3

Intrinsic coagulation activation and the risk of myocardial infarction and ischaemic stroke in young women

Bob Siegerink, Jose WP Govers-Riemslag, Frits R Rosendaal, Hugo ten Cate and Ale Algra

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Abstract

Introduction Classically, intrinsic coagulation proteins are thought to have a minor role in haemostasis. Recently, these proteins, especially FXII, were implicated as possible key players in the aetiology of thrombosis. This study aims to determine the risks of myocardial infarction and ischaemic stroke conferred by increased activation of intrinsic coagulation proteins in young women and the effect of oral contraceptive use on this association.

Methods To do so, intrinsic coagulation protein activation was determined in the RATIO study, a population based case control study including young women (18-50 years) with myocardial infarction (MI; N=205), ischaemic stroke (IS; N=175) and 638 healthy controls. Activated protein-inhibitor complexes were determined as measures of protein activation. These complexes consist of activated proteins bound to C1-esterase inhibitor (FXIIa:C1-inh, FXIa:C1-inh, Kallikrein-C1-inh) or antitrypsin inhibitor (FXIa:AT-inh). Odds Ratios (OR) and corresponding confidence intervals (95%CI) were calculated with logistic regression.

Results High levels of protein activation (>90th percentile of controls) showed an increased risk of IS: FXIIa:C1-inh (OR 2.1; 95%CI 1.3-3.5), FXIa:C1-inh (2.8; 1.6-4.7), FXIa:AT-inh (2.3; 1.4-4.0) Kallikrein:C1-inh (4.3; 2.6-7.2). If anything, MI risk was only increased by Kallikrein:C1-inh (1.5; 0.9-2.5). Oral contraceptive use further increased the risks.

Discussion High levels of activated proteins of the intrinsic coagulation system are associated with IS, but not with MI. This contradicts similar analyses among men in the Northwick Park Heart Study. Together with the finding that oral contraceptive use further increases the risks, the question whether the role of intrinsic coagulation proteins in the aetiology of ischaemic stroke thrombosis is sex-specific is raised.

Introduction

Arterial thrombosis occurs when an artery is occluded by a thrombus. Classical risk factors are diabetes, hypercholesterolemia, hypertension, smoking, obesity and oral contraceptive use. The most common manifestations are peripheral arterial disease, myocardial infarction and ischaemic stroke. Arterial thrombosis is common among the elderly and the most common cause of death in high income countries.[1]

A thrombus is formed upon activation of the coagulation system. Historically, this system has been characterised by two separate activating pathways, i.e. the extrinsic and intrinsic. The intrinsic coagulation pathway consists of the serine proteases coagulation factor XII (FXII), coagulation factor XI (FXI) and prekallikrein (PK). It further includes one non-enzymatic protein, i.e. High Molecular Weight Kininogen (HMWK).[2]

FXII has the capacity of autoactivation upon binding on negatively charged surfaces.[3,4] After activation, activated FXII (FXIIa) activates both FXI into activated factor XI (FXIa) and PK into its active form kallikrein (KAL). HMWK acts as a co-factor in both steps, during which the potent vasodilator bradykinin is released. FXIa further activates coagulation factor IX which forms together with cofactor VIIIa, Ca^{2+} ions and phospholipids the tenase complex, thereby activating the common pathway of the coagulation cascade.[5] A subform of FXIIa (also known as βFXIIa) is also known to activate coagulation factor VII, initiating the common pathway by priming the extrinsic pathway.[6] The intrinsic coagulation proteins are also involved in other biologic processes such as fibrinolysis (activation of plasminogen by FXIIa, FXIa, and KAL), vasoconstriction, inflammation and blood pressure control (KAL and bradykinin). Upon activation, the serine proteases of the intrinsic coagulation pathway are quickly bound by an inhibitor such as the C1-esterase inhibitory forming a protein-inhibitor complex which lacks the serine protease function.[7,8] An excess of these inhibitors is readily available in plasma, so the availability of activated intrinsic coagulation proteins forms the limiting step in the formation of these protein-inhibitor complexes. This makes these protein-inhibitor complexes a good measure of the level of activated proteins.

