 (allele)	1.09	1.14	1.05 (0.72 - 1.52)
214	FII SNP 7530 (allele)	1.10	1.15	1.05 (0.73 - 1.5)
319	FII SNP G20210A (dominant)	4.00	1.60	0.4 (0 - 77.58)
48	FII SNP G20210A (dominant)	1.70	1.10	0.65 (0.19 - 2.2)
285	FII SNP G20210A (dominant)	5.42	4.00	0.74 (0 - 112.99)
303	FII SNP G20210A (dominant)	1.00	1.00	1 (0.12 - 8.33)
242	FII SNP G20210A (allele)	1.39	1.54	1.11 (0.41 - 3.03)
297	FII SNP G20210A (dominant)	0.80	1.10	1.38 (0.31 - 6.05)
45	FII SNP G20210A (dominant)	0.88	1.40	1.59 (0.16 - 15.37)
152	FIX SNP 10948 (allele)	1.08	1.01	0.94 (0.4 - 2.2)
114	FIX SNP 12806 (allele)	1.54	0.99	0.64 (0.24 - 1.74)
250	FIX SNP 16171 (allele)	0.76	0.85	1.12 (0.44 - 2.84)
235	FIX SNP 21554 (allele)	0.99	1.08	1.09 (0.66 - 1.8)
185	FIX SNP 21975 (allele)	0.96	0.96	1 (0.6 - 1.66)
240	FIX SNP 27226 (allele)	1.04	1.15	1.11 (0.62 - 1.97)
124	FIX SNP 30893 (allele)	0.82	0.63	0.77 (0.28 - 2.08)

241	FIX SNP 35124 (allele)	1.04	1.15	1.11 (0.64 - 1.92)
136	FIX SNP 4135 (allele)	1.17	1.02	0.87 (0.55 - 1.39)
246	FIX SNP 6347 (allele)	0.94	1.05	1.12 (0.68 - 1.84)
223	FIX SNP 716 (allele)	1.00	1.06	1.06 (0.66 - 1.71)
318	FV Leiden (dominant)	2.40	0.00	#NULL!
46	FV Leiden (dominant)	0.81	0.42	0.52 (0.14 - 1.88)
47	FV Leiden (dominant)	0.80	0.51	0.64 (0.08 - 5)
295	FV Leiden (dominant)	1.50	1.00	0.67 (0.14 - 3.13)
49	FV Leiden (dominant)	0.83	0.68	0.82 (0.38 - 1.77)
50	FV Leiden (dominant)	0.91	0.88	0.97 (0.31 - 2.98)
51	FV Leiden (dominant)	0.85	0.96	1.13 (0.41 - 3.11)
286	FV Leiden (dominant)	3.75	4.28	1.14 (0.05 - 24.1)
334	FV Leiden (dominant)	0.77	1.12	1.45 (0.16 - 13.07)
305	FV Leiden (dominant)	1.10	1.80	1.64 (0.45 - 6)
105	FV Leiden (allele)	1.22	2.98	2.44 (0.6 - 10)
294	FV Leiden (dominant)	0.76	2.60	3.42 (0.11 - 104)
149	FV SNP 17557 (allele)	1.04	0.97	0.93 (0.7 - 1.25)
264	FV SNP lower 29565 (allele)	0.96	1.16	1.21 (0.87 - 1.69)
239	FV SNP lower 30539 (allele)	1.05	1.16	1.1 (0.72 - 1.7)
176	FV SNP lower 3578 (allele)	1.02	0.99	0.97 (0.74 - 1.27)
182	FV SNP lower 35788 (allele)	1.15	1.13	0.98 (0.58 - 1.65)
74	FV SNP Rs2269648 (allele)	1.11	0.86	0.77 (0.38 - 1.57)
87	FV SNP Rs2420369 (allele)	1.17	1.26	1.08 (0.51 - 2.28)
100	FV SNP Rs3753305 (allele)	0.98	1.36	1.39 (0.67 - 2.86)
96	FV SNP Rs6013 (allele)	1.12	1.41	1.26 (0.47 - 3.37)
82	FV SNP Rs6019 (allele)	1.13	1.13	1 (0.21 - 4.68)
97	FV SNP Rs6030 (allele)	1.11	1.41	1.27 (0.61 - 2.66)
102	FV SNP Rs6035 (allele)	1.26	2.19	1.74 (0.56 - 5.4)
104	FV SNP Rs7542281 (allele)	1.11	2.46	2.22 (0.65 - 7.56)
72	FV SNP Rs9332575 (allele)	1.05	0.68	0.65 (0.28 - 1.5)
84	FV SNP Rs9332590 (allele)	1.03	1.10	1.07 (0.55 - 2.08)
95	FV SNP Rs9332591 (allele)	1.14	1.39	1.22 (0.51 - 2.92)
90	FV SNP Rs9332618 (allele)	0.93	1.02	1.1 (0.51 - 2.38)
92	FV SNP Rs9332640 (allele)	1.22	1.40	1.15 (0.52 - 2.55)
76	FV SNP Rs9332695 (allele)	0.85	0.71	0.84 (0.24 - 2.85)
91	FV SNP Rs970741 (allele)	1.04	1.19	1.14 (0.55 - 2.4)
275	FV SNP upper 38592 (allele)	1.03	1.53	1.49 (0.69 - 3.21)
147	FV SNP upper 42713 (allele)	1.10	1.02	0.93 (0.7 - 1.23)
180	FV SNP upper 45765 (allele)	1.00	0.98	0.98 (0.56 - 1.71)
219	FV SNP upper 45888 (allele)	0.91	0.96	1.05 (0.78 - 1.42)
113	FV SNP upper 46058 (allele)	1.74	1.07	0.61 (0.21 - 1.83)
137	FV SNP upper 66464 (allele)	1.06	0.94	0.89 (0.51 - 1.53)
169	FV SNP upper 66872 (allele)	0.94	0.90	0.96 (0.68 - 1.35)
217	FV SNP upper 68717 (allele)	0.97	1.02	1.05 (0.8 - 1.37)
116	FV SNP upper 72877 (allele)	1.20	0.82	0.68 (0.35 - 1.32)
271	FVII SNP 115 (allele)	1.07	1.40	1.31 (0.28 - 6.14)

222	FVII SNP 15386 (allele)	1.00	1.06	1.06 (0.7 - 1.61)
112	FVII SNP 16826 (allele)	1.75	0.49	0.28 (0.03 - 2.4)
170	FVII SNP 18311 (allele)	0.97	0.93	0.96 (0.73 - 1.26)
198	FVII SNP 185 (allele)	0.95	0.97	1.02 (0.74 - 1.4)
192	FVII SNP 2643 (allele)	0.99	1.00	1.01 (0.76 - 1.34)
274	FVIII SNP 139972 (allele)	0.83	1.20	1.45 (0.68 - 3.08)
282	FVIII SNP 165293 rs6655259 (allele)	0.54	2.55	4.72 (0.62 - 35.73)
280	FVIII SNP 25167 (allele)	0.75	2.10	2.8 (0.7 - 11.2)
276	FVIII SNP 55941 (allele)	1.04	1.56	1.5 (0.9 - 2.5)
278	FVIII SNP 95826 (allele)	0.94	1.70	1.81 (1.02 - 3.2)
143	FVIII SNP 95910 (allele)	1.28	1.16	0.91 (0.42 - 1.96)
161	FX SNP 11962 (allele)	0.99	0.94	0.95 (0.73 - 1.24)
258	FX SNP 14881 (allele)	0.97	1.11	1.14 (0.79 - 1.65)
164	FX SNP 16893 (allele)	1.00	0.95	0.95 (0.74 - 1.22)
228	FX SNP 17396 (allele)	0.98	1.05	1.07 (0.8 - 1.44)
172	FX SNP 18352 (allele)	1.02	0.98	0.96 (0.64 - 1.45)
279	FX SNP 22739 (allele)	0.69	1.31	1.9 (0.21 - 16.96)
168	FX SNP 26242 (allele)	0.92	0.88	0.96 (0.66 - 1.38)
268	FX SNP 4544 (allele)	0.96	1.20	1.25 (0.83 - 1.87)
197	FX SNP 8946 (allele)	0.99	1.01	1.02 (0.78 - 1.33)
123	FX SNP 9501 (allele)	0.99	0.76	0.77 (0.51 - 1.17)
265	FXI SNP 10942 (allele)	1.06	1.29	1.22 (0.83 - 1.78)
202	FXI SNP 20423 (allele)	0.91	0.93	1.02 (0.72 - 1.46)
205	FXI SNP 228771 (allele)	1.00	1.03	1.03 (0.78 - 1.35)
165	FXI SNP 25455 (allele)	1.01	0.96	0.95 (0.71 - 1.26)
238	FXI SNP 26011 (allele)	1.06	1.17	1.1 (0.75 - 1.62)
128	FXI SNP 3450 (allele)	1.00	0.81	0.81 (0.59 - 1.11)
166	FXI SNP 3543 (allele)	1.04	0.99	0.95 (0.73 - 1.24)
277	FXI SNP 4197 (allele)	0.96	1.52	1.58 (0.61 - 4.1)
248	FXI SNP 6783 (allele)	1.10	1.23	1.12 (0.69 - 1.81)
337	FXII SNP (dominant)	4.80	4.10	0.85 (0.06 - 12.73)
122	FXII SNP 6570 (allele)	1.16	0.89	0.77 (0.34 - 1.75)
195	FXII SNP 7532 (allele)	1.04	1.06	1.02 (0.77 - 1.34)
247	FXIIIA SNP 148318 (allele)	1.02	1.14	1.12 (0.8 - 1.55)
140	FXIIIA SNP 165306 (allele)	1.12	1.01	0.9 (0.65 - 1.25)
157	FXIIIA SNP 165399 (allele)	1.04	0.98	0.94 (0.71 - 1.26)
144	FXIIIA SNP 170779 (allele)	1.12	1.02	0.91 (0.64 - 1.29)
167	FXIIIA SNP 176866 (allele)	1.08	1.03	0.95 (0.68 - 1.34)
281	FXIIIA SNP 177424 rs3024462 (allele)	0.49	1.82	3.71 (0.62 - 22.35)
178	FXIIIA SNP 177778 (allele)	1.12	1.09	0.97 (0.57 - 1.67)
269	FXIIIA SNP 4377 (allele)	0.92	1.16	1.26 (0.93 - 1.71)
115	FXIIIA SNP 72060 (allele)	1.00	0.67	0.67 (0.27 - 1.66)
301	FXIIIA SNP Pro564Leu (dominant)	1.40	0.89	0.64 (0.31 - 1.29)
324	FXIIIA SNP Pro564Leu (dominant)	0.80	0.99	1.24 (0.33 - 4.63)
328	FXIIIA SNP Tir204Phe (dominant)	1.02	1.95	1.91 (0.2 - 18.29)
317	FXIIIA SNP Tyr204phe (dominant)	0.82	9.10	11.1 (5.64 - 21.82)

302	FXIIIA SNP Val34Leu (dominant)	1.07	0.77	0.72 (0.36 - 1.43)
111	FXIIIA SNP Val34Leu (dominant)	1.00	1.33	1.33 (0.62 - 2.84)
325	FXIIIA SNP Val34Leu (Val/Leu vs Val/Val)	0.80	1.19	1.49 (0.4 - 5.5)
331	FXIIIA SNP Val34Leu (Leu/Leu vs Val/Val)	0.77	3.59	4.66 (0.44 - 49.1)
224	FXIIIB SNP 17686 (allele)	1.08	1.15	1.06 (0.78 - 1.45)
181	FXIIIB SNP 29759 (allele)	1.11	1.09	0.98 (0.75 - 1.28)
179	FXIIIB SNP 5995 (allele)	0.92	0.90	0.98 (0.75 - 1.28)
225	FXIIIB SNP 7319 (allele)	0.90	0.96	1.07 (0.69 - 1.66)
141	FXIIIB SNP 9706 (allele)	1.05	0.95	0.9 (0.64 - 1.29)
307	FXIIIB SNP His95Arg (dominant)	0.79	1.70	2.15 (0.88 - 5.25)
327	GPIa SNP C807T (recessive)	1.26	2.24	1.78 (0.45 - 7.04)
322	GPIa SNP glu/Lys (recessive)	1.06	0.96	0.91 (0.18 - 4.58)
330	GPIb SNP thr/Met (recessive)	0.58	1.48	2.55 (0.48 - 13.69)
109	GPIb-alpha SNP HPA-2 (allele)	2.09	2.40	1.15 (0.23 - 5.65)
110	GPIb-alpha VNTR (allele)	1.71	2.23	1.3 (0.31 - 5.43)
320	GPIIb SNP ile/Ser (recessive)	1.85	1.20	0.65 (0.18 - 2.37)
321	GPIIIa SNP Leu/pro (recessive)	1.14	1.01	0.89 (0.24 - 3.27)
88	ICAM1 SNP Rs281432 (allele)	1.22	1.32	1.08 (0.51 - 2.3)
70	ICAM1 SNP Rs3093030 (allele)	1.04	0.57	0.55 (0.26 - 1.17)
85	ICAM1 SNP Rs3093032 (allele)	1.08	1.16	1.07 (0.48 - 2.42)
94	ICAM1 SNP Rs5030341 (allele)	1.25	1.52	1.22 (0.57 - 2.59)
81	ICAM1 SNP Rs5030347 (allele)	0.97	0.97	1 (0.93 - 1.08)
98	ICAM1 SNP Rs5030390 (allele)	1.23	1.59	1.29 (0.3 - 5.61)
199	PAI-1 SNP 10381 (allele)	0.94	0.96	1.02 (0.63 - 1.66)
191	PAI-1 SNP 12219 (allele)	1.01	1.02	1.01 (0.79 - 1.3)
190	PAI-1 SNP 4588 (allele)	1.01	1.02	1.01 (0.68 - 1.49)
323	PAI-1 SNP 4G/5G (4G/4G vs 5G/5G)	0.40	0.49	1.23 (0.24 - 6.14)
326	PAI-1 SNP 4G/5G (allele)	0.50	0.84	1.68 (0.45 - 6.24)
329	PAI-1 SNP 4G/5G (4G/5G vs 4G/4G)	0.52	1.10	2.12 (0.51 - 8.69)
283	PAI-1 SNP 4G/5G promotor (4G/4G vs 5G/5G)	0.96	0.49	0.51 (0.11 - 2.4)
44	PAI-1 SNP 4G/5G promotor (allele)	0.93	1.10	1.18 (0.55 - 2.53)
218	PAI-1 SNP 5878 (allele)	0.95	1.00	1.05 (0.75 - 1.47)
245	PAI-1 SNP 664 (allele)	0.99	1.10	1.11 (0.84 - 1.48)
237	plasminogen SNP 1470 (allele)	1.00	1.10	1.1 (0.77 - 1.58)
251	plasminogen SNP 15255 (allele)	0.96	1.08	1.13 (0.87 - 1.46)
131	plasminogen SNP 18114 (allele)	1.21	1.01	0.83 (0.63 - 1.11)
155	plasminogen SNP 1983 (allele)	1.02	0.96	0.94 (0.67 - 1.33)
220	plasminogen SNP 2967 (allele)	1.04	1.10	1.06 (0.81 - 1.38)
203	plasminogen SNP 31439 (allele)	0.83	0.85	1.02 (0.75 - 1.39)
272	plasminogen SNP 34158 (allele)	1.20	1.58	1.32 (0.7 - 2.49)
146	plasminogen SNP 406 (allele)	0.97	0.89	0.92 (0.7 - 1.2)
236	plasminogen SNP 41108 (allele)	0.92	1.01	1.1 (0.77 - 1.57)
120	plasminogen SNP 41494 (allele)	1.15	0.87	0.76 (0.31 - 1.87)

153	plasminogen SNP 54925 (allele)	1.09	1.02	0.94 (0.51 - 1.72)
273	prot C receptor SNP 3600 (allele)	0.74	0.99	1.34 (0.6 - 2.97)
175	prot C receptor SNP 6196 (allele)	0.98	0.95	0.97 (0.73 - 1.28)
118	prot C receptor SNP 837 (allele)	1.00	0.74	0.74 (0.46 - 1.2)
196	prot C receptor SNP rs1415772 (allele)	1.02	1.04	1.02 (0.74 - 1.4)
208	prot C SNP 10454 (allele)	0.93	0.96	1.03 (0.74 - 1.44)
266	prot C SNP 11310 (allele)	0.95	1.16	1.22 (0.92 - 1.62)
216	prot C SNP 2583 (allele)	1.01	1.06	1.05 (0.8 - 1.37)
135	prot C SNP 3220 (allele)	1.00	0.87	0.87 (0.42 - 1.79)
130	prot C SNP 4515 (allele)	1.07	0.89	0.83 (0.63 - 1.1)
174	prot C SNP 4732 (allele)	0.97	0.94	0.97 (0.7 - 1.33)
133	prot C SNP 4919 (allele)	1.05	0.91	0.87 (0.67 - 1.13)
193	prot C SNP 5867 (allele)	0.98	0.99	1.01 (0.73 - 1.39)
101	prot C SNP Rs1401296 (allele)	1.04	1.48	1.42 (0.67 - 3.03)
78	prot C SNP Rs1799810 (allele)	1.13	1.08	0.96 (0.49 - 1.86)
103	prot C SNP Rs2069920 (allele)	0.79	1.52	1.92 (0.93 - 3.96)
80	prot C SNP Rs2069923 (allele)	1.11	1.09	0.98 (0.22 - 4.3)
73	prot C SNP Rs2069928 (allele)	0.96	0.73	0.76 (0.37 - 1.56)
77	prot C SNP Rs5937 (allele)	1.21	1.04	0.86 (0.44 - 1.66)
40	prot S rs 867186 (GG vs AA)	1.27	1.14	0.9 (0.24 - 3.4)
43	prot S rs2069948 (CC vs TT)	1.08	1.27	1.18 (0.8 - 1.74)
142	prot S SNP 13154 (allele)	1.06	0.96	0.91 (0.65 - 1.25)
233	prot S SNP 26890 (allele)	0.98	1.06	1.08 (0.7 - 1.67)
210	prot S SNP 288 (allele)	1.05	1.09	1.04 (0.74 - 1.45)
187	prot S SNP 430 (allele)	0.94	0.94	1 (0.76 - 1.31)
150	prot S SNP 66205 (allele)	1.07	1.00	0.93 (0.72 - 1.21)
206	prot S SNP 66847 (allele)	1.00	1.03	1.03 (0.66 - 1.62)
108	PSGL-1 VNTR (allele)	0.89	0.51	0.57 (0.25 - 1.33)
158	TAFI SNP 10152 (allele)	1.07	1.01	0.94 (0.7 - 1.28)
20	TAFI SNP 1040C/T (CC vs TT)	0.48	0.86	1.79 (0.45 - 7.11)
119	TAFI SNP 18857 (allele)	1.17	0.87	0.74 (0.35 - 1.59)
163	TAFI SNP 2103 (allele)	0.99	0.94	0.95 (0.65 - 1.39)
200	TAFI SNP 31427 (allele)	0.94	0.96	1.02 (0.77 - 1.35)
201	TAFI SNP 32627 (allele)	0.94	0.96	1.02 (0.77 - 1.35)
145	TAFI SNP 35605 (allele)	1.04	0.95	0.91 (0.69 - 1.21)
117	TAFI SNP 36326 (allele)	1.10	0.81	0.74 (0.37 - 1.47)
17	TAFI SNP -438G/A (GG vs AA)	0.91	0.96	1.05 (0.21 - 5.39)
230	TAFI SNP 47956 (allele)	1.05	1.13	1.08 (0.72 - 1.62)
188	TAFI SNP 48100 (allele)	0.87	0.87	1 (0.63 - 1.58)
132	TAFI SNP 4947 (allele)	1.30	1.11	0.85 (0.51 - 1.42)
15	TAFI SNP 505G/A (GG vs AA)	1.44	0.84	0.58 (0.14 - 2.41)
256	TAFI SNP 51208 (allele)	1.03	1.17	1.14 (0.86 - 1.5)
129	TAFI SNP 54691 (allele)	1.10	0.91	0.83 (0.61 - 1.12)
151	TAFI SNP 7826 (allele)	0.92	0.86	0.93 (0.72 - 1.22)
221	TF SNP 11185 (allele)	0.86	0.91	1.06 (0.52 - 2.16)
160	TF SNP 13925 (allele)	1.11	1.05	0.95 (0.71 - 1.27)

189	TF SNP 5334 (allele)	1.08	1.09	1.01 (0.74 - 1.37)
186	TF SNP 599 (allele)	0.92	0.92	1 (0.76 - 1.31)
213	TF SNP 7877 (allele)	1.12	1.17	1.04 (0.58 - 1.9)
159	TFPI SNP 1502 (allele)	1.10	1.04	0.95 (0.71 - 1.25)
138	TFPI SNP 21164 (allele)	1.09	0.97	0.89 (0.51 - 1.56)
262	TFPI SNP 2418 (allele)	0.90	1.05	1.17 (0.9 - 1.51)
243	TFPI SNP 34214 (allele)	0.91	1.01	1.11 (0.83 - 1.48)
209	TFPI SNP 3437 (allele)	1.08	1.12	1.04 (0.57 - 1.89)
207	thombomodulin SNP 5110 (allele)	0.96	0.99	1.03 (0.77 - 1.37)
232	thombomodulin SNP 4007 (allele)	1.02	1.10	1.08 (0.78 - 1.49)
231	thombomodulin SNP 5318 (allele)	1.03	1.11	1.08 (0.78 - 1.49)
127	thombomodulin SNP 6235 (allele)	1.09	0.88	0.81 (0.58 - 1.13)
83	thombomodulin SNP Rs1042580 (allele)	0.96	0.98	1.02 (0.49 - 2.14)
93	thombomodulin SNP Rs1962 (allele)	1.03	1.25	1.21 (0.56 - 2.64)
86	thombomodulin SNP Rs3176119 (allele)	0.79	0.85	1.08 (0.28 - 4.09)
71	thombomodulin SNP Rs3176123 (allele)	0.94	0.58	0.62 (0.3 - 1.28)
75	thombomodulin SNP Rs3216183 (allele)	1.02	0.81	0.79 (0.35 - 1.78)
99	thombomodulin SNP Rs6048519 (allele)	0.98	1.28	1.31 (0.63 - 2.69)
79	thombomodulin SNP Rs6082986 (allele)	1.00	0.98	0.98 (0.46 - 2.07)
89	thombomodulin SNP Rs6113909 (allele)	0.98	1.07	1.09 (0.48 - 2.49)
154	t-PA SNP 12047 (allele)	0.99	0.93	0.94 (0.73 - 1.21)
156	t-PA SNP 12264 (allele)	1.02	0.96	0.94 (0.6 - 1.47)
229	t-PA SNP 16039 (allele)	0.98	1.05	1.07 (0.82 - 1.41)
204	t-PA SNP 17825 (allele)	1.01	1.04	1.03 (0.79 - 1.35)
249	t-PA SNP 22323 (allele)	0.93	1.04	1.12 (0.79 - 1.59)
126	t-PA SNP 2586 (allele)	0.93	0.73	0.78 (0.41 - 1.51)
125	t-PA SNP 30619 (allele)	1.07	0.83	0.78 (0.51 - 1.18)
254	t-PA SNP 35171 (allele)	1.00	1.13	1.13 (0.85 - 1.51)
263	t-PA SNP 6388 (allele)	0.92	1.10	1.2 (0.74 - 1.92)
171	t-PA SNP 6971 (allele)	0.99	0.95	0.96 (0.6 - 1.53)
211	t-PA SNP 9823 (allele)	0.98	1.02	1.04 (0.8 - 1.36)
270	t-PA SNP 9944 (allele)	0.84	1.08	1.29 (0.66 - 2.5)
18	VWF SNP rs1063857 (dominant)	1.15	1.35	1.17 (0.64 - 2.16)
336	VWF SNP P475S (dominant)	0.56	0.98	1.75 (0.1 - 31.18)
19	VWF SNP rs216293 (dominant)	1.26	1.50	1.19 (0.69 - 2.05)
52	WWF SNP sma I (recessive)	1.81	3.29	1.82 (0.32 - 10.34)

ID, identification number; RR IS, relative risk for ischaemic stroke; RR MI, relative risk for myocardial infarction; RRR relative risk ratio.

S4 Table. All prothrombotic factors sorted by ID.

ID	Factor(contrast)	RR MI	RR IS	RRR (95% CI)
1	fibrinogen (Q4 vs Q1)	2.18	1.26	0.58
2	FXII (Q4 vs Q1)	1.17	1.16	0.99 (0.27 - 3.7)
3	FVII:c (Q4 vs Q1)	1.00	1.00	1
4	FIX (Q4 vs Q1)	0.93	0.96	1.03 (0.27 - 3.92)
5	FXI (Q4 vs Q1)	1.27	1.62	1.28 (0.33 - 4.86)
6	FV (Q4 vs Q1)	1.22	1.59	1.3 (0.34 - 5.02)
7	VWF (Q4 vs Q1)	1.21	1.71	1.41
8	FX (Q4 vs Q1)	0.61	0.88	1.44 (0.37 - 5.66)
9	plasminogen (Q4 vs Q1)	0.81	1.20	1.48 (0.34 - 6.4)
10	prot C (high) (Q4 vs Q1)	1.03	1.54	1.50
11	FII (Q4 vs Q1)	0.89	1.34	1.51 (0.32 - 7.04)
12	FVIII:c (Q4 vs Q1)	1.22	1.93	1.58
13	prot C (Q1 vs Q5)	0.92	1.52	1.65 (1.05 - 2.6)
14	alpha2 antiplasmin (Q4 vs Q1)	0.81	1.36	1.68 (0.39 - 7.21)
15	TAFI SNP 505G/A (GG vs AA)	1.44	0.84	0.58 (0.14 - 2.41)
16	CLT (fibrinolytic potential) (>90 percentile)	2.60	1.90	0.73 (0.24 - 2.26)
17	TAFI SNP -438G/A (GG vs AA)	0.91	0.96	1.05 (0.21 - 5.39)
18	VWF SNP rs1063857 (dominant)	1.15	1.35	1.17 (0.64 - 2.16)
19	VWF SNP rs216293 (dominant)	1.26	1.50	1.19 (0.69 - 2.05)
20	TAFI SNP 1040C/T (CC vs TT)	0.48	0.86	1.79 (0.45 - 7.11)
21	d-dimer (T3 vs T1)	1.39	1.56	1.12 (0.52 - 2.42)
22	t-PA (T3 vs T1)	0.92	1.38	1.5 (0.69 - 3.27)
23	VWF (T3 vs T1)	1.24	1.93	1.56 (0.72 - 3.34)
24	aggregation (whole blood) (Q5 vs Q1)	1.00	0.25	0.25 (0.07 - 0.93)
25	PLT aggregation (first) (Q5 vs Q1)	1.31	0.64	0.49 (0.18 - 1.31)
26	TAT (T3 vs T1)	0.97	0.71	0.73 (0.27 - 1.95)
27	VWF (T3 vs T1)	1.09	0.97	0.89 (0.43 - 1.86)
28	F1+2 fragment (T3 vs T1)	1.03	0.96	0.93 (0.35 - 2.46)
29	FVIII:c (T3 vs T1)	1.12	1.10	0.98 (0.4 - 2.41)
30	APC ratio (T3 vs T1)	0.70	0.73	1.04 (0.42 - 2.59)
31	fibrinogen (T3 vs T1)	1.26	1.51	1.2 (0.56 - 2.55)
32	t-PA (T3 vs T1)	1.10	1.33	1.21 (0.54 - 2.73)
33	PLT aggregation (irreversible) (high vs low)	1.04	1.26	1.21 (0.47 - 3.15)
34	PAI-1 (T3 vs T1)	1.30	1.61	1.24 (0.49 - 3.13)
35	PLT retention (Q5 vs Q1)	0.80	1.05	1.31 (0.39 - 4.43)
36	FVII:c (T3 vs T1)	0.65	0.91	1.4 (0.54 - 3.6)
37	d-dimer (T3 vs T1)	1.45	2.09	1.44 (0.63 - 3.32)
38	aPTT (T3 vs T1)	0.76	1.11	1.46 (0.59 - 3.62)
39	bleeding time (T1 vs T3)	0.90	1.42	1.58 (0.19 - 12.93)
40	prot S rs 867186 (GG vs AA)	1.27	1.14	0.9 (0.24 - 3.4)

41	FVIII:c (SD)	1.20	1.16	0.97 (0.74 - 1.26)
42	FVIII:c (SD)	1.13	1.15	1.02 (0.78 - 1.32)
43	prot S rs2069948 (CC vs TT)	1.08	1.27	1.18 (0.8 - 1.74)
44	PAI-1 SNP 4G/5G promotor (allele)	0.93	1.10	1.18 (0.55 - 2.53)
45	FII SNP G20210A (dominant)	0.88	1.40	1.59 (0.16 - 15.37)
46	FV Leiden (dominant)	0.81	0.42	0.52 (0.14 - 1.88)
47	FV Leiden (dominant)	0.80	0.51	0.64 (0.08 - 5)
48	FII SNP G20210A (dominant)	1.70	1.10	0.65 (0.19 - 2.2)
49	FV Leiden (dominant)	0.83	0.68	0.82 (0.38 - 1.77)
50	FV Leiden (dominant)	0.91	0.88	0.97 (0.31 - 2.98)
51	FV Leiden (dominant)	0.85	0.96	1.13 (0.41 - 3.11)
52	WWF SNP sma I (recessive)	1.81	3.29	1.82 (0.32 - 10.34)
53	fibrinopeptide A (T3 vs T1)	1.36	0.85	0.63 (0.31 - 1.26)
54	VWF (T3 vs T1)	1.53	1.02	0.67 (0.31 - 1.44)
55	FVII (T3 vs T1)	1.12	0.89	0.79 (0.31 - 2.05)
56	t-PA (T3 vs T1)	1.80	1.65	0.92 (0.38 - 2.22)
57	d-dimer (T3 vs T1)	1.59	1.62	1.02 (0.43 - 2.41)
58	F1+2 fragment (T3 vs T1)	0.92	1.00	1.09 (0.45 - 2.64)
59	FVII (SD (log scale))	0.98	1.08	1.1 (0.63 - 1.93)
60	VWF (SD (log scale))	0.95	1.15	1.21 (0.76 - 1.92)
61	fibrinogen (T3 vs T1)	1.66	2.06	1.24 (0.56 - 2.75)
62	t-PA (SD (log scale))	1.25	1.69	1.35 (0.81 - 2.25)
63	fibrinogen (SD (log scale))	1.04	1.52	1.46 (0.96 - 2.22)
64	d-dimer (SD (log scale))	1.04	1.96	1.88 (0.81 - 4.4)
65	fibrinogen (Q4 vs Q1)	2.30	2.50	1.09 (0.61 - 1.93)
66	plasminogen (SD)	1.41	1.10	0.78 (0.42 - 1.44)
67	FVII:ag (SD)	1.11	1.01	0.91 (0.57 - 1.44)
68	FVII:c (SD)	1.01	0.93	0.92 (0.58 - 1.47)
69	fibrinogen (SD)	1.02	1.05	1.03 (0.69 - 1.53)
70	ICAM1 SNP Rs3093030 (allele)	1.04	0.57	0.55 (0.26 - 1.17)
71	thombomodulin SNP Rs3176123 (allele)	0.94	0.58	0.62 (0.3 - 1.28)
72	FV SNP Rs9332575 (allele)	1.05	0.68	0.65 (0.28 - 1.5)
73	prot C SNP Rs2069928 (allele)	0.96	0.73	0.76 (0.37 - 1.56)
74	FV SNP Rs2269648 (allele)	1.11	0.86	0.77 (0.38 - 1.57)
75	thombomodulin SNP Rs3216183 (allele)	1.02	0.81	0.79 (0.35 - 1.78)
76	FV SNP Rs9332695 (allele)	0.85	0.71	0.84 (0.24 - 2.85)
77	prot C SNP Rs5937 (allele)	1.21	1.04	0.86 (0.44 - 1.66)
78	prot C SNP Rs1799810 (allele)	1.13	1.08	0.96 (0.49 - 1.86)
79	thombomodulin SNP Rs6082986 (allele)	1.00	0.98	0.98 (0.46 - 2.07)
80	prot C SNP Rs2069923 (allele)	1.11	1.09	0.98 (0.22 - 4.3)
81	ICAM1 SNP Rs5030347 (allele)	0.97	0.97	1 (0.93 - 1.08)
82	FV SNP Rs6019 (allele)	1.13	1.13	1 (0.21 - 4.68)
83	thombomodulin SNP Rs1042580 (allele)	0.96	0.98	1.02 (0.49 - 2.14)
84	FV SNP Rs9332590 (allele)	1.03	1.10	1.07 (0.55 - 2.08)

85	ICAM1 SNP Rs3093032 (allele)	1.08	1.16	1.07 (0.48 - 2.42)
86	thombomodulin SNP Rs3176119 (allele)	0.79	0.85	1.08 (0.28 - 4.09)
87	FV SNP Rs2420369 (allele)	1.17	1.26	1.08 (0.51 - 2.28)
88	ICAM1 SNP Rs281432 (allele)	1.22	1.32	1.08 (0.51 - 2.3)
89	thombomodulin SNP Rs6113909 (allele)	0.98	1.07	1.09 (0.48 - 2.49)
90	FV SNP Rs9332618 (allele)	0.93	1.02	1.1 (0.51 - 2.38)
91	FV SNP Rs970741 (allele)	1.04	1.19	1.14 (0.55 - 2.4)
92	FV SNP Rs9332640 (allele)	1.22	1.40	1.15 (0.52 - 2.55)
93	thombomodulin SNP Rs1962 (allele)	1.03	1.25	1.21 (0.56 - 2.64)
94	ICAM1 SNP Rs5030341 (allele)	1.25	1.52	1.22 (0.57 - 2.59)
95	FV SNP Rs9332591 (allele)	1.14	1.39	1.22 (0.51 - 2.92)
96	FV SNP Rs6013 (allele)	1.12	1.41	1.26 (0.47 - 3.37)
97	FV SNP Rs6030 (allele)	1.11	1.41	1.27 (0.61 - 2.66)
98	ICAM1 SNP Rs5030390 (allele)	1.23	1.59	1.29 (0.3 - 5.61)
99	thombomodulin SNP Rs6048519 (allele)	0.98	1.28	1.31 (0.63 - 2.69)
100	FV SNP Rs3753305 (allele)	0.98	1.36	1.39 (0.67 - 2.86)
101	prot C SNP Rs1401296 (allele)	1.04	1.48	1.42 (0.67 - 3.03)
102	FV SNP Rs6035 (allele)	1.26	2.19	1.74 (0.56 - 5.4)
103	prot C SNP Rs2069920 (allele)	0.79	1.52	1.92 (0.93 - 3.96)
104	FV SNP Rs7542281 (allele)	1.11	2.46	2.22 (0.65 - 7.56)
105	FV Leiden (allele)	1.22	2.98	2.44 (0.6 - 10)
106	fibrinogen (T1 vs T3)	0.62	0.40	0.65
107	fibrinogen (T1 vs T3)	0.56	0.94	1.68
108	PSGL-1 VNTR (allele)	0.89	0.51	0.57 (0.25 - 1.33)
109	GPIb-alpha SNP HPA-2 (allele)	2.09	2.40	1.15 (0.23 - 5.65)
110	GPIb-alpha VNTR (allele)	1.71	2.23	1.3 (0.31 - 5.43)
111	FXIIIA SNP Val34Leu (dominant)	1.00	1.33	1.33 (0.62 - 2.84)
112	FVII SNP 16826 (allele)	1.75	0.49	0.28 (0.03 - 2.4)
113	FV SNP upper 46058 (allele)	1.74	1.07	0.61 (0.21 - 1.83)
114	FIX SNP 12806 (allele)	1.54	0.99	0.64 (0.24 - 1.74)
115	FXIIIA SNP 72060 (allele)	1.00	0.67	0.67 (0.27 - 1.66)
116	FV SNP upper 72877 (allele)	1.20	0.82	0.68 (0.35 - 1.32)
117	TAFI SNP 36326 (allele)	1.10	0.81	0.74 (0.37 - 1.47)
118	prot C receptor SNP 837 (allele)	1.00	0.74	0.74 (0.46 - 1.2)
119	TAFI SNP 18857 (allele)	1.17	0.87	0.74 (0.35 - 1.59)
120	plasminogen SNP 41494 (allele)	1.15	0.87	0.76 (0.31 - 1.87)
121	FGA 3807 (allele)	1.07	0.82	0.77 (0.52 - 1.12)
122	FXII SNP 6570 (allele)	1.16	0.89	0.77 (0.34 - 1.75)
123	FX SNP 9501 (allele)	0.99	0.76	0.77 (0.51 - 1.17)
124	FIX SNP 30893 (allele)	0.82	0.63	0.77 (0.28 - 2.08)
125	t-PA SNP 30619 (allele)	1.07	0.83	0.78 (0.51 - 1.18)
126	t-PA SNP 2586 (allele)	0.93	0.73	0.78 (0.41 - 1.51)
127	thombomodulin SNP 6235 (allele)	1.09	0.88	0.81 (0.58 - 1.13)
128	FXI SNP 3450 (allele)	1.00	0.81	0.81 (0.59 - 1.11)
129	TAFI SNP 54691 (allele)	1.10	0.91	0.83 (0.61 - 1.12)
130	prot C SNP 4515 (allele)	1.07	0.89	0.83 (0.63 - 1.1)

131	plasminogen SNP 18114 (allele)	1.21	1.01	0.83 (0.63 - 1.11)
132	TAFI SNP 4947 (allele)	1.30	1.11	0.85 (0.51 - 1.42)
133	prot C SNP 4919 (allele)	1.05	0.91	0.87 (0.67 - 1.13)
134	FGB 9487 (allele)	1.00	0.87	0.87 (0.62 - 1.23)
135	prot C SNP 3220 (allele)	1.00	0.87	0.87 (0.42 - 1.79)
136	FIX SNP 4135 (allele)	1.17	1.02	0.87 (0.55 - 1.39)
137	FV SNP upper 66464 (allele)	1.06	0.94	0.89 (0.51 - 1.53)
138	TFPI SNP 21164 (allele)	1.09	0.97	0.89 (0.51 - 1.56)
139	FGB 1643 (allele)	1.02	0.91	0.89 (0.64 - 1.24)
140	FXIIIA SNP 165306 (allele)	1.12	1.01	0.9 (0.65 - 1.25)
141	FXIIIB SNP 9706 (allele)	1.05	0.95	0.9 (0.64 - 1.29)
142	prot S SNP 13154 (allele)	1.06	0.96	0.91 (0.65 - 1.25)
143	FVIII SNP 95910 (allele)	1.28	1.16	0.91 (0.42 - 1.96)
144	FXIIIA SNP 170779 (allele)	1.12	1.02	0.91 (0.64 - 1.29)
145	TAFI SNP 35605 (allele)	1.04	0.95	0.91 (0.69 - 1.21)
146	plasminogen SNP 406 (allele)	0.97	0.89	0.92 (0.7 - 1.2)
147	FV SNP upper 42713 (allele)	1.10	1.02	0.93 (0.7 - 1.23)
148	FGG 902 (allele)	1.10	1.02	0.93 (0.7 - 1.22)
149	FV SNP 17557 (allele)	1.04	0.97	0.93 (0.7 - 1.25)
150	prot S SNP 66205 (allele)	1.07	1.00	0.93 (0.72 - 1.21)
151	TAFI SNP 7826 (allele)	0.92	0.86	0.93 (0.72 - 1.22)
152	FIX SNP 10948 (allele)	1.08	1.01	0.94 (0.4 - 2.2)
153	plasminogen SNP 54925 (allele)	1.09	1.02	0.94 (0.51 - 1.72)
154	t-PA SNP 12047 (allele)	0.99	0.93	0.94 (0.73 - 1.21)
155	plasminogen SNP 1983 (allele)	1.02	0.96	0.94 (0.67 - 1.33)
156	t-PA SNP 12264 (allele)	1.02	0.96	0.94 (0.6 - 1.47)
157	FXIIIA SNP 165399 (allele)	1.04	0.98	0.94 (0.71 - 1.26)
158	TAFI SNP 10152 (allele)	1.07	1.01	0.94 (0.7 - 1.28)
159	TFPI SNP 1502 (allele)	1.10	1.04	0.95 (0.71 - 1.25)
160	TF SNP 13925 (allele)	1.11	1.05	0.95 (0.71 - 1.27)
161	FX SNP 11962 (allele)	0.99	0.94	0.95 (0.73 - 1.24)
162	FGA 9205 (allele)	0.99	0.94	0.95 (0.66 - 1.37)
163	TAFI SNP 2103 (allele)	0.99	0.94	0.95 (0.65 - 1.39)
164	FX SNP 16893 (allele)	1.00	0.95	0.95 (0.74 - 1.22)
165	FXI SNP 25455 (allele)	1.01	0.96	0.95 (0.71 - 1.26)
166	FXI SNP 3543 (allele)	1.04	0.99	0.95 (0.73 - 1.24)
167	FXIIIA SNP 176866 (allele)	1.08	1.03	0.95 (0.68 - 1.34)
168	FX SNP 26242 (allele)	0.92	0.88	0.96 (0.66 - 1.38)
169	FV SNP upper 66872 (allele)	0.94	0.90	0.96 (0.68 - 1.35)
170	FVII SNP 18311 (allele)	0.97	0.93	0.96 (0.73 - 1.26)
171	t-PA SNP 6971 (allele)	0.99	0.95	0.96 (0.6 - 1.53)
172	FX SNP 18352 (allele)	1.02	0.98	0.96 (0.64 - 1.45)
173	antithrombin SNP 7199 (allele)	1.05	1.01	0.96 (0.62 - 1.49)
174	prot C SNP 4732 (allele)	0.97	0.94	0.97 (0.7 - 1.33)
175	prot C receptor SNP 6196 (allele)	0.98	0.95	0.97 (0.73 - 1.28)
176	FV SNP lower 3578 (allele)	1.02	0.99	0.97 (0.74 - 1.27)