FXI deficiency causes mild bleeding in patients, whereas FXII, KAL and HMWK deficiencies do not.[2] These observations led to the traditional view that the intrinsic coagulation proteins do not play a major role in haemostasis, and are not likely to be a risk factor for thrombosis. However, recent evidence, both laboratory and clinical, indicates that these proteins may play a role in thrombus formation. Murine studies with FXII and FXI knockout

mice show that initiation of clot forming is not FXII dependent, but that propagation of clot formation is. [9,10] FXI deficient patients showed apart from their mild bleeding diathesis also a decreased risk of ischaemic stroke and venouos thrombosis, but this decrease is not observed for myocardial infarction.[11–13] In middle aged men, high levels of FXI and low levels of FXII increased the risk of MI.[14] An Austrian record-linkage study suggested that low FXII levels were associated with reduced overall death rates.[15] In the Northwick Park Heart Study, which included middle-aged men, low levels of activation from selected intrinsic coagulation proteins caused an increase in risk for both myocardial infarction and ischaemic stroke.[16] Since estrogens could have an impact on transcription levels of coagulation proteins, especially FXII due to an oestrogen receptive element in the promoter of the F12 gene, the relation between (intrinsic) coagulation proteins and the risk of arterial thrombosis is potentially different in females.[4,17]

It is unknown to what extent intrinsic coagulation proteins are involved in the pathophysiologic processes that lead to the different forms of arterial thrombosis, especially in young women. This patient group is of particular interest because of their use of oestrogen containing medication (i.e. oral contraceptives). We therefore set out to determine whether high levels of activation of the intrinsic coagulation system are associated with myocardial infarction or ischaemic stroke in young women and whether oral contraceptive use further increases this risk.

Methods

Study design & participants The Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study is a multicenter population-based case-control study. The study consists of three substudies, including patients with confirmed myocardial infarction, ischaemic stroke or peripheral arterial disease. One control group was frequency-matched to all three case groups. The study was initiated to evaluate the risk of arterial thrombosis due to the changing composition of oral contraceptive pills (1990 – 1995).[18–20] Blood and DNA were collected during the second phase of the study (1998 – 2002). Informed consent was obtained from all participants and the study was approved by the medical ethics committees of the participating hospitals. For the current study we report data from the myocardial infarction and ischaemic stroke substudies.

Patient selection has been described in detail previously.[18,19,21] In short, all women aged 18 to 50 years old who presented with a first event of myocardial infarction or ischaemic

stroke to one of the sixteen participating hospitals in the Netherlands between 1990 and 1995 were eligible and approached for study participation. A standardised questionnaire on patient characteristics and possible cardiovascular risk factors such as (familial) medical history, use of oral contraceptives and smoking habits was filled in by both cases and controls. Some of these questions were targeted to the year prior of diagnosis (cases) or the matched index year (controls). All participants were reapproached to donate blood or buccal swab for DNA analyses during the second phase of the study.

Measurements We determined protein-inhibitor complexes of the serine proteases of the intrinsic coagulation system as a measure of enzyme activation in citrated plasma. The inhibitor could either be a C1-esterase inhibitor for FXIIa, FXIa of kallikrein (FXIIa:C1-inh, FXIa:C1-inh, KAL:C1-inh) or antitrypsin inhibitor (FXIa:AT-inh). These complexes were measured by an ELISA, as described earlier.16 In short, for the FXIIa:C1-inh ELISA we used mAB KOK 12 which is specific for complexed C1-esterase inhibitor as antigen and mAb F3 which recognises FXII as well as α-FXIIa and β-FXIIa subsequently as conjugate. The KAL:C1-inh assay uses the same antigen, but uses mAb K15 which is directed against prekallikrein and kallikrein as conjugate.22,23 The FXIa protein-inhibitor assays both use the XI-5 mAB

Table 1. Characteristics of the RATIO participants

	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%)
Hypercholesterolaemia	21 (10%)	14 (8%)	19 (3%)
Oral contraceptives use *	81 (40%)	92 (53%)	213 (33%)
Smoking *	169 (82%)	101 (58%)	270 (42%)
FXIIa:C1-inh, median (Q1-Q3)	0.23 (0.13-0.33)	0.28 (0.15-0.45)	0.24 (0.12-0.33)
FXIa:C1-inh, median (Q1-Q3)	0.23 (0.14-0.60)	0.37 (0.18-0.76)	0.23 (0.15-0.52)
FXIa:AT-inh, median (Q1-Q3)	0.24 (0.20-0.29)	0.29 (0.22-0.42)	0.25 (0.21-0.31)
KAL:C1-inh, median (Q1-Q3)	0.05 (0.03-0.13)	0.11 (0.03-0.27)	0.04 (0.03-0.12)