177	FGB 11079 (allele)	1.02	0.99	0.97 (0.73 - 1.28)
178	FXIIIA SNP 177778 (allele)	1.12	1.09	0.97 (0.57 - 1.67)
179	FXIIIB SNP 5995 (allele)	0.92	0.90	0.98 (0.75 - 1.28)
180	FV SNP upper 45765 (allele)	1.00	0.98	0.98 (0.56 - 1.71)
181	FXIIIB SNP 29759 (allele)	1.11	1.09	0.98 (0.75 - 1.28)
182	FV SNP lower 35788 (allele)	1.15	1.13	0.98 (0.58 - 1.65)
183	FGG 129 (allele)	0.94	0.93	0.99 (0.73 - 1.34)
184	antithrombin SNP 1734 (allele)	1.02	1.01	0.99 (0.64 - 1.54)
185	FIX SNP 21975 (allele)	0.96	0.96	1 (0.6 - 1.66)
186	TF SNP 599 (allele)	0.92	0.92	1 (0.76 - 1.31)
187	prot S SNP 430 (allele)	0.94	0.94	1 (0.76 - 1.31)
188	TAFI SNP 48100 (allele)	0.87	0.87	1 (0.63 - 1.58)
189	TF SNP 5334 (allele)	1.08	1.09	1.01 (0.74 - 1.37)
190	PAI-1 SNP 4588 (allele)	1.01	1.02	1.01 (0.68 - 1.49)
191	PAI-1 SNP 12219 (allele)	1.01	1.02	1.01 (0.79 - 1.3)
192	FVII SNP 2643 (allele)	0.99	1.00	1.01 (0.76 - 1.34)
193	prot C SNP 5867 (allele)	0.98	0.99	1.01 (0.73 - 1.39)
194	FGA 6534 (allele)	0.96	0.97	1.01 (0.77 - 1.33)
195	FXII SNP 7532 (allele)	1.04	1.06	1.02 (0.77 - 1.34)
196	prot C receptor SNP rs1415772 (allele)	1.02	1.04	1.02 (0.74 - 1.4)
197	FX SNP 8946 (allele)	0.99	1.01	1.02 (0.78 - 1.33)
198	FVII SNP 185 (allele)	0.95	0.97	1.02 (0.74 - 1.4)
199	PAI-1 SNP 10381 (allele)	0.94	0.96	1.02 (0.63 - 1.66)
200	TAFI SNP 31427 (allele)	0.94	0.96	1.02 (0.77 - 1.35)
201	TAFI SNP 32627 (allele)	0.94	0.96	1.02 (0.77 - 1.35)
202	FXI SNP 20423 (allele)	0.91	0.93	1.02 (0.72 - 1.46)
203	plasminogen SNP 31439 (allele)	0.83	0.85	1.02 (0.75 - 1.39)
204	t-PA SNP 17825 (allele)	1.01	1.04	1.03 (0.79 - 1.35)
205	FXI SNP 228771 (allele)	1.00	1.03	1.03 (0.78 - 1.35)
206	prot S SNP 66847 (allele)	1.00	1.03	1.03 (0.66 - 1.62)
207	thombomodulin SNP 5110 (allele)	0.96	0.99	1.03 (0.77 - 1.37)
208	prot C SNP 10454 (allele)	0.93	0.96	1.03 (0.74 - 1.44)
209	TFPI SNP 3437 (allele)	1.08	1.12	1.04 (0.57 - 1.89)
210	prot S SNP 288 (allele)	1.05	1.09	1.04 (0.74 - 1.45)
211	t-PA SNP 9823 (allele)	0.98	1.02	1.04 (0.8 - 1.36)
212	FGB 1083 (allele)	0.97	1.01	1.04 (0.71 - 1.52)
213	TF SNP 7877 (allele)	1.12	1.17	1.04 (0.58 - 1.9)
214	FII SNP 7530 (allele)	1.10	1.15	1.05 (0.73 - 1.5)
215	FII SNP 5467 (allele)	1.09	1.14	1.05 (0.72 - 1.52)
216	prot C SNP 2583 (allele)	1.01	1.06	1.05 (0.8 - 1.37)
217	FV SNP upper 68717 (allele)	0.97	1.02	1.05 (0.8 - 1.37)
218	PAI-1 SNP 5878 (allele)	0.95	1.00	1.05 (0.75 - 1.47)
219	FV SNP upper 45888 (allele)	0.91	0.96	1.05 (0.78 - 1.42)
220	plasminogen SNP 2967 (allele)	1.04	1.10	1.06 (0.81 - 1.38)
221	TF SNP 11185 (allele)	0.86	0.91	1.06 (0.52 - 2.16)
222	FVII SNP 15386 (allele)	1.00	1.06	1.06 (0.7 - 1.61)

223	FIX SNP 716 (allele)	1.00	1.06	1.06 (0.66 - 1.71)
224	FXIIIB SNP 17686 (allele)	1.08	1.15	1.06 (0.78 - 1.45)
225	FXIIIB SNP 7319 (allele)	0.90	0.96	1.07 (0.69 - 1.66)
226	antithrombin SNP 9089 (allele)	1.01	1.08	1.07 (0.81 - 1.41)
227	FII SNP 21239 (allele)	1.00	1.07	1.07 (0.82 - 1.4)
228	FX SNP 17396 (allele)	0.98	1.05	1.07 (0.8 - 1.44)
229	t-PA SNP 16039 (allele)	0.98	1.05	1.07 (0.82 - 1.41)
230	TAFI SNP 47956 (allele)	1.05	1.13	1.08 (0.72 - 1.62)
231	thombomodulin SNP 5318 (allele)	1.03	1.11	1.08 (0.78 - 1.49)
232	thombomodulin SNP 4007 (allele)	1.02	1.10	1.08 (0.78 - 1.49)
233	prot S SNP 26890 (allele)	0.98	1.06	1.08 (0.7 - 1.67)
234	FGG 9340 (allele)	0.94	1.02	1.09 (0.81 - 1.46)
235	FIX SNP 21554 (allele)	0.99	1.08	1.09 (0.66 - 1.8)
236	plasminogen SNP 41108 (allele)	0.92	1.01	1.1 (0.77 - 1.57)
237	plasminogen SNP 1470 (allele)	1.00	1.10	1.1 (0.77 - 1.58)
238	FXI SNP 26011 (allele)	1.06	1.17	1.1 (0.75 - 1.62)
239	FV SNP lower 30539 (allele)	1.05	1.16	1.1 (0.72 - 1.7)
240	FIX SNP 27226 (allele)	1.04	1.15	1.11 (0.62 - 1.97)
241	FIX SNP 35124 (allele)	1.04	1.15	1.11 (0.64 - 1.92)
242	FII SNP G20210A (allele)	1.39	1.54	1.11 (0.41 - 3.03)
243	TFPI SNP 34214 (allele)	0.91	1.01	1.11 (0.83 - 1.48)
244	antithrombin SNP 2415 (allele)	1.00	1.11	1.11 (0.75 - 1.65)
245	PAI-1 SNP 664 (allele)	0.99	1.10	1.11 (0.84 - 1.48)
246	FIX SNP 6347 (allele)	0.94	1.05	1.12 (0.68 - 1.84)
247	FXIIIA SNP 148318 (allele)	1.02	1.14	1.12 (0.8 - 1.55)
248	FXI SNP 6783 (allele)	1.10	1.23	1.12 (0.69 - 1.81)
249	t-PA SNP 22323 (allele)	0.93	1.04	1.12 (0.79 - 1.59)
250	FIX SNP 16171 (allele)	0.76	0.85	1.12 (0.44 - 2.84)
251	plasminogen SNP 15255 (allele)	0.96	1.08	1.13 (0.87 - 1.46)
252	FII SNP 4992 (allele)	1.03	1.16	1.13 (0.83 - 1.52)
253	FII SNP 280 (allele)	0.94	1.06	1.13 (0.7 - 1.82)
254	t-PA SNP 35171 (allele)	1.00	1.13	1.13 (0.85 - 1.51)
255	FGG 5836 (allele)	1.13	1.28	1.13 (0.57 - 2.24)
256	TAFI SNP 51208 (allele)	1.03	1.17	1.14 (0.86 - 1.5)
257	FII SNP 5389 (allele)	1.01	1.15	1.14 (0.86 - 1.51)
258	FX SNP 14881 (allele)	0.97	1.11	1.14 (0.79 - 1.65)
259	FII SNP 3696 (allele)	1.03	1.18	1.15 (0.51 - 2.58)
260	FGA 251 (allele)	0.93	1.07	1.15 (0.87 - 1.52)
261	antithrombin SNP 5403 (allele)	0.91	1.06	1.16 (0.54 - 2.51)
262	TFPI SNP 2418 (allele)	0.90	1.05	1.17 (0.9 - 1.51)
263	t-PA SNP 6388 (allele)	0.92	1.10	1.2 (0.74 - 1.92)
264	FV SNP lower 29565 (allele)	0.96	1.16	1.21 (0.87 - 1.69)
265	FXI SNP 10942 (allele)	1.06	1.29	1.22 (0.83 - 1.78)
266	prot C SNP 11310 (allele)	0.95	1.16	1.22 (0.92 - 1.62)
267	FGA 5498 (allele)	0.92	1.15	1.25 (0.84 - 1.86)
268	FX SNP 4544 (allele)	0.96	1.20	1.25 (0.83 - 1.87)

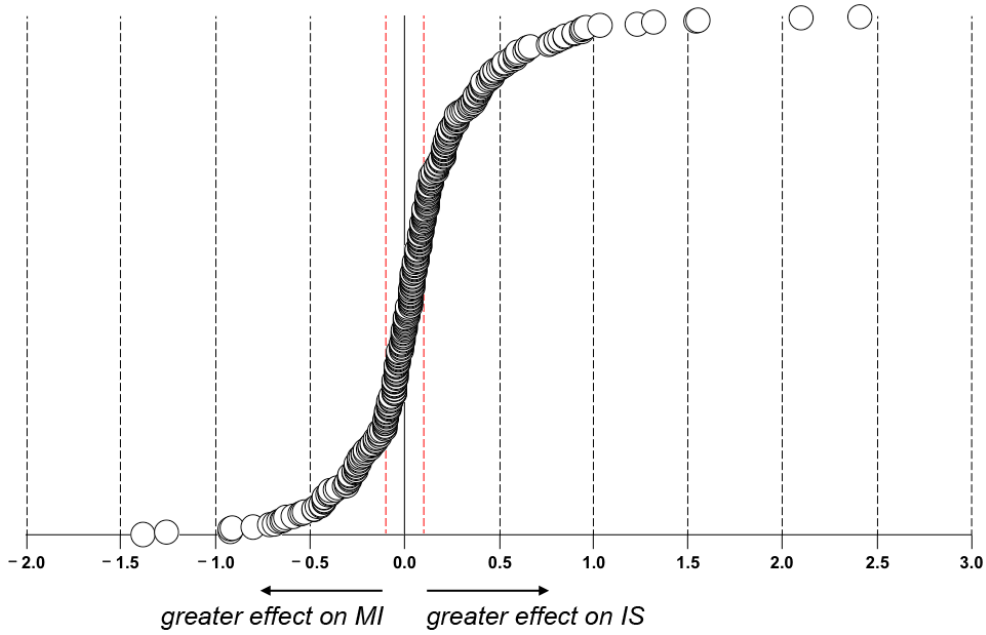
269	FXIIIA SNP 4377 (allele)	0.92	1.16	1.26 (0.93 - 1.71)
270	t-PA SNP 9944 (allele)	0.84	1.08	1.29 (0.66 - 2.5)
271	FVII SNP 115 (allele)	1.07	1.40	1.31 (0.28 - 6.14)
272	plasminogen SNP 34158 (allele)	1.20	1.58	1.32 (0.7 - 2.49)
273	prot C receptor SNP 3600 (allele)	0.74	0.99	1.34 (0.6 - 2.97)
274	FVIII SNP 139972 (allele)	0.83	1.20	1.45 (0.68 - 3.08)
275	FV SNP upper 38592 (allele)	1.03	1.53	1.49 (0.69 - 3.21)
276	FVIII SNP 55941 (allele)	1.04	1.56	1.5 (0.9 - 2.5)
277	FXI SNP 4197 (allele)	0.96	1.52	1.58 (0.61 - 4.1)
278	FVIII SNP 95826 (allele)	0.94	1.70	1.81 (1.02 - 3.2)
279	FX SNP 22739 (allele)	0.69	1.31	1.9 (0.21 - 16.96)
280	FVIII SNP 25167 (allele)	0.75	2.10	2.8 (0.7 - 11.2)
281	FXIIIA SNP 177424 rs3024462 (allele)	0.49	1.82	3.71 (0.62 - 22.35)
282	FVIII SNP 165293 rs6655259 (allele)	0.54	2.55	4.72 (0.62 - 35.73)
283	PAI-1 SNP 4G/5G promotor (4G/4G vs 5G/5G)	0.96	0.49	0.51 (0.11 - 2.4)
284	fibrinogen (SD)	1.30	1.01	0.78 (0.61 - 0.98)
285	FII SNP G20210A (dominant)	5.42	4.00	0.74 (0 - 112.99)
286	FV Leiden (dominant)	3.75	4.28	1.14 (0.05 - 24.1)
287	t-PA (Q4 vs Q1)	5.89	2.32	0.39 (0.07 - 2.07)
288	PAI-1 (Q4 vs Q1)	3.35	1.32	0.39 (0.08 - 1.88)
289	KAL-C1-INH (T3 vs T1)	0.73	0.67	0.92 (0.28 - 2.99)
290	FXIa-AT-INH (T3 vs T1)	1.31	1.22	0.93 (0.23 - 3.82)
291	FXIIa-C1-INH (T3 vs T1)	0.73	0.86	1.18 (0.37 - 3.77)
292	FXIa-C1-INH (T3 vs T1)	1.05	1.51	1.44 (0.41 - 5.02)
293	fibrinogen (>300 mg/dl)	3.68	2.72	0.74 (0.11 - 5.05)
294	FV Leiden (dominant)	0.76	2.60	3.42 (0.11 - 104)
295	FV Leiden (dominant)	1.50	1.00	0.67 (0.14 - 3.13)
296	fibrinogen SNP C148T (or G455A) (allele)	1.10	0.93	0.85 (0.45 - 1.6)
297	FII SNP G20210A (dominant)	0.80	1.10	1.38 (0.31 - 6.05)
298	anti-cardiolipin IgG (>95 percentile)	1.80	0.90	0.5 (0.11 - 2.25)
299	FGA SNP Thr312Ala rs6050 (allele)	0.82	0.43	0.52 (0.15 - 1.8)
300	CLT (hyperfibrinolysis vs normofibrinolysis) (T3 vs T2)	2.82	1.50	0.53 (0.16 - 1.8)
301	FXIIIA SNP Pro564Leu (dominant)	1.40	0.89	0.64 (0.31 - 1.29)
302	FXIIIA SNP Val34Leu (dominant)	1.07	0.77	0.72 (0.36 - 1.43)
303	FII SNP G20210A (dominant)	1.00	1.00	1 (0.12 - 8.33)
304	VWF (Q4 vs Q1)	4.20	6.70	1.6 (0.4 - 6.38)
305	FV Leiden (dominant)	1.10	1.80	1.64 (0.45 - 6)
306	FGB SNP 455G/A rs1800790 (allele)	0.98	1.76	1.8 (0.32 - 10.06)
307	FXIIIB SNP His95Arg (dominant)	0.79	1.70	2.15 (0.88 - 5.25)
308	ADAMTS13 (Q1 vs Q4)	1.40	3.10	2.21 (0.65 - 7.51)
309	anti-prothrombin IgG (>95 percentile)	0.80	1.80	2.25 (0.38 - 13.5)

310	FXIa-AT-INH (>90 percentile)	0.94	2.18	2.32 (0.68 - 7.95)
311	anti-beta2GP (>95 percentile)	1.20	2.80	2.33 (0.63 - 8.71)
312	KAL-C1-INH (>90 percentile)	2.12	5.14	2.42 (0.77 - 7.64)
313	FXIIa-C1-INH (>90 percentile)	0.74	1.87	2.53 (0.74 - 8.6)
314	CLT (hypofibrinolysis vs. normofibrinolysis) (T1 vs T2)	1.60	4.07	2.54 (0.71 - 9.09)
315	FXIa-C1-INH (>90 percentile)	1.13	2.92	2.58 (0.77 - 8.72)
316	lupus anticoagulant (ratio >=1.15)	5.30	43.10	8.13 (0.61 - 108.76)
317	FXIIIA SNP Tyr204phe (dominant)	0.82	9.10	11.1 (5.64 - 21.82)
318	FV Leiden (dominant)	2.40	0.00	0
319	FII SNP G20210A (dominant)	4.00	1.60	0.4 (0 - 77.58)
320	GPIIb SNP ile/Ser (recessive)	1.85	1.20	0.65 (0.18 - 2.37)
321	GPIIIa SNP Leu/pro (recessive)	1.14	1.01	0.89 (0.24 - 3.27)
322	GPIa SNP glu/Lys (recessive)	1.06	0.96	0.91 (0.18 - 4.58)
323	PAI-1 SNP 4G/5G (4G/4G vs 5G/5G)	0.40	0.49	1.23 (0.24 - 6.14)
324	FXIIIA SNP Pro564Leu (dominant)	0.80	0.99	1.24 (0.33 - 4.63)
325	FXIIIA SNP Val34Leu (Val/Leu vs Val/Val)	0.80	1.19	1.49 (0.4 - 5.5)
326	PAI-1 SNP 4G/5G (allele)	0.50	0.84	1.68 (0.45 - 6.24)
327	GPIa SNP C807T (recessive)	1.26	2.24	1.78 (0.45 - 7.04)
328	FXIIIA SNP Tir204Phe (dominant)	1.02	1.95	1.91 (0.2 - 18.29)
329	PAI-1 SNP 4G/5G (4G/5G vs 4G/4G)	0.52	1.10	2.12 (0.51 - 8.69)
330	GPIb SNP thr/Met (recessive)	0.58	1.48	2.55 (0.48 - 13.69)
331	FXIIIA SNP Val34Leu (Leu/Leu vs Val/Val)	0.77	3.59	4.66 (0.44 - 49.1)
332	VWF (Q4 vs Q1)	1.39	1.25	0.9 (0.41 - 1.97)
333	d-dimer (high vs low)	2.10	2.60	1.24 (0.27 - 5.63)
334	FV Leiden (dominant)	0.77	1.12	1.45 (0.16 - 13.07)
335	aPTT (for Protein C) (Q1 vs Q5)	1.53	2.43	1.59 (0.35 - 7.26)
336	VWF SNP P475S (dominant)	0.56	0.98	1.75 (0.1 - 31.18)
337	FXII SNP (dominant)	4.80	4.10	0.85 (0.06 - 12.73)
338	VWF (SD)	1.04	0.97	0.93 (0.65 - 1.34)
339	fibrinogen (SD)	1.13	1.14	1.01 (0.68 - 1.51)
340	d-dimer (SD)	1.02	1.27	1.25 (0.72 - 2.16)
341	t-PA (SD)	1.04	1.60	1.54 (0.55 - 4.34)
342	fibrinogen (Q5 vs Q1)	2.45	1.63	0.67 (0.14 - 3.25)
343	VWF (Q5 vs Q1)	1.52	1.06	0.7 (0.16 - 3.06)
344	d-dimer (Q5 vs Q1)	1.49	1.09	0.73 (0.12 - 4.38)
345	trombin generation (PEAK) (SD)	1.71	1.31	0.77 (0.38 - 1.53)
346	trombin generation (PEAK) (SD)	1.04	1.31	1.26 (0.76 - 2.1)
347	trombin generation (PEAK) (SD)	1.03	1.31	1.27 (0.83 - 1.95)