** in the year prior to event/index year, # levels are expressed as a proportion of fully activated normal pooled plasma. AT-inh, alpha 1-antitrypsin inhibitor; C1-inh, C1-esterase inhibitor; FXII, coagulation factor XII; FXI, coagulation factor XI; KAL, kallikrein; Q1-Q3, range of first and third quartile.*

as antigen, which recognises both the native and activated FXI form of FXI.24 R11 mAB which binds to native, complexed and inactive C1-inhibitor, was used as conjugate for the FXIa:C1-inh assay; mAb AT-15, which is directed against complexed AT, was used as conjugate in the FXIa:AT-inh assay.25,26 All conjugates where biotinylated with EZLink N-hydroxysuccinimide ester-biotin according to instructions from the manufacturer (Pierce, Rockford, IL, USA). Absorbance was read at 450 nm on an EL 808 Ultra microplate reader (Bio-tek Instruments Inc.,Winooski, VT, USA).

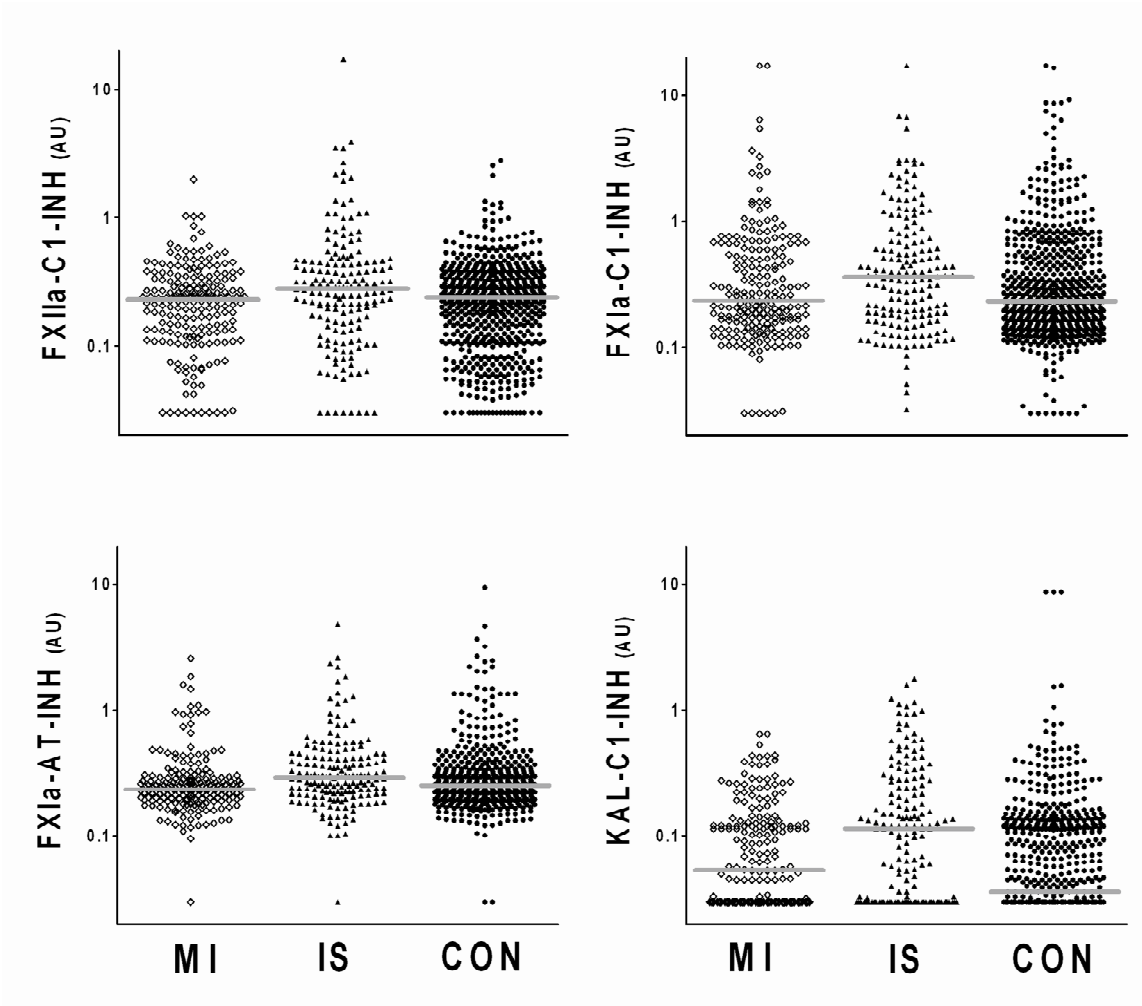
Results were expressed as a proportion of fully activated normal pooled plasma; activation was performed by adding an equal volume of 0.2 mg mL^{-1} dextran sulphate (Mr 500 000; Sigma Chemical Co., St Louis, MO, USA) in the FXIIa:C1-inh and KAL:C1-inh assay. Normal pooled plasma for the FXIa protein-inhibitor assays was fully activated by adding kaolin (final concentration 5 mg mL^{-1}). Activation was stopped by adding three volumes of phosphate-buffered saline (PBS) containing 0.1 mg mL–1 soybean trypsin inhibitor (Sigma Chemical Co.) and 0.05% (w/v) polybrene (Sigma Chemical Co.). Kaolin was removed by centrifuging the reaction mixture for 5 min at 13 000 x g. No signal was detected in FXII-, FXI- or kallikrein-deficient plasmas as a control for the specific ELISA.