348	fibrinogen (SD (log scale))	1.52	1.36	0.89 (0.65 - 1.24)
349	FVII:c (unit log) (SD (log scale))	0.98	1.07	1.09 (0.74 - 1.6)
350	t-PA (Q4 vs Q1)	3.20	1.42	0.44 (0.17 - 1.15)
351	d-dimer (Q4 vs Q1)	1.70	1.52	0.89 (0.39 - 2.06)

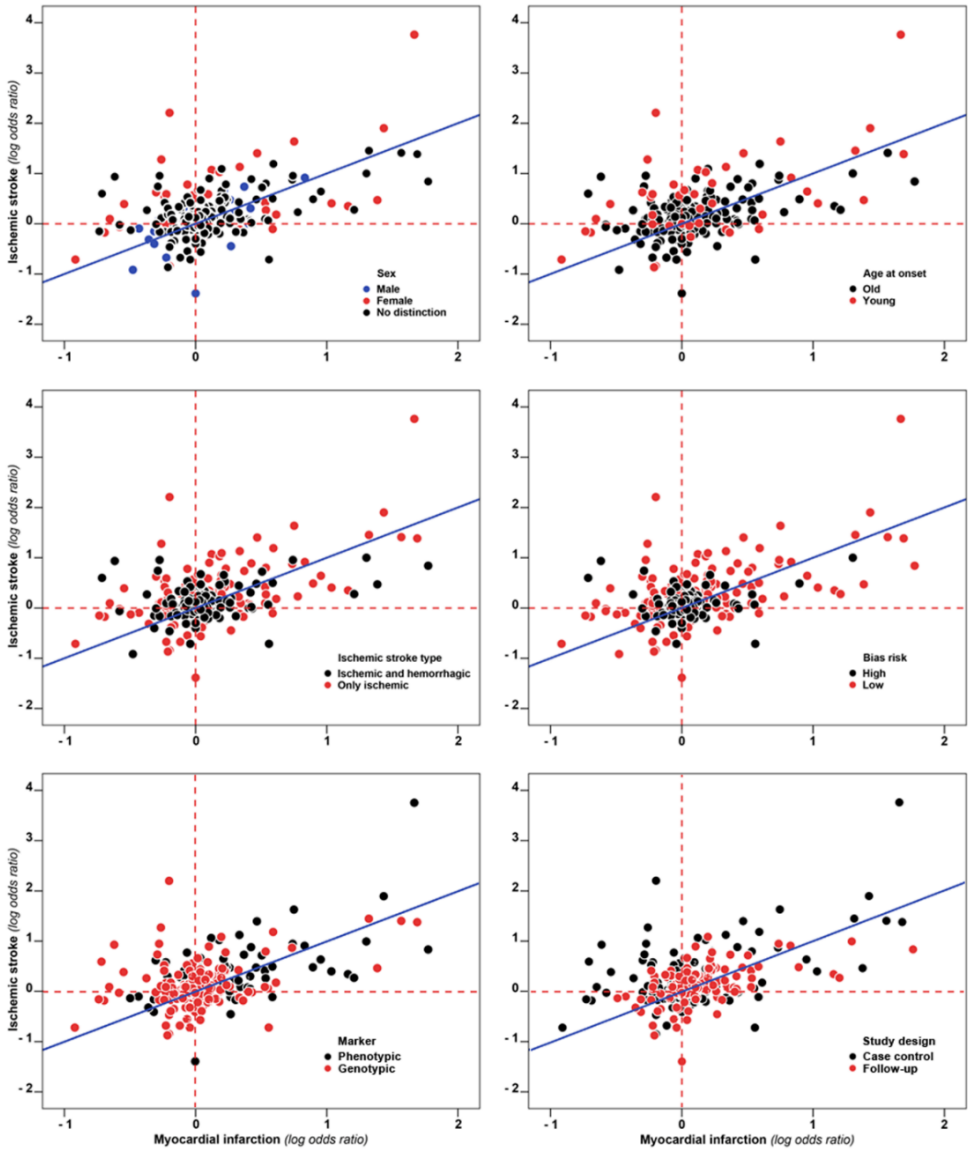
ID, identification number; RR IS, relative risk for ischaemic stroke; RR MI, relative risk for myocardial infarction; RRR relative risk ratio.

S1 Fig. Schematic representation of all the relative risk ratios (RRR) of the markers of hypercoagulability.



The bars indicate the RRR for each marker of prothrombotic state. Scale is logarithmic. RRR>0 (right) greater effect on ischaemic stroke; RRR<0 (left) greater effect on myocardial infarction. 135 out of 351 markers (38%) had an RRR between 0.9 and 1.1 (red dashed lines).

S2 Fig. Prothrombotic risk factors and their effect on myocardial infarction and ischaemic stroke by subgroups.



Each point depicts the log odds ratio as a measure of effect 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.

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Chapter 6

Concomitant headache influences long-term prognosis after acute cerebral ischaemia of non-cardioembolic origin

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Stroke, 2013

Abstract

Background and Purpose: Acute cerebral ischaemia is frequently associated with headache. It is unknown whether concomitant headache reflects a partly different pathogenesis and thus may influence long-term prognosis after stroke. Here we compared the long-term risk of recurrent vascular events in patients in whom a transient ischaemic attack (TIA) or minor ischaemic stroke of non-cardioembolic origin was associated with headache with those without headache.

Methods: We used data from the LiLAC (Life Long After Cerebral ischaemia) cohort. Participants were grouped based on the presence or absence of headache at presentation. We calculated the hazard ratios (HRs) and corresponding 95% confidence intervals (CI) for any first vascular event (primary outcome) or any cardiac or cerebral event (secondary outcomes). Adjustments were made for baseline clinical characteristics.

Results: Of 2473 participants, 420 (17%) experienced headache during the acute event. Median follow-up was 14.1 years. For the primary outcome, the crude HR of headache vs. no headache was 0.75 (95% CI 0.66-0.89) and the adjusted HR 0.83 (95% CI 0.71-0.97). For cardiac events the adjusted HR was 0.88 (95% CI 0.67-1.14) and for cerebral events, 0.97 (95% CI 0.76-1.24). The ratio of cardiac versus cerebral events, however, did not differ between the two groups. Participants with headache were at lower risk of vascular death (adjusted HR 0.73; 95% CI 0.61-0.87).

Conclusions: Patients who experienced headache in association with a TIA or minor ischaemic stroke have a better vascular prognosis than those without concomitant headache. This may, at least partly, reflect a different pathogenesis.

Introduction

Ischaemic strokes are associated with headache in over a quarter of cases.[1-5] This might be due to stimulation of sensory afferents of the trigeminovascular system, either directly by ischaemia or indirectly by cortical spreading depression (SD) secondary to cerebral ischaemia.[6-8] In rare cases, headache may reflect migrainous stroke.[9, 10] Alternatively, it has been estimated that up to 30% of patients with a presumed transient ischaemic attack (TIA) in fact had a migraine attack with headache and neurological aura symptoms.[11-13] It is thus conceivable that distinct pathophysiological mechanisms are, at least partly, involved in cerebral ischaemic events with and without associated headache. If true, there might also be a different prognosis for recurring vascular events.[14] Here we compared, in a large cohort of patients with established TIA or minor ischaemic stroke, the long-term risk of recurrent vascular events in patients with and without associated headache.

Methods

Patients and study design

For this study we used data of 2473 participants who were included in the LiLAC (Life Long After Cerebral ischaemia) cohort, which is based on the Dutch TIA Trial (DTT) that started in 1986. The background, design, and results of this multicenter trial have been described in detail elsewhere.[15] In brief, participants with a TIA (symptoms for less than 24 hours) or minor ischaemic stroke (symptoms for more than 24 hours) and who were still independent in most daily activities (modified Rankin scale <= 3), were, within three months from onset, randomly assigned to 30 mg or 283 mg of aspirin, or 50 mg of atenolol or its placebo, in a factorial design. Participants with a cardiac source of embolism or a clotting disorder were excluded. In the LiLAC study, follow-up of all the participants who were still alive at the end of the DTT (spring 1990) was extended up to the period between March 2001 and December 2003.[16] For logistical reasons only patients from the 24 hospitals which had enrolled at least 50 patients in the DTT were included in LiLAC (2473 of the original 3150). Follow-up data were obtained from the neurologists who had included patients in the DTT and the patients' general practitioner. If data were still incomplete, the participant (or, if unavailable, a relative or acquaintance) was contacted directly. All the participants were informed about the background and procedures of the trial, both through discussion and by means of a printed information sheet, and all gave their explicit consent. The protocol of the LiLAC study was approved by the ethics committee of the University Medical Centre Utrecht.

Baseline characteristics

Extensive baseline characteristics were recorded in the DTT by neurologists using a checklist that was specifically worded to be understood by patients. The list contained several multiple-choice questions about the nature and time course of symptoms, including the presence and onset of any kind of headache, and whether the headache was throbbing or continuous. Because the primary aim of the DTT was to assess treatment effects of aspirin and atenolol, no other headache details, nor the presence of associated (migraine) symptoms were recorded. Headache was taken as any headache reported by the patient occurring simultaneous with the onset of TIA or minor stroke.[17] Apart from the specific history, records

included demographic data, vascular risk factors, vascular history, blood pressure, physical examination, laboratory tests, electrocardiogram (ECG), and medications. A brain CT scan was obtained in all participants, apart from those with only transient monocular blindness. Cerebral infarcts were defined as circumscribed hypodense lesions and subdivided into lacunar small deep lesions and cortical infarcts. Infarcts were further subdivided according to their location. Depending on the clinical details, the scans were classified as showing relevant infarcts (lesions concordant with the symptoms) or irrelevant infarcts (lesions not concordant with the symptoms).

Outcome event

Our primary outcome measure was the composite event of death from all vascular causes, non-fatal stroke (caused by ischaemia or haemorrhage) or non-fatal myocardial infarction, whichever occurred first. Separate analyses were performed for the outcomes cardiac events (fatal or non-fatal myocardial infarction, death from congestive heart failure and sudden death), cerebral events (fatal or non-fatal ischaemic or haemorrhagic stroke) and deaths. Deaths were furthermore classified as vascular or non-vascular deaths. The definition of vascular death included sudden death (unexpected cardiac death within an hour of onset of symptoms, or within 24 hours if the patient was unexpectedly found dead), or death from stroke, myocardial infarction, congestive heart failure, or systemic bleeding. All events were classified independently by three physicians specialized in the field of cerebrovascular disease according to criteria described previously.[16]

Statistical analysis

Median follow-up was calculated using the estimates of the censoring distribution as described previously.[18] The occurrence of outcome events in patients with onset headache and those without onset headache was compared in terms of hazard ratios (HRs). HRs were determined with the cause-specific Cox proportional hazards model, with corresponding 95% confidence intervals (CI), and adjusted in bivariable analyses for differences in baseline characteristics between patients with or without headache. A final multivariable model was built, which included all variables that changed the crude HR by at least 5% in the bi-variable

analyses. Pre-specified subgroups analyses were performed for the primary outcome, and a forest plot of adjusted HRs was drawn. For a visual comparison of the two groups, nonparametric cumulative incidence functions were drawn.[19] They are a crude representation of the data and show the competing risks of death, cardiovascular and cerebral event over time. Cumulative incidence ratios (CIRs) of cardiac events to cerebral events were calculated from these functions with corresponding bootstrap 95% CI. Differences in CIRs between the two groups were tested at various times (5, 10 and 15 years of follow-up) by means of a permutation test. Significance level was set at $p<0.05$.

Table 1. Baseline characteristics of the 2473 patients included in the study.

Characteristics	headache N=420	no headache N=2053
Demographics		
Age (years; SD)	63.3 (10.5)	65.6 (10)
Male	258 (61%)	1350 (66%)
History		
Myocardial infarction	54 (13%)	205 (10%)
Intermittent claudication	22 (5%)	106 (5%)
Diabetes mellitus	28 (7%)	174 (8%)
Hypertension	151 (36%)	889 (43%)
Angina	56 (13%)	198 (10%)
Hyperlipidaemia	17 (4%)	77 (4%)
Smoking	181 (43%)	944 (46%)
Event		
Minor ischaemic stroke	314 (75%)	1400 (68%)
TIA	106 (25%)	653 (32%)
Rankin grade >=2	106 (25%)	466 (23%)
Visual disturbances only	23 (5%)	139 (7%)
Pure motor symptoms	159 (38%)	866 (42%)
Pure sensory symptoms	24 (6%)	103 (5%)
Sensory-motor symptoms	136 (32%)	668(33%)
Dizziness	77 (18%)	184 (9%)
Paresis	276 (66%)	1487 (73%)
Dysarthria	94 (22%)	494 (24%)
Aphasia	79 (19%)	400 (20%)
Lacunar syndrome	188 (45%)	1182 (58%)
Vertebrobasilar syndrome	70 (17%)	212 (10%)
12 lead ECG		
Q wave	56 (13%)	299 (15%)
ST-depression	36 (9%)	175 (9%)
Negative T wave	52 (12%)	197 (10%)
Acute phase hypertension		
Systolic blood pressure > 160 mmHg	184 (44%)	1077 (52%)
Diastolic blood pressure > 90 mmHg	254 (60%)	1297 (63%)

Results

Headache was recorded in 420/2473 (17%) participants. Baseline characteristics and CT findings of the headache and non-headache groups are summarized in Tables 1 and 2. Follow-up was complete for all participants until close-out of the DTT. After that, 26 patients were lost to follow-up.

Table 2. CT characteristics at the time of the initial TIA or minor stroke.

CT findings (N=2362)	**headache** N=404	**no headache** N=1958
Any infarct	165 (41%)	820 (42%)
Irrelevant infarct only	33 (8%)	198 (10%)
Relevant infarct	132 (33%)	622 (32%)
Type and location of relevant infarct		
Anterior circulation	66 (50%)	462 (74%)
Posterior circulation	51 (39%)	90 (14%)
Cortical infarcts	51 (39%)	145 (23%)
Lacunar infarcts	40 (30%)	367 (59%)

CT, computer tomography.

The overall median follow-up was 14.1 years (IQR 13.1 - 15.1). The crude hazard ratio for vascular events for patients with headache as compared with those without headache was 0.75 (95% CI 0.66-0.89). After multivariable adjustment for all relevant variables the HR slightly increased to 0.83 (95% CI 0.71-0.97) (Table 3).

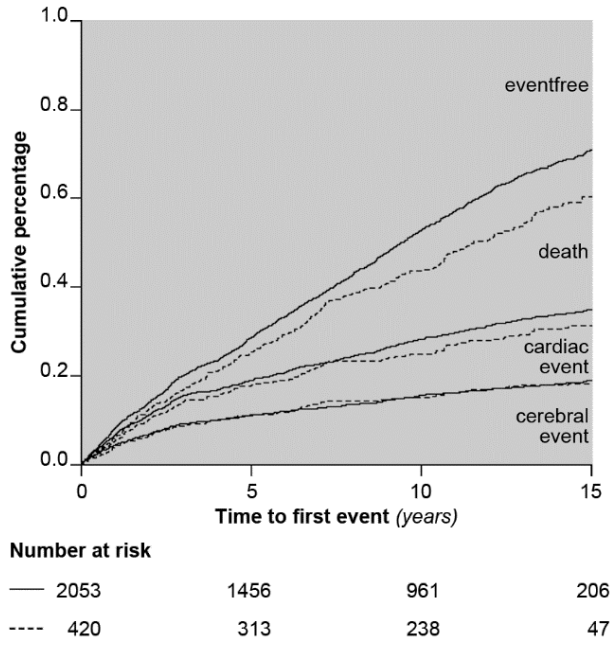
Table 3. Crude and adjusted hazard ratios for long-term vascular events.

Outcome event	Headache N=420	No headache N=2053	Crude HR (95% CI)	Adjusted HR (95% CI)[1]
First vascular event	196 (47%)	1144 (56%)	0.75 (0.66 - 0.89)	0.83 (0.71 - 0.97)
Cerebral event (fatal and non-fatal)	79 (19%)	395 (19%)	0.91 (0.72 - 1.16)	0.97 (0.76 - 1.24)
Cardiac event (fatal and non-fatal)	66 (16%)	364 (18%)	0.81 (0.63 - 1.06)	0.88 (0.67 - 1.14)
Death	210 (50%)	1279 (62%)	0.72 (0.62 - 0.83)	0.78 (0.68 - 0.91)
Vascular death	140 (33%)	936 (45%)	0.66 (0.55 - 0.79)	0.73 (0.61 - 0.87)
Non-vascular death	70 (17%)	343 (17%)	0.89 (0.69 - 1.15)	0.93 (0.72 - 1.21)

[1] *HRs are adjusted for age, sex, history of hypertension, history of angina, Modified Rankin Score (MRS), negative T wave on ECG and lacunar syndrome at presentation.*

Patients with headache tended to have a slightly reduced risk of fatal or non-fatal myocardial infarction (adjusted HR 0.88; 95% CI 0.67-1.14). No difference between the two groups was found in the risk of fatal or non-fatal stroke (adjusted HR 0.97; 95% CI 0.76-1.24). Patients with headache had a lower risk of death from vascular events than those without headache (adjusted HR 0.73; 95% CI 0.61-0.87), whereas no difference was found in the risk of non-vascular death.

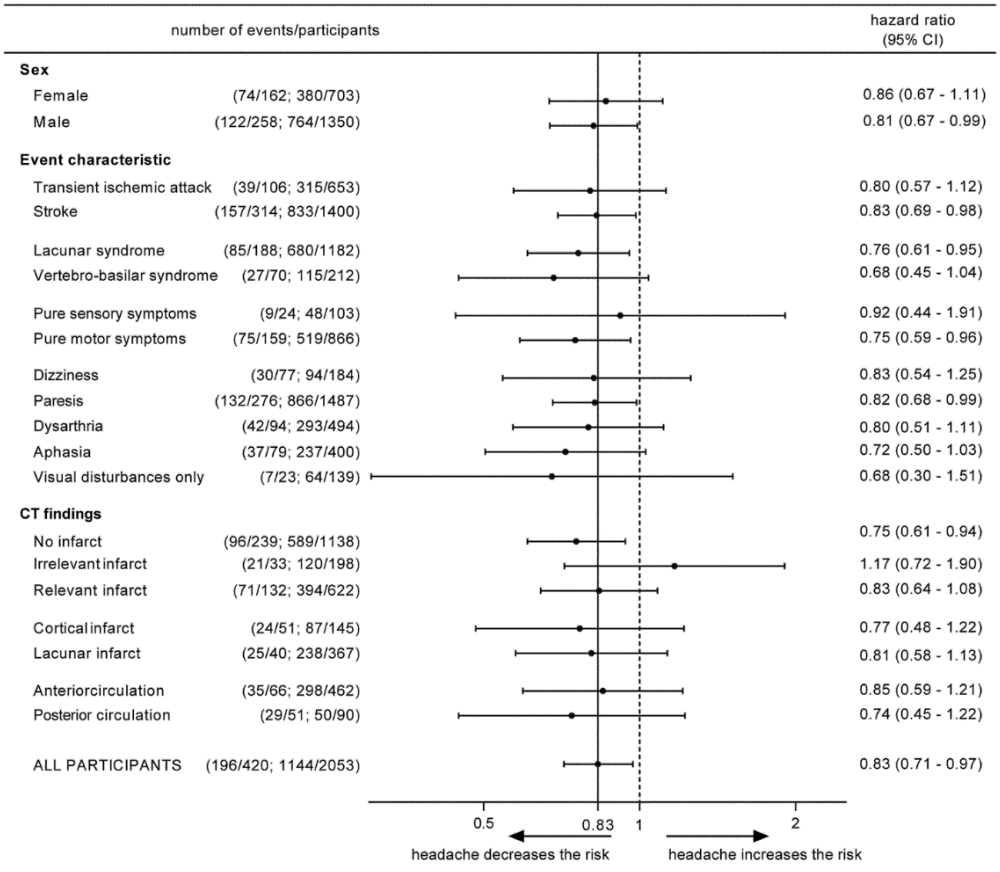
Figure 1. Non parametric cumulative incidence functions for cardiac events (fatal and non-fatal myocardial infarction), cerebral events (fatal and non-fatal ischaemic stroke) and death.



Solid and dashed lines represent participants without headache and with headache respectively.

The non-parametric cumulative incidence curves in Figure 1 illustrate the crude cumulative percentages of patients with and without headache who had a cardiac event or a recurrent cerebral event. At 5, 10 and 15 years of follow-up the cumulative incidence ratios of cardiac events to cerebral events were respectively 0.62 (95% CI 0.35-0.96), 0.64 (95% CI 0.41-0.94), 0.71 (95% CI 0.47-0.99) for the headache group and 0.70 (95% CI 0.57-0.86), 0.81 (95% CI 0.69-0.96), 0.84 (95% CI 0.73-0.97) for the no headache group. Cumulative incidence ratios did not differ significantly between the two groups at any time period (at 5, 10 and 15 years p=0.60; p=0.25; p=0.35, respectively).

Figure 2. Forest plot of hazard ratios and 95% confidence intervals for first vascular events by subgroups.



Numbers between parentheses indicate: number of patients with headache who experienced the event/total number of patients at risk; number of patients without headache who experienced the event/total number of patients at risk. Hazard ratios are adjusted for age, sex, history of angina, history of hypertension, modified Rankin Scale, negative T wave on ECG and lacunar syndrome at presentation. Horizontal line represents 95% confidence limits. Solid vertical line represents the overall adjusted hazard ratio for first vascular event in the entire population.

In a separate analysis, we assessed which of the demographic or clinical characteristics implied a particularly reduced risk of vascular events. Patients presenting with headache and vertebro-basilar syndrome (HR 0.68; 95% CI 0.45-1.04), aphasia (HR 0.72; 95% CI 0.50-1.03) or visual disturbances only (HR 0.68; 95% CI 0.30-1.51) were found to have the lowest risk (Figure 2), but hazard ratios did not differ in a statistically significant way between subgroups.

Discussion

We found that participants in whom a TIA or minor ischaemic stroke was associated with headache had a reduced long-term risk of recurrent vascular events compared with those without headache. This effect seems mainly due to a reduced risk of vascular death. Besides the lower risk of vascular death, our results also suggested a lower risk of cardiac events in participants with headache, whereas the risk of cerebral events seemed comparable to participants without headache. This different trend in cardiac events opposed to cerebral events, however, was not statistically significant.

Our study is the first comparing the long-term vascular prognosis of ischaemic stroke with and without concomitant headache. Onset headache was present in 17% of our population, more frequent among women, and more often associated with lesions involving the cortical and posterior circulation, as reported in other studies.[17, 20] Previous studies that focused on short-term outcome found no relationship between concomitant headache and stroke severity, in hospital mortality and 6 months outcome.[21, 22] The long duration of follow-up, the large number of participants and the large number of outcome events enabled us to adjust for several covariates. The hazard ratio for first vascular event remained statistically significant after adjustment for potential confounders.

The present study also has limitations. Detailed headache profiles, such as history of migraine, severity or location, were not recorded. Therefore we could not relate these characteristics to the outcomes. In addition, the selection of participants was restricted to non-cardio embolic TIA or minor stroke. The exclusion of large infarcts reduces the bias related to survival in the acute phase but could also limit the generalizability of the findings.

The Dutch TIA Trial was performed before the TOAST criteria were published, and no formal classification of stroke source was recorded. In the trial, patients with cerebral ischaemia due to identifiable causes other than arterial thrombosis or arterial embolism, including atrial fibrillation, were excluded and therefore, the participants of our study have cerebral ischaemia of *presumed* arterial origin. We cannot exclude, however, that some of our participants had a cryptogenic source of stroke which in some studies is related to a different outcome than other subtypes of stroke.[23, 24]

It is unlikely that the lower vascular event rate in the participants with headache is caused by a higher number of TIA mimics in this group, because there were no differences in hazard ratios between participants presenting with a TIA or a minor ischaemic stroke and participants with or without ischaemic lesions on the CT scan (Figure 2).[13, 25]

The more benign long-term vascular prognosis in patients with headache points to a possible different pathophysiology of the presenting TIA or minor stroke. Certain subtypes of stroke, including arterial dissections and the reversible cerebral vasoconstriction syndrome (RCVS), present more often with headache than others and are also associated with better long term prognosis after the event. [23, 26] Although arterial dissection and RCVS are relative rare causes of ischaemic stroke, we cannot exclude that they have played a role in our results.

It has been proposed that headache related to stroke is due to stimulation of sensory afferents of the trigeminovascular system.[8] The stimulation could be either directly by ischaemia or indirectly by factors associated with ischaemia. One factor that might play a role in the indirect stimulation of the trigeminovascular system is cortical spreading depression (SD).[7] SD is the likely mechanism for migraine aura and is characterized by slowly spreading waves of neuronal depolarization and associated changes in cerebral blood flow.[27] SD is related to stroke in different ways. First, it may increase susceptibility to stroke. Transgenic mice harboring the human familial hemiplegic migraine type 1 *CACNA1A* calcium channel gene mutation are highly susceptible to SD and have increased sensitivity to ischaemia, predisposing them to strokes during mild ischaemic events.[28, 29] Second, SD was found in the penumbra of non migrainous patients with middle cerebral artery infarction increasing the infarct lesion size.[30] It is unknown whether the occurrence of SD depends on subtype or cause of stroke and whether this has influence on the long-term prognosis of stroke patients.[31] Headache could be a marker of the presence of SD, even in patients without a history of migraine.

Blood pressure is another factor that is involved in the pathophysiology of stroke related headache. It was previously reported that one of the independent predictors of headache in patients with stroke is the absence of a history of hypertension.[32] This was also confirmed in our cohort and could suggest that atherosclerosis plays a less important role in the pathogenesis of stroke with headache.

We hypothesize that ischaemic stroke with concomitant headache reflects a different subtype of stroke compared to stroke related to other mechanisms, such as atherosclerosis. Future studies are required to confirm this.

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Chapter 7

Recurrence and mortality in young women with myocardial infarction or ischaemic stroke: long term follow-up of the RATIO study

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Abstract

Background: Little information is available on long term clinical outcome of young patients who survived a cardiovascular event.

Objective: To investigate the long term mortality and cardiovascular recurrences in young women who suffered from myocardial infarction or ischaemic stroke.

Methods: A cohort began in 1995 and completed in 1997 was followed up to 2012. Participants were young women (<50 years) with either myocardial infarction (n=226) or ischaemic stroke (n=160) or no history of arterial thrombosis (controls, n=782). Incidence rates (IR) and IR ratios (IRR) were calculated for arterial events and mortality during follow-up. Hazard ratios (HR) obtained from Cox proportional models were used to adjust for several cardiovascular risk factors. To determine whether hypercoagulability affects the risk or recurrence, a coagulation score based on procoagulant markers was compiled and used in a quartile analysis.

Results: During a median follow-up of 19 years, 83 deaths occurred. Mortality rates per 1000 person-years were 8.8 (95% Confidence Interval (CI) 6.2-12.3) in myocardial infarction patients, 4.4 (95% CI 2.4-7.6) in ischaemic stroke patients, and 2.4 (95% CI 1.7-3.4) in controls. Cardiovascular events occurred in 44 myocardial infarction patients, 37 ischaemic stroke patients and 13 controls, leading to IR per 1000 person-year of 12.1 (95% CI 8.7-16.2), 14.1 (95% CI 9.9-19.4) and 0.9 (95% CI 0.5-1.5), respectively. Adjusted HRs for cardiovascular events were 9.8 (95% CI, 5.0-19.4) in myocardial infarction cases and 12.9 (95% CI, 6.7-25.0) in ischaemic stroke cases compared with controls. A moderate relationship between the coagulation score and cardiovascular recurrences was observed in ischaemic stroke patients but not in myocardial infarction patients.

Conclusions: Patients who survived a myocardial infarction or ischaemic stroke at a young age have a high long term mortality and risk of recurrence. An increased coagulation tendency seems to play a role on the recurrences of ischaemic stroke but not of myocardial infarction. These findings provide a direct insight in the consequences of cardiovascular diseases in young women, which persist for decades after the initial event.

Introduction

The rates of death attributable to acute cardiovascular events have declined in the last decades, but the burden of the disease remains high in the increasing number of survivors.[1] This might be particularly important for those affected at a young age, in whom the impact on quality of life and on socioeconomic costs, considering their life expectancy, is the highest.[2, 3] Recent evidence showed that despite a better short-term prognosis in young patients, the long term mortality is unexpectedly high when compared with the elderly; nevertheless, only limited data exist on long-term follow-up in this age category.[4, 5]

Research into myocardial infarction and ischaemic stroke have traditionally focused on male and aging populations, leaving women and the young underrepresented in cardiovascular research.[6, 7] Therefore, knowledge on young onset cardiovascular disease is limited and it is unknown to what extent the results of the studies that include elderly males are applicable to women and the young.[8] Second, age-related risk factors are highly prevalent in elderly patients groups and could obscure the effects of non-age-related risk factors.[1] This makes studies focused on patients with a young age of onset more suitable to study non age-related risk factors.

Of those risk factors, many are shared by both myocardial infarction and ischaemic stroke, whereas there is emerging evidence that others are not. Previous research in young onset disease demonstrated that an increased clotting propensity is a risk factor for ischaemic stroke, whereas the risk of myocardial infarction is only affected marginally.[9-12] The difference in the role of coagulation in the aetiology of myocardial infarction and ischaemic stroke might also be relevant for the recurrence and prevention of these two diseases, and raises the question whether secondary prevention for ischaemic stroke and myocardial infarction should differ more than it does now.

In this study we determined long term mortality and morbidity in young women who survived myocardial infarction or ischaemic stroke compared with a control group. Moreover, we assessed the impact of known cardiovascular risk factors and of an increased coagulation propensity on the risk of recurrent arterial thrombotic events.

Methods

Patients

Subjects who participated in the Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) case-control study form the basis of the RATIO follow-up study. For the present study two groups of patients (subjects who survived either a myocardial infarction or an ischaemic stroke) and controls (subjects without an arterial thrombotic event) were included. Patient selection within the RATIO case-control study has been described in detail previously.[13-15] In short, all women 18 to 50 years of age who presented with a first event of myocardial infarction or ischaemic stroke (index events) to one of the 16 participating hospitals in the Netherlands between 1990 and 1995 were eligible and approached for study participation. Diagnosis was made on the basis of clinical symptoms and confirmed by appropriate tests. Myocardial infarction was diagnosed by the presence of clinical symptoms, elevated cardiac enzyme levels, and corresponding electrocardiographic changes. Clinical symptoms of ischaemic stroke were confirmed by either computed tomography or magnetic resonance imaging. Ischaemic stroke of cardioembolic origin was excluded by the presence of atrial fibrillation or suggestive cardiac ultrasound imaging. All cases were included from 1995 to 1998. Women were approached to participate as a control subject by random digit dialling and were matched according to age, area of residence, and year of event. A standardized questionnaire on patient characteristics and possible cardiovascular risk factors such as familial medical history, hypertension, diabetes, hypercholesterolemia, and smoking habits was filled in by both cases and controls. Some of these questions were targeted to the year before diagnosis (cases) or the matched index year (controls). All participants were requested to donate blood or buccal swab for DNA analyses. Blood draw procedures and laboratory procedures are described in details in the previous publications. Blood samples, together with a questionnaire on patient characteristics, were collected after a median of 69 months (range, 38 to 117 months) for myocardial infarction cases and 95 months (range, 23 to 146 months) for ischaemic stroke cases, thereby ensuring blood was drawn after the acute phase. All participants gave informed consent and the study was approved by the ethics committees of the participating hospitals.[13]

Follow-up data

In 2013, data on participants of the RATIO case-control study were linked to the Dutch Registry of death certificates and to the Dutch Hospital Data registry by the Central Bureau of Statistics of the Netherlands. The first provides both primary and secondary causes of deaths coded according to The International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) classification. The second provides nationwide electronic coverage of data on all hospital admissions since 1995. Data are collected in virtually all general and university hospitals and most specialized clinics. For each hospital admission, information on the date of admission and discharge, diagnoses, and surgical procedures is available. These diagnoses are encoded according to ICD 9th Revision (ICD-9). A previous study comparing a random sample of hospital admissions in the Dutch Hospital Data registry to information from hospital records showed that 99% of the personal (age, sex, date of birth and postal code) and administrative data (date of admission, discharge and death) and 84% of principal diagnoses were correctly encoded.[16] For myocardial infarction, the percentage of correctly encoded diagnosis has since been found to be almost 100%.[17]

Participants of the RATIO case-control study were linked to this registry through date of birth, sex, and postal code. Individuals with information leading to more than one person (e.g., twins or individuals with the same date of birth in the same postal area) or to nobody at all, were excluded.

Outcomes

After linkage to the to the Dutch Registry of death certificates and to the Dutch Hospital Data registry, death, class of death and any further acute arterial thrombotic event were identified. For this study prespecified outcomes were 1) overall mortality and 2) the first occurrence of an acute cardiovascular event, either myocardial infarction or ischaemic stroke, whichever occurred first. Cardiovascular events were further analysed separately as the first occurrence of myocardial infarction and the first occurrence of ischaemic stroke. Mortality was classified as vascular (codes ICD-10 I20 to I95) and non-vascular. The outcome myocardial infarction includes the diagnoses defined by the ICD-9-CM codes 4100 to 4109 and the cause of death

defined by the ICD-10 code I21. The outcome ischaemic stroke includes the diagnosis defined by the ICD-9-CM codes 433, 4330 to 4333, 4338, 4339, 434, 4340, 4341, and 4349 and codes 4350- to 4359 (transient ischaemic stroke), and the cause of death defined by the ICD-10 codes I63 and I64. We chose to include these two acute cardiovascular diseases only, as these will invariably lead to hospitalization and were therefore likely to be captured by the Dutch Hospital Data registry.

Statistical Analyses

Rates of the prespecified outcomes were measured by dividing the number of events by the observation time, which was defined as the time between the index date and the end of follow-up. Follow-up ended on the date of a first incident cardiovascular event, the date of death, or 31st December 2012, whichever came first. Median follow-up was calculated using the estimates of the censoring distribution for overall mortality. The crude incidence rates (IR) and their 95% confidence intervals (CI) were based on a Poisson distribution and expressed per 1000 persons-years at risk. Kaplan-Meier curves were used to plot the survival for patients and controls subjects. The relative risk of cardiovascular event for patients vs control subjects was estimated by hazard ratios (HR) and corresponding 95% CI with a Cox proportional hazard model. Potential sources of confounding, i.e., age, sex, body mass index (BMI), alcohol consumption, smoking history, diabetes mellitus, hypertension, hyperlipidaemia and family history of a cardiovascular event (i.e., any acute cardiovascular event before 60 years old in a first relative) were included as covariables.

Coagulation score

To explore the influence of an increased coagulation propensity on the risk of a recurrent cardiovascular event we compiled an individual prothrombotic score (coagulation score). The score was constructed with coagulation markers measured in both patient groups and in control subjects, being: 1) tissue factor/tissue plasminogen activator induced clot-lysis time (CLT) as a measure of fibrinolytic potential [11]; 2) antigen levels of coagulation factors of the intrinsic coagulation system (factor XII, FXII, and FXI and prekallikrein) [18]; 3) inhibitor complexes of the serine proteases of the intrinsic coagulation system (C1 esterase inhibitor

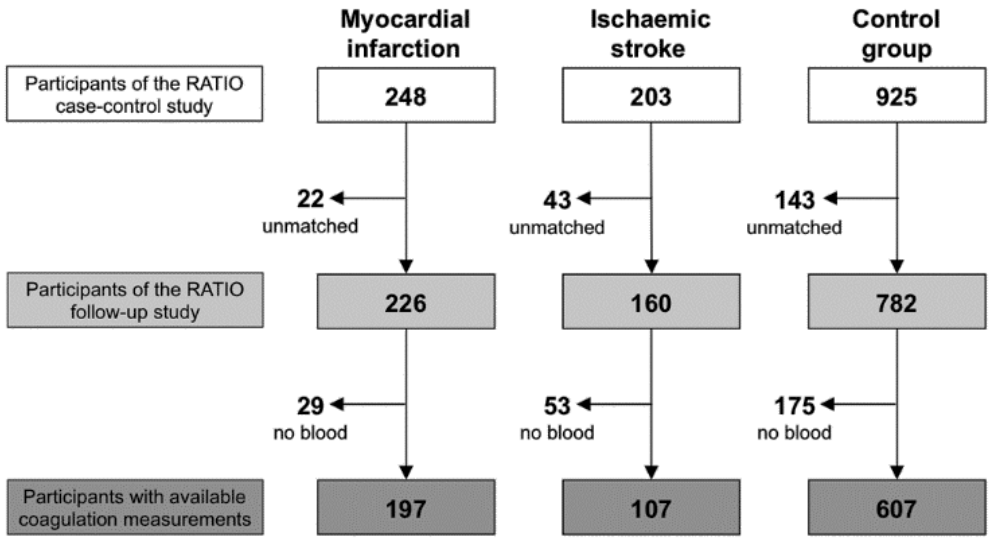
for FXIIa, FXIa, Kallikrein, and antitrypsin inhibitor) as measures of the activation of intrinsic coagulation factors [12]; 4) a disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13) antigen levels [19]; 5) von Willebrand factor (VWF) antigen levels [19]; 6) antiphospholipid antibody tests (presence of lupus anticoagulant detected with dilute Russell's viper venom time (dRVVT) reagents, IgG anticardiolipin antibody concentrations, IgG anti-β2-glycoprotein I concentrations and anti-prothrombin antibodies concentrations) [9]; 7) presence of factor V Leiden (either heterozygous or homozygous) [20, 21]; 8) presence of G20210A mutation in the prothrombin gene (either heterozygous or homozygous) [20, 21]; 9) presence of the homozygous form of the methylenetetrahydrofolate reductase (MTHFR) C677T [20, 21]. The score was based on the beta coefficients, adjusted for matching variables, obtained from logistic regression models for the association between each coagulation marker and index ischaemic stroke. These analyses were based on dichotomous exposures, with the 90th percentile of the control group distribution as cut off value, or on the presence of the genetic variant, or on the test positivity, whichever was the most appropriate. These beta coefficients were summed so that the compiled score represents the coagulation weighted prothrombotic potential for each patients, with higher values of the score corresponding to a higher levels of thrombotic propensity.