Statistical analyses With the 90th percentile of the controls as a predefined cut-off point, we applied a logistic regression model to obtain odds ratios as measures for rate ratios associated with high levels of activation of the intrinsic coagulation factors. All odds ratios were adjusted for the frequency matching factors area of residence, year of event and age. Further adjustments were made for potential confounders (diabetes, hypertension and hypercholesterolemia and smoking) in a fully adjusted model. To assess the intrinsic coagulation protein system as a whole, a dummy variable was created which counted the number of proteins with high activation levels. Interaction of all intrinsic coagulation proteins with OC use in the year prior to the event was assessed by comparing the risk in those with either or both exposures with those with no exposures.

Results

Table 1 displays the baseline characteristics of the study participants. As expected, cases had more cardiovascular risk factors such as smoking, hypercholesterolemia, diabetes and hypertension than controls. The median levels of activated protein-inhibitor complexes of the intrinsic coagulation proteins are displayed in table 1. Figure 1 shows the distribution of the four protein-inhibitor complexes for cases and controls separately.

Figure 1. Levels of activated intrinsic coagulation proteins



Individual levels of protein activation are expressed as a proportion of fully activated normal pooled plasma. Medians are displayed with horizontal bars. Patients with Myocardial infarction are depicted with open squares (◇), ischaemic stroke patients with triangles (◢) and controls with circles (●).

AT-inh, alpha 1-antitrypsin inhibitor; AU, arbitrary units; C1-inh, C1-esterase inhibitor; FXII, coagulation factor XII; FXI, coagulation factor XI; KAL, kallikrein; MI, myocardial infarction; IS, ischaemic stroke; CON, controls.

Table 2. Risk of arterial thrombosis and high levels of activated intrinsic coagulation factors

FXIIa:C1-inh	≤ 90th percentile	> 90th percentile	OR (95% CI)	OR fully adjusted (95% CI)
Control	568 (90%)	63 (10%)	*1 [ref]*	*1 [ref]*
Myocardial infarction	182 (90%)	21 (10%)	0.82 (0.46-1.47)	0.74 (0.38-1.46)
Ischaemic stroke	130 (75%)	39 (23%)	2.10 (1.27-3.48)	1.87 (1.07-3.26)
FXIa:C1-inh				
Control	568 (90%)	63 (10%)	*1 [ref]*	*1 [ref]*
Myocardial infarction	184 (91%)	19 (9%)	0.96 (0.54-1.71)	1.13 (0.60-2.15)
Ischaemic stroke	133 (79%)	35 (21%)	2.77 (1.63-4.73)	2.92 (1.63-5.22)
FXIa:AT-inh				
Control	568 (90%)	63 (10%)	*1 [ref]*	*1 [ref]*
Myocardial infarction	185 (91%)	18 (9%)	0.94 (0.53-1.68)	0.94 (0.49-1.82)
Ischaemic stroke	134 (80%)	34 (20%)	2.33 (1.37-3.96)	2.18 (1.22-3.87)
KAL:C1-inh				
Control	570 (90%)	63 (10%)	*1 [ref]*	*1 [ref]*
Myocardial infarction	174 (85%)	30 (15%)	1.50 (0.91-2.47)	2.12 (1.18-3.81)
Ischaemic stroke	123 (72%)	47 (28%)	4.34 (2.62-7.18)	5.14 (2.93-9.00)