To investigate if high values of the coagulation score increased the risk of a recurrent event, we applied a Cox proportional hazard model based on the quartiles of the prothrombotic score distribution as exposure categories, with the lowest quartile as reference. The model was adjusted for age, sex, BMI, alcohol consumption, smoking history, diabetes mellitus, hypertension, hyperlipidaemia and family history of a cardiovascular event.

Results

1376 women (248 myocardial infarction cases, 203 ischaemic stroke cases and 925 controls) participated into the RATIO case-control study. Of these women, 1168 (87%) were successfully linked to the registries (Figure 1). The follow-up therefore included 226 patients with myocardial infarction, 160 patients with ischaemic stroke and 782 control subjects. Median follow-up was 18.7 years (IQR 17.5-20.5).

Figure 1. Flow chart for study participants.



RATIO, Risk of Arterial Thrombosis in Relation to Oral Contraceptives.

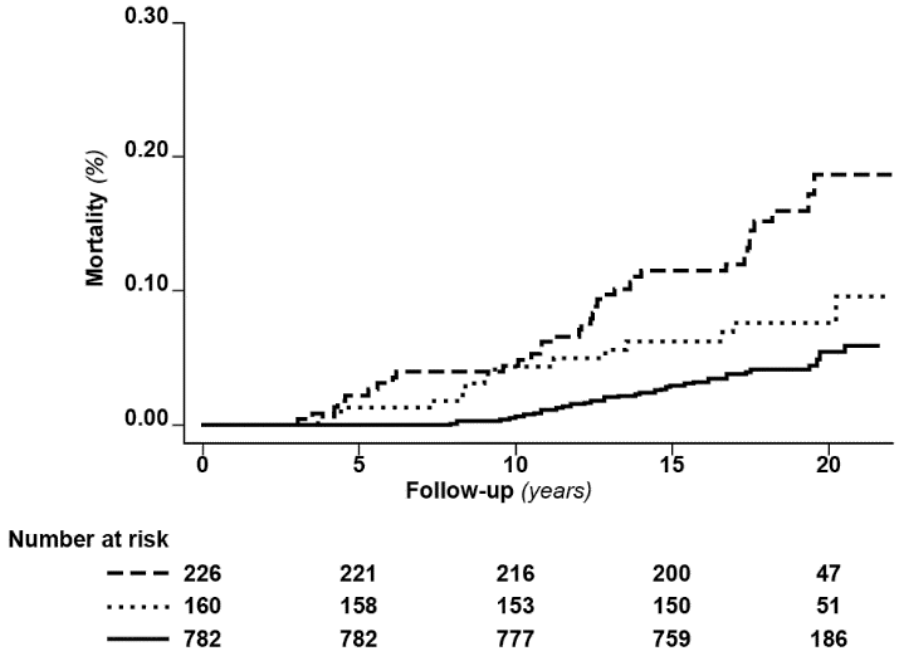
Table 1. Baseline characteristics for patients and control subjects.

	Myocardial infarction n=226	**Ischaemic stroke** n=160	**Control group** n=782
Demographic characteristics			
Age at the event (SD)	42.4 (6.1)	40.0 (7.5)	48.4 (7.9)
BMI (SD)	27.1 (5.4)	25.5 (5.7)	24.3 (4)
Median follow-up (years) (IQR)	18.6 (17.2-20.1)	18.9 (17.3-20.7)	18.7 (17.5-20.5)
History of, n (%)			
Diabetes mellitus	11 (5%)	9 (6%)	12 (2%)
Hypertension	56 (25%)	41 (26%)	48 (6%)
Hyperlipidaemia	25 (11%)	9 (6%)	20 (3%)
Alcohol consumption	138 (61%)	90 (57%)	532 (69%)
Smoking	188 (83%)	100 (63%)	333 (43%)
Cardiovascular family history*	145 (66%)	82 (56%)	269 (37%)

* Data available for 97% of the subjects in the myocardial infarction group, 92% of the subjects in the ischaemic stroke group, and 94% of the subjects in the control group. SD, standard deviation; IQR, interquartile range.

Clinical characteristics of patients and control subjects at the start of follow-up are depicted in Table 1. Mean age was 44 years, and was similar between patients subgroups. Both myocardial infarction and ischaemic stroke patients had a markedly higher prevalence of traditional cardiovascular risk factors than control subjects. The coagulation score could be calculated for 197 patients with myocardial infarction and 107 patients with ischaemic stroke, for whom blood was available (Figure 1). Mean values of the score were 1.08 (min -0.36, max 5.73) in patients with myocardial infarction and 2.06 (min -0.28, max 9.03) in patients with ischaemic stroke.

Figure 2. Kaplan-Meier curves for overall mortality.



The dashed line represents women with myocardial infarction, the dotted line represents women with ischaemic stroke and the solid line represents controls.

Mortality

During follow-up, 35 (16%) patients with myocardial infarction, 13 (8%) patients with ischaemic stroke, and 35 (6%) control subjects died, leading to a mortality rate per 1000 person-year of 8.8 (95% CI 6.2-12.3) in the myocardial infarction group , 4.4 (95% CI 2.4-7.6) in the ischaemic stroke group, and 2.4 (95% CI 1.7-3.4) in the control group (Table 2). The risk of death was almost 4 times higher for patients with myocardial infarction (IRR 3.7, 95% CI 2.5-5.4) and two times higher for patients with ischaemic stroke (IRR 1.8, 95% CI 1.0-3.5) than for control subjects. Figure 2 shows Kaplan Meier curves for cumulative mortality in all three groups.

Table 2. Incidence rates for overall mortality and vascular mortality for patients and control subjects.

	Outcome Events	py	Incidence rate (95% CI) per 1000 py	Incidence rate ratio (95% CI)
Outcome: overall mortality				
Myocardial infarction n=226	35 (16%)	3959	**8.8 (6.2-12.3)**	3.7 (2.5-5.4)
Ischaemic stroke n=160	13 (8%)	2928	**4.4 (2.36-7.59)**	1.8 (1.0-3.5)
Control group n=782	35 (6%)	14436	**2.4 (1.7-3.4)**	ref
Outcome: vascular mortality				
Myocardial infarction n=226	14 (6%)	3959	**3.5 (1.9-5.9)**	12.8 (5.0-32.4)
Ischaemic stroke n=160	6 (4%)	2928	**2.1 (0.75-4.46)**	7.4 (2.6-26.2)
Control group n=782	4 (1%)	14436	**0.3 (0.1-0.7)**	ref

Overall mortality includes deaths from any cause, whereas vascular mortality includes only deaths from an acute vascular event. Total persons-year depends on the analysis. Incident rate ratios are not adjusted. CI, confidence interval; py, persons-year; ref, reference.

In patients, almost one in two deaths were related to a recurrent vascular event (15/35 for patients with myocardial infarction, leading to a vascular mortality rate of 3.5, 95% CI 1.9-5.9 per 1000 person-year; 6/13 for patients with ischaemic stroke, yielding a vascular mortality rate of 2.1, 95% CI 0.8-4.5 per 1000 person-year), whereas in the control group vascular deaths were less than one seventh of the overall deaths (vascular mortality rate 0.3, 95% CI 0.1-0.7 per 1000 person-year). So, the risk of cardiovascular death was 13 times higher for myocardial infarction patients (IRR 12.8, 95% CI 5.1-32.4) and 7 times higher for ischaemic stroke patients (IRR 7.4, 95% CI 2.6-26.2) than for control subjects.

Incident cardiovascular events

Overall, 94 participants experienced a cardiovascular event during follow-up (either myocardial infarction or ischaemic stroke). 44 patients with myocardial infarction and 37 patients with ischaemic stroke had a recurrent event during follow-up, leading to an incidence rate per 1000 person-year of 12.1 (95% CI 8.7-16.2) and 14.1 (95% CI 9.9-19.4), respectively (Table 3), whereas the incidence rate for cardiovascular event in the control group was 0.9 (95% CI 0.5-1.5). Compared with the control group, myocardial infarction patients had a 12-fold increased risk of a cardiovascular event (age adjusted HR 12.4, 95% CI 6.6-23.1), and ischaemic stroke patients a 15-fold increased risk (age adjusted HR 15.0, 95% CI 7.9-28.1). These relative risks slightly decreased when BMI and a history of alcohol consumption and smoking were taken into account (Table 3). These estimates remained essentially the same upon additional adjustments for chronic diseases such as diabetes, hypertension and hypercholesterolemia, and for a positive family history of cardiovascular disorder.

There was a strong relationship between the type of recurrent event (myocardial infarction or ischaemic stroke) and the index thrombotic event. The incidence rate per 1000 person-years of myocardial infarction during follow-up was higher in myocardial infarction patients (IR 10.1, 95% CI, 7.5-13.8) than in ischaemic stroke patients (IR 2.7, 95% CI, 1.2-5.4) or control subjects (IR 0.4, 95%CI 0.2-0.9). Similarly, incidence rate per 1000 person-years of ischaemic stroke was higher in ischaemic stroke patients (IR 11.1, 95% CI 7.5-15.9) than in myocardial infarction patients (IR 1.9, 95% CI, 0.8-3.8) or control subjects (IR 0.5, 95% CI, 0.2-1.0).

Table 3. Incidence rates and hazard ratios for cardiovascular events.

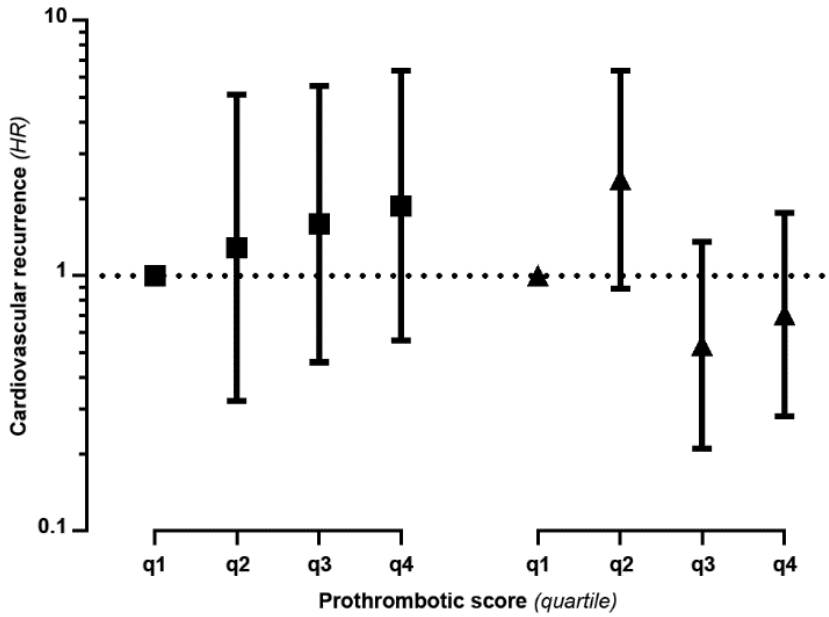
	Events, n (%)	py	Incidence rate per 1000 py (95% CI)	HR (95% CI)	HR_1 (95% CI)	HR_2 (95% CI)	HR_3 (95% CI)
Outcome: any cardiovascular event							
Myocardial infarction n=226	44 (20%)	3654	**12.0 (8.7-16.2)**	12.4 (6.6-23.1)	10.1 (5.14-19.72)	9.6 (4.9-18.9)	9.8 (5.0-19.4)
Ischaemic stroke n=160	37 (23%)	2627	**14.1 (9.9-19.4)**	15.0 (7.9-28.2)	13.3 (10.0-25.3)	12.8 (6.6-24.7)	13.0 (6.7-25.0)
Control group n=782	13 (2%)	14557	**0.9 (0.5-1.5)**	ref	ref	ref	ref
Outcome: myocardial infarction							
Myocardial infarction n=226	37 (16%)	3696	**10.0 (7.1-13.1)**	22.4 (9.3-53.9)	20.9 (8.1-54.0)	18.5 (7.1-48.0)	19.6 (8.0-51.0)
Ischaemic stroke n=160	8 (5%)	2941	**2.7 (1.2-5.4)**	6.5 (2.6-18.7)	6.0 (2.1-17.6)	5.4 (1.8-16.2)	5.6 (1.9-16.8)
Control group n=782	6 (1%)	14588	**0.4 (0.2-0.9)**	ref	ref	ref	ref
Outcome: ischaemic stroke							
Myocardial infarction n=226	8 (4%)	4152	**1.9 (0.8-3.8)**	3.8 (1.4-10.5)	2.8 (1.0-8.3)	2.8 (1.0-8.2)	2.7 (0.9-8.1)
Ischaemic stroke n=160	30 (19%)	2707	**11.1 (7.5-15.8)**	21.9 (9.6-49.9)	19.1 (8.3-44.3)	18.2 (7.7-42.8)	17.9 (7.6-42.2)
Control group n=782	7 (1%)	14601	**0.5 (0.2-1.0)**	ref	ref	ref	ref

Hazard ratios (HR) are obtained by multivariable Cox regression models and are all adjusted for age. HR_1 are also adjusted for smoking, alcohol consumption and BMI. HR_2 are adjusted for #1 and additionally for history of diabetes, hypertension, and hypercholesterolemia. HR_3 are adjusted for #1 and #2 and additionally for family history of a cardiovascular event. CI, confidence interval; py, persons-year; ref, reference.

Coagulation score

Figure 3 shows the association between quartiles of the coagulation score and the risk of a recurrent cardiovascular event, with the first quartile as reference category. Patients with myocardial infarction and high values of the coagulation score (i.e., high thrombotic propensity) had no increase in the risk of a recurrent event compared with patients with low values of the score (i.e., low thrombotic propensity; fourth quartile vs first quartile HR 0.7, 95% CI, 0.3-1.8).

Figure 3. Hazard ratios for cardiovascular recurrence by quartiles of the coagulation score.



Squares indicate hazard ratios for cardiovascular recurrences in patients with ischaemic stroke by quartile of the coagulation score, whereas triangles indicate hazard ratios for cardiovascular recurrences in patients with myocardial infarction by quartile of the coagulation score. Hazard ratios are obtained by Cox proportional hazard models and are all adjusted for age, smoking, alcohol consumption, BMI, history of diabetes, hypertension, and hypercholesterolemia and family history of a cardiovascular event. Dashed lines indicate 95% confidence interval. The y axis scale is logarithmic. q indicates quartile of the coagulation score. The first quartile (q1) is the reference category.

On the contrary, a doubling of the risk of cardiovascular recurrences was observed in patients with ischaemic stroke and high values of the coagulation score compared with patients with low values (fourth quartile vs first quartile HR 1.9, 95% CI 0.6-6.3), with evidence of a “dose response” relationship (second quartile vs first quartile HR 1.3, 95% CI, 0.3-5.1, and third quartile vs first quartile HR 1.6, 95% CI, 0.5-5.6).

Discussion

In the present study we demonstrated that young women who survived myocardial infarction or ischaemic stroke have a high long term mortality, four times higher for patients with myocardial infarction (IRR 3.7, 95% CI 2.5-5.4) and two times higher for patients with ischaemic stroke (IRR 1.8, 95% CI 1.0-3.5) than control subjects. This high mortality is mainly due to a high incidence of fatal vascular events.

Data on long term mortality in young patients who suffered from an arterial thrombotic event are scarce and in our knowledge this is the study with the longest follow-up and the first comparing directly patients with myocardial infarction, ischaemic stroke and control subjects. The longest follow-up available in literature for young ischaemic stroke patients originated from a Dutch and an Italian cohort (median follow-up 8.3 and 11.7 years respectively), in which mortality rates ranged from 12 to 18 per 1000 persons-year.[5, 22] Similar mortality rates in young survivors of myocardial infarction have been reported in two USA cohorts (median follow-up 3.7 and 12.7 years).[23, 24] We found mortality rates slightly lower than the ones reported in those cohorts for both myocardial infarction and ischaemic stroke (mortality rate per 1000 persons-year 8.8, 95% CI, 6.2-12.3 for myocardial infarction and 4.4, 95% CI, 2.4-7.6 for ischaemic stroke). This could have several explanations. First, mortality rates in females after a thrombotic event are expected to be lower than the corresponding rates in males.[5, 25] Second, because patients enrolled in our study after a median time of 1.5 years from the event, mortality rates might be underestimated due to immortal time bias (i.e., a period of follow-up during which, by design, death cannot occur). Mortality is known to be higher in the first year after a thrombotic event, and then become stable over the following years.[5] Nevertheless, we found that long term mortality was twice as high in myocardial infarction patients than in ischaemic stroke patients. This difference is unlikely to be explained by immortal time bias, because it should affect both diseases equally, and we believe it may reflect a true difference in long term prognosis between the two diseases. We should note, however, that cardioembolic strokes, that are associated with the worst prognosis among ischaemic stroke subtypes, were excluded from our cohort.[5]

Despite the difference in mortality rates, cardiovascular recurrence rates were similar between myocardial infarction and ischaemic stroke patients (IR per 1000 person-years 12.1, 95% CI, 8.7-16.2 and 14.1, 95% CI 9.9-19.4 respectively), and were in line with previous

separately published results for ischaemic stroke and myocardial infarction.[24, 26-28] Myocardial infarction patients had a 12-fold increased risk of any arterial thrombotic event (HR 12.4, 95% CI 6.6-23.1) and ischaemic stroke patients a 15-fold increased risk (HR 15.0, 95% CI 7.9-28.1) compared with the control group. These relative risks attenuated only slightly when modifiable cardiovascular risk factors such as smoking, alcohol consumption and BMI were taken into account, but did not reduce further when chronic diseases such as diabetes, hyperlipidemia or hypertension and family history of cardiovascular diseases were added to the regression model (fully adjusted HRs 9.8, 95% CI, 5.0-19.4 and 12.9, 95% CI 6.7-25.0, respectively). This suggests that even when other classical cardiovascular risk factors are taken into account, the risk of a subsequent vascular event remains high in patients with myocardial infarction or ischaemic stroke at young age compared with the general population. Type of recurrence (cardiac or cerebral) was found to be true to type, i.e., the recurrence risk for cerebrovascular disease was highest in stroke patients, whereas the risk of cardiac events was highest in patients with a myocardial infarction. This finding is supported by other studies that investigated ischaemic stroke and myocardial infarction separately.[24, 29]

Previous studies showed that classical risk factors for the first episode, such as hypertension and hyperlipidaemia, were not associated with recurrences in both stroke and myocardial infarction.[24, 26] It is well known that the risk profile of a recurrent event generally can be entirely different from that of a first event and that often a risk factor is found to be numerically weaker for a second event than for a first.[30, 31] Here we investigated if procoagulant status, affects the risk of recurrent cardiovascular events. For this purpose we compiled a coagulation score that included several markers of hypercoagulability, both acquired and inherited. The score was weighted on the risk of the index ischaemic stroke event, because index ischaemic stroke has been shown to be the arterial event in which hypercoagulability plays the greatest role.[32, 33] In this way, the coagulation score represents the individual prothrombotic propensity. When we analyzed the relationship between the score and recurrence we found that high prothrombotic propensity was associated with the risk of vascular recurrences in ischaemic stroke patients, but not in myocardial infarction patients (HR for the fourth quartile vs the lowest quartile 0.7, 95% CI, 0.3-1.8 in patients with myocardial infarction and 1.9, 95% CI 0.6-6.3 in patients with ischaemic stroke). Although women with ischaemic stroke and an overt cardiac-embolic-source were excluded from this study, all other subtypes were combined as data needed for classification were not

available.[34] This hampers the ability to better elucidate the pathophysiological mechanisms beyond our observation. However, we believe our finding may have clinical relevance, given its possible implications on secondary prevention, especially in the era of the direct oral anticoagulants (inhibitors of factor IIa, and factor Xa), that represent new treatment options to establish a more targeted anticoagulation.

As this is an observational study in which blood samples were collected after the index event, one could argue that levels of procoagulant markers in our study may have been affected by acute-phase reactions. However, we consider it unlikely that the effect of procoagulant markers was a result of the acute-phase because procoagulant markers were obtained at least 1.5 year after the index event for both groups of patients.

Strengths of our study are the long follow-up and the homogeneity of data collection. It was therefore possible to compare the risk of death and recurrences in myocardial infarction patients, ischaemic stroke patients and control subjects.