Odds ratios are obtained from logistic regression and are adjusted for the stratification factors age, area of residence and year of event. Odds ratios depicted as 'fully adjusted' are additionally adjusted for diabetes, hypertension and hypercholesterolaemia and smoking.
AT-inh, alpha 1-antitrypsin inhibitor; C1-inh, C1-esterase inhibitor; FXII, coagulation factor XII; FXI, coagulation factor XI; KAL, kallikrein; OR, odds ratio; CI, confidence interval; ref, reference.

The relative risks for both myocardial infarction and ischaemic stroke conferred by high levels of activated intrinsic coagulation protein levels, as well as the corresponding cut-offs, are displayed in table 2. By our definition, 10% of controls have high levels of protein-inhibitor complexes. No association between myocardial infarction and FXIIa:C1-inh was found (10% of cases exposed, Odds Ratio 0.82; 95% Confidence Interval 0.46-1.47). Also FXIa:C1-inh (9%, OR 0.96; 95% CI 0.54-1.71) and FXIa:AT-inh (9%, OR 0.94; 95% CI 0.53-1.68) were not associated with myocardial infarction. KAL:C1-inh slightly increased the risk of myocardial infarction (15%, OR 1.50; 95% CI 0.91-2.47). In ischaemic stroke patients, high levels of FXIIa:C1-inh were more frequent than in controls (23% exposed, OR 2.10; 95% CI 1.27-3.48). Also high levels of FXIa:C1-inh (21%, OR 2.77; 95% CI 1.63-4.73) and FXIa:AT-inh (20%, OR 2.33; 95% CI 1.37-3.96) were more frequent. KAL:C1-inh conferred a fourfold increase in ischaemic stroke (28%, OR 4.34; 95% CI 2.62-7.18). Further adjustments for hypertension, diabetes, hypercholesterolemia and smoking did not change the overall pattern, as is displayed in table 2 ('fully adjusted' odds ratios).

The use of an oral contraceptive in the year prior to the event roughly doubled the risk of both myocardial infarction (OR 2.3; 95% CI 1.6-3.4) and ischaemic stroke (OR 2.8; 95% CI 1.8-4.2).[16, 17] The risk of ischaemic stroke conferred by a combination of high levels of intrinsic coagulation protein activation (i.e. >p90) and prior oral contraceptive use was higher than could be expected by the separate effects as can be seen in table 3. The risk of myocardial infarction was not further increased by the combination of high levels of intrinsic coagulation protein activation and oral contraceptives, except for KAL:C1-inh. The combination of high levels of KAL:C1-inh and oral contraceptives use when compared with women who had neither risk factor increased the risk of myocardial infarction six fold (OR 6.1; 95% CI 2.5- 15). This combined effect was also present in the ischaemic stroke analysis: women with both high levels of KAL:C1-inh and oral contraceptive use had a 17-fold increase in ischaemic stroke risk (OR 17; 95% CI 7.4 – 41). Further adjustments for potential confounders (hypertension, diabetes, hypercholesterolemia and smoking) did not change the overall pattern, as is displayed in table 3 ('fully adjusted' odds ratios).

Table 4 shows the odds ratios per number of proteins with high levels of activation. For each additional high level of protein-inhibitor complexes the risk of ischaemic stroke is increased 2-fold (OR 2.11; 95%CI 1.31-3.38) whereas the risk for myocardial infarction was not affected (OR 1.14; 95%CI 0.75-1.73). When compared to those without a high level of intrinsic coagulation protein activation, people with two or more high levels had a fourfold increase in risk for ischaemic stroke (OR 4.39; 95%CI 2.44-7.90), but no such association was found between multiple high levels and myocardial infarction (OR 1.30; 95% CI 0.66-2.57).