Some caveats should be made. A possible limitation to our study is that arterial cardiovascular events (both myocardial infarction and ischaemic stroke) were not objectively confirmed, but obtained from the Dutch Hospital Data registry. However, a previous study showed that the percentage of correctly encoded myocardial infarctions in this registry was almost 100%.[17] In addition, although the exact percentage of correctly encoded ischaemic strokes is unknown, it is unlikely that any misclassification in this diagnosis would have occurred differently in our two patient groups and the control group. Another limitation is that, despite the long follow-up, numbers of arterial cardiovascular events in some subgroups were small, leading to imprecision of the estimated rates. Finally, in our study we did not have information on medication use during the follow-up period.

In conclusion, we showed that women who survived from an arterial thrombotic event at young age have a high mortality and morbidity. Mortality was higher for myocardial infarction survivors than ischaemic stroke survivors despite the same risk of vascular recurrences, and was mainly due to a high incidence of fatal vascular event during follow-up. Cardiovascular recurrences were true to type and an increased coagulation tendency played a role on the recurrences in woman with ischaemic stroke but not in women with

myocardial infarction. These findings provide a direct insight in the consequences of cardiovascular diseases in young women, which persist for decades after the initial event.

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Chapter 8

General Discussion

Abstract

This thesis reports on studies that have investigated the role of several factors on the risk of arterial thrombosis. The thesis is organised in two sections. The first section (Chapters 2-5) deals with risk factors for the first arterial thrombotic event whereas the second section (Chapters 6 and 7) concerns risk factors for recurrence. The investigations touch several aspects of epidemiology, and make use of several observational study designs, encompassing case control, cohort and meta-analysis. Several statistical models were also applied ranging from logistic regression models, Cox proportional hazards time to event regression models as well as non-parametric statistics. Because of the heterogeneous nature, each investigation had specific problems in study design, data collection and analyses. A common line, however, is the haemostatic balance and its markers.

The studies are here summarised, and details of the methodology along with the possible implications in understanding the pathophysiology of arterial thrombosis are discussed. Additionally, a comparison between myocardial infarction and ischaemic stroke as well as hypothetic links between arterial and venous thrombosis are taken into consideration.

Summary and discussion about aetiology of arterial thrombosis

Anyone intuitively senses that explaining and predicting are different.[1] Aetiological research aims to assess the presence or absence of a presumed causal relationship between a putative risk factor and a specific clinical condition. In this particular perspective, confounding and knowledge of the pathophysiology of the disease play a fundamental role.[2,3]

Arterial thrombosis is a multicausal disease, in which a single complete causal mechanism (also called sufficient cause by Kenneth Rothman) requires the joint action of many component factors (component causes).[4] A set of component causes occurring together may complete the causal mechanism, creating a sufficient cause and thus initiating the disease process.

Based on this model, the starting point to understand the aetiology of a disease is finding risk factors (component causes) for that particular disease. However, to find an association as a component cause of a disease, it is fundamental that we establish whether the association between this factor and the outcome is causal. In 1964, Hill proposed some criteria, which in turn were anticipated by the inductive canons of John Stuart Mill, that subsequently have widely been adopted to evaluate whether an association might be interpreted as causal.[5] Those are (1) strength, (2) consistency, (3) specificity, (4) temporality, (5) biological gradient, (6) plausibility, (7) coherence, (8) experimental evidence, and (9) analogy.[5] Rothman contends that Hill's criteria fail to clearly distinguish causal from non-causal relations.[4] The problem of causality can rarely, if ever, be resolved only on the basis of these criteria considered in an isolated manner, and establishing the nature of a given relationship (causal vs non causal) is a multidimensional process, demanding detailed knowledge on pathophysiological pathways.[2] In summary, the problem of causality in biomedical research usually demands application of various kind of studies, that is experimental and observational studies as well. Nevertheless, a well performed epidemiological study can provide the starting point to assess the potential causal role of putative risk factors in human diseases.

Risk factors for new arterial thrombotic events

The first part of the thesis deals with risk factors for a first arterial thrombotic event and faces the problems related to aetiological research.

Chapter 2 reports a study on the association between low levels of ADAMTS13 and the risk of a first myocardial infarction. The design is that of a case-control study based on individual patient data meta-analysis. The large sample size achieved allowed to investigate extreme levels of ADAMTS13, showing that only very low levels of ADMATS13 are associated with an increased risk of myocardial infarction. This meta-analytical approach based on individual participant data has many potential advantages over meta-analysis of aggregate data, both statistically and clinically, that have been extensively described in Chapter 2.[6,7] In order to establish if the observed association is causal, a further discussion is necessary. Firstly, because all included studies had a case-control design, we faced some limitations. In a case-control design blood samples can only be collected after the event, making it difficult to establish the temporality between the presumed cause and the effect.[8] Secondly, publication bias, residual confounding, recall bias and survivor bias all play a role in the over- or underestimation of the causal effects of interest, even though particular attention has been given to the selection of the studies included in the meta-analysis and to the inclusion of possible sources of confounding in the statistical models. Therefore, even with a well-designed epidemiological study, how can it be said that ADAMTS13 is part of the causal mechanism of myocardial infarction? The previous knowledge of the disease pathophysiology could help. The hypothesis that ADAMTS13 is implicated in the pathophysiology of myocardial infarction is related to its regulatory function on von Willebrand Factor (VWF), which previously has been strongly associated with arterial thrombosis.[9,10] Therefore, there is a strong biological plausibility that ADAMTS13 is involved in the pathophysiology of myocardial infarction. Furthermore, there is experimental evidence that supports a causal relationship between ADAMTS13 and myocardial infarction. ADAMTS13 knockout mice developed larger myocardial infarctions after coronary occlusion and showed decreased left ventricular function when compared with wild-type mice. Also, treatment with recombinant ADAMTS13 (rADAMTS13) reduced infarct size in wild-type mice.[11-13] Finally, similar results were found in the Rotterdam prospective cohort, so our findings meet the criteria of consistency.[14] All these considerations support a causal

relationship in the association between ADAMTS13 and myocardial infarction. Thanks to the IPD design, we also could perform additional analyses, i.e., of mediation and interaction between ADAMTS13 and VWF, in order to better elucidate the causal role of ADAMTS13 in myocardial infarction. We found that the effect of ADAMTS13 on the risk of myocardial infarction is not mediated by VWF, as might be expected. Therefore this study suggests that ADAMTS13 and VWF are both risk factors with different pathways, as also was shown in other studies.[15,16] These observations can support the following sufficient cause model: even though ADAMTS13 activity below the lowest bound of the normal range (i.e. ~50%) is high enough for cleavage of plasma VWF (as it has been shown in ADAMTS13 heterozygous individuals), locally, at sites of endothelial injury (component cause), the reduced ADAMTS13 activity may lead to a reduced cleavage of secreted large VWF multimers, and thereby contribute to local thrombus formation.

Chapter 3 reports a study on the association between pregnancy loss and both forms of arterial thrombosis in the frame of the original RATIO case-control study. This chapter is based on a case-control design. In this study, the concept of causation is more controversial. Indeed, pregnancy loss has a link with several comorbidities, including risk factors for arterial thrombosis (such as diabetes, hypertension, hyperlipidaemia, obesity and tobacco use).[17] Moreover, pregnancy loss is also associated with hypercoagulability, and especially with the presence of antiphospholipid antibodies, a well-known prothrombotic condition.[17-19] The aim of this chapter was to provide an improved understanding of the link between pregnancy loss and arterial thrombosis. Pregnancy loss itself, however, cannot be viewed as a cause of arterial thrombosis, but rather a proxy of a series of component causes. Being a composite measure, it violates the consistency assumption and causal inference on the exact aetiologic mechanism cannot be made. Indeed, even after adjustment for several variables (i.e., cardiovascular risk factors, cardiovascular family history and the presence of antiphospholipid antibodies) it is likely that residual confounding plays a role in the direction and strength of the association. We found that recurrent miscarriages and stillbirth were associated with ischaemic stroke whereas the risk of myocardial infarction was only marginally affected. When myocardial infarction and ischaemic stroke are two similar diseases with the same aetiology, it is likely that these markers would have similar associations. Therefore, even though no formal causal assertion can be made, these data at

least suggest that myocardial infarction and ischaemic stroke have some differential aetiologic mechanisms. This concept is further investigated in the following chapters.

The coagulation balance is the main topic of the central part of the thesis. Chapter 4 and Chapter 5 deal both with the same question: does hypercoagulability play a similar role on the risk of the two main forms of arterial thrombosis, myocardial infarction and ischaemic stroke? The term hypercoagulability stands for an increased prothrombotic tendency, caused either by an elevated procoagulant activity or by a decreased anticoagulant activity. In these two chapters the aetiological question is crucial, but rather than referring to a single marker, it refers to the whole spectre of hypercoagulability. In **Chapter 4** the question is investigated in the frame of the RATIO case-control study. The role of almost 30 markers of hypercoagulability on the risk of myocardial infarction is directly compared with their role on the risk of ischaemic stroke by using the relative odds ratio. We found that prothrombotic factors increased the risk of ischaemic stroke more than what they did for the risk of myocardial infarction, suggesting a different role of hypercoagulability in the aetiology of these two diseases, at least in young women. In **Chapter 5** the same question is addressed in a meta-analysis on all studies available in the literature that investigated markers of hypercoagulability as a risk factor for myocardial infarction and ischaemic stroke. By applying a strict methodology, in order to obtain unbiased comparisons, we were able to collect data from 351 markers of hypercoagulability, derived from 31 study populations. The data analysis was similar to that in Chapter 4, with the calculation of the relative risk ratio, but now the picture of the coagulation system was much more complete. Results from this meta-analysis support the hypothesis that hypercoagulability plays a greater role on the risk of ischaemic stroke than on the risk of myocardial infarction, which was particularly true in the young populations. When we talk about causation in the comparison between two diseases, there are some major sources of bias that deserve to be discussed. Firstly, the presence of different study designs and statistical analyses should be taken into account. Differences in study design, data acquisition, data analyses and the underlying research questions in the separate studies can hamper the comparability between the two diseases. One strategy to overcome this problem is to include only studies that investigated both endpoints, and do within study comparisons. In this way data acquisition, control group recruitment, composition and data analysis are identical, minimising problems with comparability. We adopted this method in both chapters, restricting the analyses to the RATIO study in Chapter

4 and to studies in which both diseases had been investigated in Chapter 5. This had as the result that study design, control group composition, questionnaires and sample measurement, analyses and research questions were similar for the two relative risks. The second problem that had to be taken into consideration is that a different relative risk might represent differences in background absolute risks of diseases. Similar absolute risk differences between the two diseases for the marker of interests can lead to higher relative risk in the disease with the lower occurrence. However, myocardial infarction and ischaemic stroke have approximately the same incidence in the general population.[20] Nevertheless, this is not the case in specific subgroups, for example, in patients with atrial fibrillation. This problem does not occur in Chapter 4, since patients with atrial fibrillation were not included, but the prevalence of patients with atrial fibrillation in the population investigated in Chapter 5 might have influenced the results. Nevertheless, we show that the hypothesis that the two diseases have different aetiological mechanisms is consistent among different populations and under various circumstances.

Risk factors for recurrent arterial thrombotic events

The second part of this thesis dealt with the risk of subsequent events and to some extent with prognosis. The aim was to give unbiased estimates of the probability of vascular recurrence and mortality after an arterial thrombotic event and to explore risk factors associated with the recurrent event. In order to address these two scopes, designs and approaches used have changed compared with the previous chapters. Chapter 6 and Chapter 7 were both based on a cohort study design, which has several advantages compared with case-controls studies, but also some drawbacks.[8,21,22] The main advantage is that the cohort design allows to calculate absolute risks of multiple outcomes, at variance with case-control studies in which only relative risks (odds ratios) can be calculated. However, especially for long-term follow-up, the results from cohort studies can be severely biased by the participants who were lost to follow-up. A substantial number of subjects lost to follow-up can raise serious doubts about the validity of a study.[23] The LiLAC cohort and the RATIO follow-up cohort did not suffer from this since in both studies we were able to follow almost all patients till the end of the study. Another issue regarding long-term follow-up is the presence of competing events. A patient may experience an event other than the one of interest, which alters the probability of

experiencing the event of interest. In the presence of many competing risk events, the Kaplan-Meier estimation procedure may not be directly applicable because a patient experiencing a competing risk event has to be censored in an informative manner (the censoring in the Kaplan-Meier is uninformative by definition).[23-25] To overcome this problem in chapter 6 we applied a nonparametric estimation of cumulative incidence of the event of interest by taking the informative nature of censoring due to competing risks into account.[24]

Chapter 6 investigated the role of concomitant headache on vascular recurrences and mortality in minor stroke and transient ischaemic attack (TIA) of non-cardioembolic origin. The study was conducted in the setting of the LiLAC cohort study. We found that patients who experienced concomitant headache at the time of a minor stroke or TIA had a lower risk of long-term vascular death. Moreover, they had a reduced vascular recurrence compared with patients without concomitant headache, even when several potential predictors were taken into account. In order to give unbiased estimations of cumulative incidence of vascular recurrences, we accounted for competing risk events (death) by a two-step process.[24,26] In the first step, we calculated the Kaplan-Meier estimate of the overall survival for any event. Note that both the events of interest as well as the competing risk events are considered 'events'. In the second step, the conditional probabilities of experiencing the event of interest were calculated. The cumulative incidences were estimated with the obtained non-parametric cumulative incidence function.[23] Therefore, the internal validity of the study was not influenced by this potential bias. However, the prognostic value of headache on the risk of recurrence and death is not strong enough to have clinical relevance, and this study does not suggest it to be a marker with clinical prognostic value. Nevertheless, because the association with the outcome persisted after taking into account several other potential confounders, the findings of this study might be used to provide insight into causality of stroke, suggesting that patients presenting with headache may have an aetiologically different type of stroke than patients with other stroke presentations. This hypothesis has been recently supported by other evidence.[27,28] Therefore, despite the absence of a formal causal question, this study also touches on the aetiological aspect of ischaemic stroke.

Chapter 7 describes a study on mortality rates after ischaemic stroke and myocardial infarction. Risk factors associated with vascular recurrences were also investigated, including the role of hypercoagulability. The analyses are based on the follow-up of the RATIO study.

We compared patients with myocardial infarction, ischaemic stroke, and subjects without arterial thrombosis for long-term mortality and vascular recurrences. This study showed that women who suffered from a major arterial thrombotic event had a high risk of death and vascular recurrences for decades after the first event. Additionally, we found that vascular recurrences are true to type (i.e., the recurrence rate for cerebrovascular events is higher in patients with ischaemic stroke whereas the rate of cardiac events is higher in patients presenting with myocardial infarction). The rates of death and recurrences were likely to be underestimated. To be able to be included in the RATIO case-control study, patients must have survived for a specific period of time (median 1.5 years) after the acute event (myocardial infarction or ischaemic stroke). This inevitably led to an underestimation of the true incidence rates, and it affected the external validity of our results. However, it is unlikely that it influenced the comparison between myocardial infarction and ischaemic stroke, because it is reasonable to assume that it acts equally on the two groups of patients. Therefore, the direct comparison between myocardial infarction and ischaemic stroke on the role of hypercoagulability on vascular recurrences is valid.

After the description of hypercoagulability in Chapters 4 and 5, we built a coagulation score in Chapter 7 in order to reflect a personal prothrombotic propensity. Despite the low statistical power of our analysis related to the relatively low number of outcomes, we found that high levels of the score (reflecting a prothrombotic state) increased the risk of vascular recurrences in ischaemic stroke patients but not in myocardial infarction patients. This finding may be interpreted together with our findings in the previous chapters: hypercoagulability plays a greater role in ischaemic stroke than in myocardial infarction, on both the risk of the first and recurrent events.

Comparison between ischaemic stroke and myocardial infarction and links with venous thrombosis

In this section, we discuss the differences between myocardial infarction and ischaemic stroke in more detail, and compare these diseases with other forms of thrombosis. Myocardial infarction and ischaemic stroke were contrasted in almost all chapters of this thesis. Strokes with evident sources of cardiac emboli were excluded, when possible, from this comparison. In Chapter 2, in which a direct comparison was not possible, we looked at other studies in the literature that reported on the association between ADAMTS13 and ischaemic stroke. This indirect comparison suggests that ADAMTS13 is a stronger risk factor for ischaemic stroke than for myocardial infarction.[15,29,30] In Chapter 3 the comparison is made with in the same study population (the RATIO case-control) and we found that pregnancy loss increases the risk of ischaemic stroke whereas that of myocardial infarction was affected only marginally. As already discussed above, pregnancy loss here is a proxy of several causes, one of which is very likely a prothrombotic state.[31-33] Chapters 4 and 5 focus both on the direct comparison between myocardial infarction and ischaemic stroke, and they conclude that there is an imbalance in the role of hypercoagulability in the two main forms of arterial thrombosis. Finally, in Chapter 7, the comparison between the two diseases focuses on recurrences, and we found that hypercoagulability has a prognostic value in predicting recurrences after stroke but not after myocardial infarction. The conclusions of all those investigations support the hypothesis that hypercoagulability plays a greater role in the pathogenesis of ischaemic stroke than on that of myocardial infarction.

Several mechanisms can explain this observation. Arterial thrombosis most frequently occurs after the rupture or erosion of an unstable atherosclerotic plaque that exposes thrombogenic elements to blood, such as collagen, von Willebrand factor, fibrinogen, fibronectin and laminin.[34-36] This results in platelet adhesion, activation and aggregation under the conditions of the rapid blood flow of arteries.[37] Activated platelets can also provide negatively-charged surfaces that harbour coagulation factors and markedly potentiate cell-based thrombin generation and blood coagulation.[36] Atherosclerosis and arterial thrombosis have traditionally been considered two distinct processes. However, it is becoming increasingly clear that the cellular and biochemical interactions underlying thrombosis are also directly relevant to atherosclerosis. Indeed, the coagulation reaction linked to fibrin generation may

contribute to the rapid progression of atherosclerotic plaque, creating an insidious cycle, which eventually leads to the catastrophic ischaemic event.[36,38] Therefore, the first explanation for the different role of hypercoagulability on myocardial infarction and ischaemic stroke can be found in differences between the vessel walls and flow patterns of the coronary arteries, the carotids, intracranial arteries and the cerebral microvasculature. These differences can influence the way that asymptomatic thrombi are formed and their promotion of the rapid progression of atherosclerotic lesions. Another consideration is the effect of flow deceleration on the blood clotting process. Once a critical stenosis has developed as a result of the atherothrombotic process and vasoconstriction, blood flow recirculation and stagnation downstream from the site of plaque injury becomes more prominent. Blood coagulation is favoured under such conditions, leading to the propagation of a fibrin-rich and red-cell-rich thrombus (the fibrin tail).[39] Also, in this case the different diameters and muscular components of the vessel walls might be implicated in the differences between the two diseases.

Ischaemic stroke has several aetiologies, in which it differs from myocardial infarction where the cause is almost invariably the atherosclerotic plaque. According to the most used classification, the TOAST, stroke can be classified into 5 categories, each with its own causes and consequences: cardioembolism, large-artery atherosclerosis, small-vessel occlusion, stroke of other determined aetiology and stroke of undetermined aetiology.[40,41] Risk-factor profiles differ across ischaemic stroke subtypes, including the most debated category of 'stroke of undetermined cause' (cryptogenic).[42,43] Cryptogenic stroke represents one third of all transient ischemic attacks (TIA) or ischaemic strokes, and half of the ischaemic strokes in the young.[44] It has been proposed that cryptogenic events might often be caused by occult arterial sources of thromboembolism, paroxysmal atrial fibrillation (AF), patent foramen ovale (PFO), or cardiac structural abnormalities.[45] However, even with a detailed investigation, only a minority of patients with cryptogenic stroke have been found to have potentially unstable plaques in arterial vessels, some of which are probably coincidental. Similarly, although long-term monitoring of heart rhythm identifies paroxysmal AF in up to a third of patients with cryptogenic events, the relevance of these mostly short episodes of AF for cryptogenic stroke is uncertain.[46,47] Unfortunately, we were not able to investigate differences between subtypes of ischaemic stroke in the projects described in this thesis, since the necessary data were not available neither in the RATIO population, nor in the available

literature included in the systematic review. However, the concept that cryptogenic events have the fewest atherosclerotic markers and no excess of cardioembolic markers made it the most interesting candidate for the observed difference in hypercoagulability between myocardial infarction and ischaemic stroke.[43] If there is such a link, it might have important implications in the management, prophylaxis and treatment of those patients and therefore, it deserves to be elucidated in further studies.