In total, 19 myocardial infarction and 21 ischaemic stroke patients were on anticoagulants. The results of the analyses when restricted to those patients who did not take oral anticoagulant therapy at the time of blood drawing did not change. Therefore, oral anticoagulant therapy does not change our results. The associations of FXIa:C1-inh, FXIa:AT-inh and KAL:C1-inh persist in ischaemic stroke patients whose blood was sampled years after the event (data not shown). The association of FXIIa:C1-inh appeared to attenuate with increasing time between event and blood drawing. However, absence of association cannot be established due to the broad confidence intervals.

Table 3. Risk of arterial thrombosis due to high levels of activated intrinsic coagulation protein and prior oral contraceptives

	> 90th percentile	OC use	# controls	Myocardial infarction			Ischaemic stroke		
				# MI cases	OR (95% CI)	OR fully adjusted (95% CI)	# IS cases	OR (95% CI)	OR fully adjusted (95% CI)
FXIIa:C1-inh	-	-	376	107	*1 [ref]*	*1 [ref]*	62	*1 [ref]*	*1 [ref]*
	-	+	192	75	2.4 (1.6-3.5)	2.0 (1.3-3.2)	68	2.8 (1.8-4.5)	2.7 (1.6-4.5)
	+	-	47	16	0.9 (0.4-1.7)	0.8 (0.4-1.7)	18	1.8 (0.9-3.6)	1.6 (0.7-3.3)
	+	+	16	5	2.4 (0.7 -	1.8 (0.5-7.2)	21	8.7 (3.7-21)	8.1 (3.1-21)
FXIa:C1-inh	-	-	382	111	*1 [ref]*	*1 [ref]*	60	*1 [ref]*	*1 [ref]*
	-	+	186	73	2.5 (1.7 -	2.2 (1.4-3.5)	73	3.2 (2.0-5.1)	3.4 (2.0-5.7)
	+	-	40	12	1.1 (0.5 -	1.5 (0.7-3.2)	20	3.8 (1.9-7.7)	4.7 (2.1-10)
	+	+	23	7	1.8 (0.7 -	1.5 (0.5-4.4)	15	6.1 (2.5-14)	5.5 (2.2-14)
FXIa:AT-inh	-	-	383	112	*1 [ref]*	*1 [ref]*	65	*1 [ref]*	*1 [ref]*
	-	+	185	73	2.5 (1.6 -	2.2 (1.4-3.5)	69	3.1 (1.9-4.9)	3.1 (1.9-5.3)
	+	-	39	11	1.0 (0.5 -	1.2 (0.5-2.8)	15	3.2 (1.5-6.6)	3.3 (1.4-7.3)
	+	+	24	7	1.9 (0.7 -	1.3 (0.4-3.8)	19	4.8 (2.1-11)	4.1 (1.7-9.8)
KAL:C1-inh	-	-	377	106	*1 [ref]*	*1 [ref]*	62	*1 [ref]*	*1 [ref]*
	-	+	193	68	2.2 (1.5- 3.3)	1.9 (1.2-3.0)	61	2.7 (1.7-4.4)	2.6 (1.5-4.5)
	+	-	46	17	1.2 (0.6 -	1.6 (0.8-3.4)	19	3.7 (1.9-7.4)	4.2 (1.9-9.0)
	+	+	17	13	6.1 (2.5 - 15)	6.8(2.5-18)	28	17 (7.4-41)	23 (9.2-59)

Relative risks are stratified to oral contraceptive use prior to the event or index year (-/+), high levels of protein activation (+/-) or both (+/+) with the -/- category as reference. Odds ratios are obtained from logistic regression and are adjusted for the stratification factors age, area of residence and year of event. 'Fully adjusted' odds ratios are additionally adjusted for diabetes, hypertension and hypercholesterolemia and smoking.
AT-inh, alpha 1-antitrypsin inhibitor; C1-inh, C1-esterase inhibitor; FXII, coagulation factor XII; FXI, coagulation factor XI; KAL, kallikrein; OC, oral contraceptive; OR, odds ratio; CI, confidence interval; ref, reference.

Discussion

Our study shows that high levels of activated proteins of the intrinsic coagulation system are associated with ischaemic stroke, and not or to a lesser extent with myocardial infarction, in young women. In general, high levels increased the risk of ischaemic stroke 2.5 fold whereas the risk of myocardial infarction was hardly affected. The increased risk of ischaemic stroke was further increased by oral contraceptive use. When the intrinsic coagulation proteins were assessed jointly, high levels were more frequent in cases with ischaemic stroke than in myocardial infarction cases.

The rate-limiting step in the formation of the protein-inhibitor complexes is the availability of activated coagulation proteins, since the inhibitors are present in excess in plasma. The cause of the elevated complexes is not clear. It could either be a relative increase in which the absolute levels of zymogens remain stable with an increased activation rate or it could be an absolute increase of zymogens with a stable activation rate. Our study has some limitations; because of the case-control study design blood was collected after the event in the case groups. This might have led to reverse causation, a process in which a consequence of an event is mistaken for the cause of the event. Because blood sampling in the RATIO study was several months after the

Table 4. Risk of arterial thrombosis with increasing numbers of high levels of activated intrinsic coagulation proteins

# of high levels	Controls	Myocardial infarction			Ischaemic stroke		
	N	N	OR (95%CI)	OR $_{\text{fully adjusted}}$ (95%CI)	N	OR (95%CI)	OR $_{\text{fully adjusted}}$ (95%CI)
0	454 (72%)	137 (69%)	*1 [ref]*	*1 [ref]*	84 (50%)	*1 [ref]*	*1 [ref]*
1	117 (19%)	44 (23%)	1.14 (0.75-1.73)	1.16 (0.72-1.88)	44 (26%)	2.11 (1.31-3.38)	2.10 (1.125-3.53)
2	42 (6.5%)	16 (7.5%)	1.27 (0.67-2.43)	1.42 (0.68-3.00)	17 (11%)	3.00 (1.50-6.00)	3.57 (1.72-7.43)
3	13 (2%)	2 (1%)	0.44 (0.08-2.33)	0.58 (0.10-3.47)	15 (8%)	4.67 (1.88-12)	5.41 (2.00-14)
4	3 (0.5%)	1 (0.5%)	1.10 (0.08-15)	3.84 (0.29-51)	7 (5%)	12.6 (2.7-58)	8.09 (1.5-42)

Odds ratios are obtained from logistic regression and are adjusted for the stratification factors age, area of residence and year of event. Odds ratios depicted as 'fully adjusted' are additionally adjusted for diabetes, hypertension and hypercholesterolemia and smoking.

OR, odds ratio; CI, confidence intervals; ref, reference

event, we can safely rule out the possibility that our findings directly reflect the transient effects of the acute phase which lasts days to weeks. However, slow subsiding effects which are induced by ischaemic stroke could explain some of the relation between FXIIa:C1-inh and ischaemic stroke. Non-transient effects (chronic effects) can still be the cause of reverse causation in our study and can only be ruled out in a prospective study. Furthermore, the necessity of a blood sample after the event implies that our results are only valid for non-fatal arterial thrombosis. Although our study can be considered to be of reasonable size, or even large due to the rare nature of cardiovascular disease in young women, the RATIO study lacks power to detect small effects. Lack of precision, which is reflected in the wide confidence intervals, hampers definite interpretation of some of our results. This is most clear when the intrinsic coagulation system is assessed as a whole; only few patients and controls were positive on three or all four assays, making it difficult to interpret the odds ratios for these strata. However, the pattern is clear: the risk increase found for ischaemic stroke was higher for each additionally elevated activated protein-inhibitor complex, whereas this pattern was absent for myocardial infarction. This observation suggests that the pathogenic role of the intrinsic coagulation proteins is not similar for all manifestations of arterial thrombosis.

Our results indicated that the risks conferred by high levels of intrinsic coagulation proteins were further increased by oral contraceptive use. This raises the question whether women who want to start with oral contraceptive use should be screened. The highest risk, after adjustment for potential confounders, was found in women who both had high levels of activated prekallikrein (>p90 of controls) and used oral contraceptives: a 23 fold increase in risk compared with women with neither risk factor (fully adjusted model). Even with this relative risk a total of ~15 000 women have to be screened for their kallikrein activation levels before the start of oral contraceptives to prevent one event. If the 10% of these women with the highest kallikrein activation levels do not start to use oral contraceptives, one ischaemic stroke case will be prevented per year. Although a formal cost-benefit analyses is likely to show a slightly different number, these calculations readily demonstrate that screening is not desirable, mainly due to the low incidence of ischaemic stroke in this population. This is even more clear when one considers the beneficial effects of oral contraceptive use in this population, such as a reduction of pregnancy-associated morbidity and mortality and ovarian and endometrial cancer.