Moreover, there is another interesting interpretation of these findings. The classical paradigm of the pathophysiology of thrombus formation began with the pathologist Virchow, who in the mid-1800s postulated three major causes of thrombosis: changes in the vessel wall, changes in the blood flow, and changes in the blood composition.[48] This broad classification is still valid. However, we are used to consider that changes in blood flow and blood composition are mainly valid for venous thrombosis, whereas changes in vessel wall (atherosclerosis) are the cause of arterial thrombosis. Partly because of the obvious anatomical differences, as well as their distinct clinical presentations, arterial thrombosis and venous thrombosis were traditionally considered separate diseases, with different pathophysiological mechanisms. In this thesis, risk factors such as pregnancy loss and markers of hypercoagulability were investigated. However, the main risk factors for arterial thrombosis include hypertension, hyperlipidaemia, smoking, and diabetes mellitus, and those are still the most important.[49,50] In the last decade, there has been an increasing awareness about the association between venous and arterial thrombosis.[51] However, the nature of this association is unclear. Several attempts have been made to find links between arterial risk factors and venous thrombosis, with diverging results.[52-58] According to the findings of a recent meta-analysis, except for cigarette smoking, traditional arterial cardiovascular risk factors are not associated with an increased risk of venous thrombosis.[59] Atherosclerosis itself might have the potential to promote the development of thrombotic disorders in the venous system and the two clinical conditions might be simultaneously triggered by the activation of coagulation, in both the arterial and the venous system. Because the effect of hypercoagulability in arterial thrombosis is usually much less pronounced than in venous thrombosis, the results of this thesis lead to the consideration that ischaemic stroke shares similarities with venous thrombosis, at least for the major role of hypercoagulability.[60] Patients with venous thrombosis have been found to have an excess rate of arterial thrombosis compared with the general population (and to lesser extent patients with arterial thrombosis

seem to have more venous thrombotic complications).[61-66] Studies that have investigated arterial events after venous thrombosis reported that rates of ischaemic stroke are higher than those of myocardial infarction.[64] This finding was also present in a meta-analysis with long term follow-up, especially for unprovoked venous thrombotic events.[63] These are intriguing findings that together with our observations may stimulate new studies to explore the hypothesis whether the excess in ischaemic stroke events after venous thrombosis might be explained by hypercoagulability.

If the relationship between venous thrombosis and ischaemic stroke is stronger than that with myocardial infarction, there may also be clinical implications. In the era of the direct oral anticoagulants (DOACs), such as dabigatran, which inhibits thrombin, and rivaroxaban, apixaban, and edoxaban, which inhibit factor Xa, the primary and secondary prophylaxis of ischaemic stroke might need to be more similar to that of venous thrombosis than of myocardial infarction.[67-70] It has been suggested that DOACs are safer than vitamin K antagonists but as efficacious for prevention of venous thrombosis, as well as for stroke in atrial fibrillation.[71] Our findings suggest that they might be also helpful in other stroke categories, for instance for the secondary prevention of stroke of undetermined origin. A few trials testing this hypothesis in patients with stroke of undetermined cause are already ongoing.[72] It remains the challenge to identify those individuals at high risk for arterial thrombosis and implement safe and effective antithrombotic strategies that can prevent thrombotic vascular occlusion both for the first event and for recurrences. This thesis overturns the one-size-fits-all approach in arterial thrombosis and suggests to tailor antithrombotic therapies.

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Addendum

English summary

Nederlandse samenvatting

Curriculum vitae

List of publications

Acknowledgements

English summary

Arterial thrombosis refers to a blood clot that develops in an artery and as such obstructs the flow of blood to major organs. Depending on where the clot forms, arterial thrombosis can cause several serious conditions, the most common and severe being myocardial infarction and ischaemic stroke. These diseases are the leading cause of morbidity and mortality worldwide, although the observation that their incidence is slightly decreasing in middle-aged individuals. In the last few years, the scientific literature has emphasised the need to increase our knowledge on the primary and secondary prevention of these two diseases, especially in young populations, whilst the young often remain understudied due to the rarity of the disease in this age category. Against this background, we designed a series of studies to investigate the role of several factors on the risk of arterial thrombosis. Moreover, we were interested in the comparison between the two major presentations of arterial thrombosis, with the aim to find similarities and differences in aetiologic mechanisms for these two diseases. In order to have a comprehensive view of the topic, part of the projects was on risk factors for the first arterial thrombotic event whereas others focussed on risk factors for recurrence. Therefore, different study designs and different approaches have been used; however, a common denominator for most analyses was the role of the haemostatic balance. Next to several coagulation markers, the investigations addressed clinical markers such as pregnancy loss and headache. We used data from three studies: the Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study, the RATIO follow-up and the Life Long After Cerebral ischaemia (LiLAC) study. RATIO is a case-control study on young women (aged 18-50) with myocardial infarction or ischaemic stroke in the Netherlands between 1990 and 1995. It enrolled 248 myocardial infarction cases, 253 ischaemic stroke cases and 925 frequency matched controls. Ischaemic strokes of a clear cardio-embolic origin were excluded. The RATIO follow-up is a cohort study based on the RATIO case-control study. With data from national databases participants in the original RATIO study were followed till 2012 for mortality and vascular recurrence. The LiLAC study is a cohort study on patients with transient ischaemic attack (TIA) or minor ischaemic stroke (modified Rankin grade $\leq$ 3) of non-cardioembolic origin in the Netherlands. It included 2473 patients followed for a median of 14 years with very little loss to follow-up. Mortality and vascular recurrence were also recorded in this cohort.

The first project of this thesis was a study on the association between low levels of ADAMTS13 and the risk of a first myocardial infarction. Whether ADAMTS13, the cleaving protease of von Willebrand Factor, increases the risk of myocardial infarction was an unresolved issue, because previous single centre studies were unable to give a definitive conclusion. We did an individual patient data meta-analysis. With this method applied to a large number of patients, we were able to look at extreme levels of ADAMTS13 and showed that only very low levels of ADAMTS13 increased the risk of myocardial infarction, at variance of what was observed for ischaemic stroke, in which also moderately low levels were associated with risk of disease. We concluded that this is likely to be a causal association since it is biologically plausible, it is supported by experimental evidence and it is consistent across different and recent studies.

Then we investigated the association between pregnancy loss and both forms of arterial thrombosis in the frame of the original RATIO case-control study. Pregnancy loss has a link with several comorbidities including hypercoagulability (for example through antiphospholipid antibodies); however, few studies have looked at an association with subsequent thrombotic diseases. With this study we showed that recurrent miscarriage and stillbirth are associated with ischaemic stroke but not with the risk of myocardial infarction. This association was unchanged when other risk factors for arterial thrombosis and the presence of antiphospholipid antibodies were taken into account.

Following the idea that there are differences in the aetiology of myocardial infarction and ischaemic stroke, we set up two studies dealing with the question: does hypercoagulability play a similar role on the risk of the two main forms of arterial thrombosis? We defined hypercoagulability as an increased prothrombotic tendency due to either an excess of procoagulant markers or a deficiency of anticoagulant markers. In a first project, the question was investigated in the frame of the RATIO case-control study. The role of almost 30 markers of hypercoagulability on the risk of myocardial infarction was directly compared with their role on the risk of ischaemic stroke with the relative odds ratio. We found that prothrombotic factors increased the risk of ischaemic stroke more than what they do for the risk of myocardial infarction, suggesting a different role of hypercoagulability in the aetiology of these two diseases, at least in young women. With a second project, the question was directed to all studies available in the literature that investigated markers of hypercoagulability as a

risk factor for myocardial infarction and ischaemic stroke. Three hundred and fifty-one markers from 31 study populations were included and, similarly to the previous study, their relative effect was analysed. Results from this meta-analysis also supported the hypothesis that hypercoagulability plays a greater role on the risk of ischaemic stroke than on the risk of myocardial infarction, and notably this was particularly true in the young.

The second part of this thesis moved to risk factors for recurrences and mortality after the first arterial thrombotic event. The two studies belonging to this section are both based on a cohort study design. The role of concomitant headache on the risk of vascular recurrences and mortality after minor stroke and transient ischaemic attack (TIA) of non-cardioembolic origin was investigated within the frame of the LiLAC cohort study. We found that patients who experienced concomitant headache at the time of a minor stroke or TIA had a reduced vascular recurrence compared with patients without concomitant headache, even when several potential confounders were taken into account. Concomitant headache was also associated with a reduced risk of vascular death. This suggests that patients presenting with headache may have an aetiologically different type of stroke than patients with other stroke presentations. Finally, we performed a project on mortality rates after ischaemic stroke and myocardial infarction, and on risk factors associated with vascular recurrences. The analysis was based on the RATIO-follow-up. Patients with myocardial infarction or ischaemic stroke were compared with subject without arterial thrombosis for long-term mortality and vascular recurrences. This study showed that women who suffered from a major arterial thrombotic event have a high risk of death and vascular recurrences for decades after the first event. We also showed that hypercoagulability, similarly to what seen for the first event, is associated with the risk of a second arterial event in patients with ischaemic stroke, but not in patients with myocardial infarction.

Taken together, the findings of this thesis lead to the conclusion that there are differences in the aetiology of the two main forms of arterial thrombosis. Indeed, they support the hypothesis that hypercoagulability plays a greater role in the pathogenesis of ischaemic stroke than in that of myocardial infarction, without taking cardio-embolic stroke into account, as was done in most of the studies presented. Hypercoagulability is usually considered a risk factor for venous thrombosis and is less well characterised in relation to arterial thrombosis. There may be several explanations, based on the differences in the vessels wall and flow

between the venous and the arterial circulation. However, if the aetiology of myocardial infarction and ischaemic stroke is more different than what we are used to consider until now, we may hypothesise that ischaemic stroke shares more similarities with venous thrombosis than other forms of arterial thrombosis. This might have clinical implications, especially for young patients with ischaemic stroke, in whom in almost 50% of cases no clear explanation of the event is found (stroke of undetermined aetiology). Further studies should explore whether those patients benefit from an approach more similar to venous thrombosis prophylaxis, with the use of direct oral anticoagulants or other novel approaches instead of antiplatelet therapies.

Nederlandse samenvatting

Wanneer een bloedstolsel een slagader blokkeert, spreekt men van arteriële trombose. De meest voorkomende vormen zijn hartinfarct (hartaanval) en herseninfarct (beroerte). Deze aandoeningen zijn wereldwijd de belangrijkste oorzaken van ziektelast en sterfte, hoewel de frequentie in mensen van middelbare leeftijd licht dalende is. De laatste jaren is herhaaldelijk opgeroepen tot meer onderzoek naar preventie van deze aandoeningen, vooral ook in jongeren, waar deze aandoeningen niet vaak voorkomen, maar daardoor ook weinig bestudeerd worden.

Wij hebben een aantal onderzoekingen naar risicofactoren voor arteriële trombose uitgevoerd, met name in een jongere populatie, waarbij we vooral geïnteresseerd waren in een vergelijking tussen hart- en herseninfarct. Een deel van ons onderzoek richtte zich op een eerste ziektegebeurtenis, en een deel op recidieven. Daarom hebben we verschillende onderzoeksbenaderingen gebruikt, waarbij een gemeenschappelijk aspect de rol is van de hemostatische balans. Daarnaast onderzochten we factoren zoals miskramen en hoofdpijn.

We hebben gegevens gebruikt van drie onderzoeken: de 'Risk of Arterial Thrombosis in relation to Oral contraceptives (RATIO) studie', de RATIO-vervolgstudie, en de 'Life Long After Cerebral Ischaemia (LILAC) studie'. RATIO is een patiënt-controle onderzoek onder vrouwen van 18-50 jaar met een hart- of herseninfarct tussen 1990 en 1995, met 248 vrouwen met een hartinfarct, 253 met een herseninfarct, en 925 controles. De RATIO-vervolgstudie is een cohortstudie gebaseerd op het RATIO patiënt-controle onderzoek, waarin de deelnemers met gegevens uit nationale registers tot 2012 werden gevolgd voor het optreden van sterfte en vasculaire recidieven. LILAC is een cohortonderzoek van patiënten met een licht herseninfarct of voorbijgaande attaque ('TIA'), waarin 2473 patiënten gedurende 14 jaar gevolgd zijn, onder meer voor sterfte en vasculaire recidieven.

Het eerste project betrof het verband tussen lage bloedspiegels van ADAMTS13, het enzym dat von Willebrandfactor inactiveert, en het risico op een hartinfarct. We voerden hiervoor een metanalyse uit waarbij de individuele patiëntengegevens van vier onderzoeken werden samengevoegd. Met deze methode konden we naar het effect van zeer lage spiegels kijken, en die bleken samen te hangen met een verhoogde kans op een hartinfarct - in tegenstelling tot eerdere bevindingen waar ook matig verlaagde concentraties de kans op een herseninfarct

bleken te verhogen. We concludeerden dat de relatie tussen ADAMTS13 niveaus en het optreden van een hartinfarct oorzakelijk is, aangezien het biologisch plausibel is en consistent met ander eerder onderzoek.

Vervolgens onderzochten we de relatie tussen miskramen en beide vormen van arteriële trombose in het RATIO patiënt-controle onderzoek. Het is bekend dat het optreden van miskramen geassocieerd is met, onder meer, hypercoagulabiliteit, zoals bijvoorbeeld optreedt bij het antifosfolipidesyndroom, maar er is weinig onderzoek waarin nagegaan is of er na miskramen vaker trombotische ziekten optreden. Wij vonden dat recidiverende miskramen en doodgeboorte samenhangen met het op latere leeftijd optreden van een herseninfarct, maar niet van een hartinfarct. Deze relatie bleek niet afhankelijk van de aanwezigheid van antifosfolipide-antistoffen.

Voortbouwend op de gedachte dat er verschillen zijn in de oorzaken van hartinfarcten en herseninfarcten ontwierpen we twee studies met als vraagstelling: heeft hypercoagulabiliteit, dus een overmaat aan stolling of stollingscapaciteit, een verschillend effect wat betreft de kans op het optreden van een hart- of herseninfarct? We definieerden hypercoagulabiliteit als hetzij een hoge concentratie van protrombotische stoffen in het bloed, hetzij een lage concentratie aan anticoagulante stoffen. In het eerste onderzoek keken we naar bijna 30 markers van hypercoagulabiliteit in het RATIO patiënt-controle onderzoek, en vergeleken hun effect op het ontstaan van hart- en herseninfarct met de relatieve odds ratio. We vonden dat deze markers het risico op een herseninfarct meer beïnvloedden dan dat van een hartinfarct. Dit suggereert een verschillende rol van hypercoagulabiliteit bij de twee vormen van arteriële trombose, althans bij jonge vrouwen. Dit gingen we vervolgens na in het tweede project, dat gebaseerd was op een literatuurstudie van alle publicaties die dit soort markers hadden gerapporteerd voor zowel hart- als herseninfarct. Zo bekeken we 351 markers uit 31 onderzoekspopulaties, en ook in deze metanalyse vonden we dat hypercoagulabiliteit een sterker effect had op het optreden van een herseninfarct dan een hartinfarct. Dit was met name zo in jongere individuen.

Het tweede deel van dit proefschrift betreft risicofactoren voor recidieven en sterfte na een eerste arteriële trombose. Twee cohortstudies vormen de basis van dit deel. De rol van hoofdpijn ten tijde van een eerst licht herseninfarct of TIA op het optreden van vasculaire recidieven of sterfte werd onderzocht in het LILAC onderzoek. We vonden dat patiënten die

hoofdpijn ervoeren ten tijde van het herseninfarct of de TIA een lager risico hadden op een recidief arteriële trombose in de jaren erna, ook wanneer mogelijk verstorende variabelen verdisconteerd werden in de analyse. Ook hadden deze patiënten een lager sterfterisico. Dit suggereert dat patiënten bij wie hoofdpijn één van de symptomen is een andere vorm van herseninfarct hebben dan patiënten zonder hoofdpijn. Het tweede onderzoek was naar sterfte na een hart- of herseninfarct, waarbij de gegevens van de RATIO vervolgstudie werden gebruikt. We vergeleken patiëntes met een hart- of herseninfarct met controles zonder deze aandoeningen wat betreft langetermijn sterfte en vasculaire aandoeningen. Hierbij bleek een verhoogd risico te blijven bestaan tot tientallen jaren na de eerste arteriële trombose, en bovendien dat hypercoagulabiliteit het risico extra verhoogde na een herseninfarct, maar niet na een hartinfarct.

De resultaten van dit proefschrift leiden tot de conclusie dat er duidelijke verschillen zijn in de etiologie van de twee belangrijkste vormen van arteriële trombose, en dat met name de stolling een belangrijker rol speelt bij het ontstaan van een herseninfarct dan een hartinfarct, en ook bij de langetermijn gevolgen hiervan. Hypercoagulabiliteit wordt doorgaans vooral als een risicofactor voor veneuze trombose gezien, en is minder onderzocht in relatie tot arteriële trombose. Er zijn ook belangrijke verschillen tussen arteriële en veneuze trombose, zoals de rol van de vaatwand en de bloedstroomsnelheid. Desniettemin, wanneer de ontstaanswijzen van hart- en herseninfarct meer van elkaar blijken te verschillen dan tot dusver werd aangenomen, kunnen we veronderstellen dat het herseninfarct meer gemeen heeft met veneuze trombose dan met andere vormen van arteriële trombose qua ontstaanswijze. Dit kan klinische consequenties hebben, met name voor jonge patiënten met een herseninfarct, bij wie in bijna de helft geen oorzaak gevonden wordt. Toekomstig onderzoek moet uitwijzen of deze patiënten baat hebben bij een benadering die overeenkomsten vertoont met de trombosepreventie bij veneuze trombose, zoals een behandeling met anticoagulantia in plaats van bloedplaatjesremming.

Curriculum vitae

Alberto Maino was born on 5 June 1981 in Busto Arsizio (Italy) and graduated from Liceo Scientifico "Arturo Tosi" in Busto Arsizio in 2000. He started his studies in Medicine at the Università degli Studi di Milano, Italy, where he graduated in 2006 with honours. He then moved to the laboratory of molecular haematology of prof. F. Peyvandi in Milan, in which he was involved in both laboratory and clinical research in the field of thrombotic microangiopathies. In 2009 he started his specialization in Internal Medicine at the Università degli Studi di Milano. He did several internships in internal medicine, haematology, cardiology, cardiovascular ultrasound, nephrology and emergency medicine. Part of his training was abroad. In 2010 he spent 4 months at the Christian Medical College, Vellore, India, at the department of Haematology, where he worked as a clinical fellow, and in 2012 he spent one year at the Leiden University Medical Center, The Netherlands in the department of Clinical Epidemiology, where he worked on coagulation and arterial thrombosis as a research fellow. After taking his specialization *cum laude* in internal medicine in 2013, he worked as a consultant at the Department of Thrombosis and Haemostasis, Fondazione IRCCS Cà Granda Hospital, Milano, while holding a position of external PhD fellow at the department of Clinical Epidemiology, Leiden University Medical Center. He had the chance to continue working on various projects about risk factors for arterial thrombosis under the supervision of dr. B. Siegerink, prof. A. Algra and prof. F.R. Rosendaal, of which he presented the results on several (inter)national meetings.

During his PhD-fellowship Alberto followed several courses on Epidemiology taught by renowned epidemiologists (among others, Vandenbroucke, Rosendaal, Rothman and Samet). He was also involved in numerous epidemiology courses both as a teaching assistant and as an instructor. He is part of the organizing board of the Master course of Clinical Research at the Università degli Studi di Milano.

From March 2016, Alberto works as a physician at the Internal Medicine Unit of Azienda Provinciale per i Servizi Sanitari, Trento, Italy, and collaborates with the Università degli Studi di Milano on several research projects in the field of cardiovascular disorders.

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