A case control study nested in the second Northwick Park Heart Study (NPHS II) also assessed the relationship between the protein-inhibitor complexes of intrinsic coagulation proteins and the risk of coronary heart disease (CHD, N=231) and stroke (N=56 of which 12 haemorrhagic). CHD was defined as definite MI (fatal and nonfatal), possible MI (fatal), angina or coronary angiographic findings requiring intervention and ECG changes at 5 years of follow up. Strokes were diagnosed and categorised on the basis of clinical presentation, computed tomography, lumbar puncture, and autopsy findings. CHD risk was decreased in the second tertile of FXIIa:C1-inh; if anything, the third tertile also showed a decrease in risk, but to a lesser extent, resulting in a U-shaped relation. Other protein-inhibitor complexes (FXI-C1-inh, FXI-AT-inh, KAL:C1-inh) did not alter CHD risk. A similar U-shaped pattern was observed for the relation between KAL:C1-inh complexes and stroke. These results are essentially similar after reanalysis with the 90th percentile as a cut-off (analyses done in collaboration with the NPHS II investigators; results not shown here). There are several possible explanations for the discrepant findings in the RATIO and the NPHS II analyses. First, the differences might be due to chance. Second, the NPHS II is a nested case control study, so blood draw was before the event. Although reverse causation in the RATIO study is not a likely explanation for our results, due to the timing of blood draw and the different effects for myocardial infarction and ischaemic stroke, it cannot be ruled out. Third, the case definitions in the RATIO study were more stringent than those in the NPHS. In the NPHS ischaemic and hemorrhagic strokes were combined and CHD also included possible MI and angina. Fourth, the largest difference between the studies is found in the sex and age of the participants: NPHS included only middle-aged men, whereas the RATIO study included only relatively young women. This difference is of particular interest because FXII transcription is oestrogen sensitive, due to an oestrogen responsive element in the promoter region of the F12 gene.[3] Furthermore, a recent murine study suggests that oestrogens influence the transcription of several coagulation proteins.[15]

The mechanisms by which these proteins are involved in the pathogenesis of ischaemic stroke in young women still have to be established. Perhaps such an increased activity of these proteins results in an imbalance of several systems in which the intrinsic coagulation proteins are involved (e.g. coagulation, fibrinolysis and inflammation). Ultimately, this imbalance then leads to a hypercoagulable state which increases the risk of ischaemic stroke. According to this hypothesis, some event, such as a plaque rupture or changes in

blood flow, acts as a trigger of the start of coagulation. The extent of the following thrombus formation is dependent on the tendency to clot: patients suffering from a hypercoaguable state are more likely to form an occlusive thrombus with clinical effects than those without such a predisposition. This mechanism predicts that a dose-dependent effect should be observed: the greater the imbalance, the larger the tendency to coagulate, the greater the risk of ischaemic stroke. Not all forms of arterial thrombosis need to be subject to this hypothesised mechanism. Possibly, exposure of the highly thrombogenic surface after rupture of a coronary plaque inevitably will start clot formation, whether the patient is more prone to coagulation or not. Although the formation of a thrombus in itself is involved in the aetiology of both myocardial infarction and ischaemic stroke, we postulate that the tendency to start a clot is an important risk factor for ischaemic stroke but not for myocardial infarction.

Conclusion These results indicate that increased levels of activated intrinsic coagulation proteins are associated with ischaemic stroke, but not myocardial infarction, in young women. These risks are further increased by oral contraceptive use. These results differ from an earlier study in middle aged men (NPHS II). This raises two questions: why are the effects of intrinsic coagulation proteins different for young myocardial infarction and ischaemic stroke, and are these effects sex specific? Answers to these questions could come from both basic and epidemiologic research. Basic research is needed to further unravel the function of activated intrinsic coagulation proteins, and perhaps identify different mechanisms for different forms of arterial thrombosis. Epidemiologic research could also provide more insight in the potentially different mechanisms for MI and IS, for example by specifying the associations per stroke subtype. Furthermore, epidemiologic research could provide more insight on the sex-specific effects, by including both men and women. A prospective study design could also diminish the risk of reverse causation, but the low incidence of myocardial infarction and ischaemic stroke in the young makes such a study unfeasible

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