Cover Page

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10 Clot lysis time and the risk of myocardial infarction and ischaemic stroke in young women

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Abstract

Introduction Reduced overall fibrinolytic capacity increases the risk of myocardial infarction (MI), as demonstrated previously in studies which included primarily men. We aimed to determine the influence of altered fibrinolysis on the risk of MI and ischaemic stroke (IS) in young women.

Methods The RATIO study is a population-based case-control study including young women with MI (n=205), IS (N=175) and 638 matched healthy controls. Fibrinolytic potential was determined with a tissue factor/tissue plasminogen activator induced clot-lysis assay. Odds ratios (OR) and corresponding confidence intervals adjusted for cardiovascular risk factors were obtained with logistic regression as measures of rate ratios. Clot-lysis time (CLT) was divided into tertiles based on the control group (T1-T3), with the middle tertile (T2) as reference.

Results *Hypofibrinolysis* (prolonged CLT) was associated with an increase in risk of MI (T3 vs T2, OR 2.8;95%CI 1.7-4.7). *Hyperfibrinolysis* (decreased CLT) only a small effect (T1 vs T2, 1.6; 0.9-2.9). *Hypofibrinolysis* did not affect the risk of IS significantly (T3 vs T2, 1.5; 0.7-3.0), whereas *hyperfibrinolysis* increased this risk substantially (T1 vs T2, 4.1; 2.1-8.0). Oral contraceptive use and smoking further increased these risks.

Discussion *Hypofibrinolysis* increases the risk for MI in young women, a finding similar to previous studies. Counter-intuitively, *hyperfibrinolysis* increased the risk of IS fourfold, which suggests that MI and IS have different aetiologies.

Introduction

Cardiovascular disease places a large burden on western societies, both on the healthcare systems as well as on the quality of life. The two major forms of arterial thrombosis are myocardial infarction and ischaemic stroke.[1] Arterial thrombosis is most prevalent in the elderly; risk factors, such as diabetes, hypertension and hypercholesterolaemia are also more common with progressing age. Because the prevalence of these 'traditional' risk factors is less pronounced in the young, new risk factors can more easily be identified in young participants.[1]

An increased capacity to form blood clots leading to a procoagulant state may increase the risk of arterial thrombosis.[2] A procoagulant state may not solely be the result of an increased propensity to initiate or propagate clot formation, but can also result from a decreased ability to dissolve these newly formed clots.[3] The main fibrinolytic factor is plasmin, which degrades fibrin and thereby dissolves the clot. The zymogen of plasmin, plasminogen, can be activated by tissue plasminogen activator (tPA) or urokinase.[4] Plasmin itself can be inhibited directly by α2-antiplasmin. Furthermore, plasminogen activator inhibitor 1 (PAI-1) decreases the formation of plasmin by direct inhibition of tPA and urokinase. Thrombin Activatable Fibrinolysis Inhibitor (TAFI) also hampers fibrinolysis by altering the fibrin structure and thereby reducing binding and activation of plasminogen. Individual factors of the fibrinolytic system, especially PAI-1 and TAFI, have been linked to myocardial infarction and ischaemic stroke although results have been inconclusive and contradictory as was reviewed by Meltzer et al.[5]

Besides individual factors, clot lysis assays can be used to asses the fibrinolytic capacity. Some studies, but not all, indicate an association between the euglobin clot lysis time or dilute whole blood clot lysis time and myocardial infarction. This association might be most pronounced in patients with minimal atherosclerosis.[5,6] Studies on ischaemic stroke are limited.[7,8] However, these global tests in these studies do not reflect all the appropriate components of the fibrinolytic system. A previously described test takes all primary components of the fibrinolytic system (plasminogen, alpha2-antiplasmin, PAI-1 and TAFI) into account and therefore reflects the true global plasma fibrinolytic potential better.[9,10] The main determinant of this test is the level of PAI-1, followed by plasminogen TAFI, prothrombin, and α2-antiplasmin levels.[10] Two studies indicate that a prolonged clot lysis

time (CLT) is associated with venous thrombosis, especially in combination with other risk factors such as oral contraceptives.[11,12]

Hypofibrinolysis is also associated with different cardiovascular diseses: prolonged CLT (fourth quartile vs. first quartile) was associated with a twofold increase in risk of myocardial infarction for men aged <50 years, whereas no such association was found for men aged ≥50.[12,13] Another case-control study with 330 young patients with coronary heart disease, ischaemic stroke (including transient ischaemic attack) and peripheral arterial disease also showed an increased risk for the combined endpoint arterial thrombosis with increasing clot lysis time.[14] The inclusion of both sexes (37% men) and the broad case definitions make it difficult to draw conclusions for some subanalyses, such as an interaction analysis focussing on oral contraceptive use and smoking. We therefore set out to determine whether abnormal fibrinolysis, both hyper- and hypofibrinolysis, is associated with both myocardial infarction and ischaemic stroke in young women and whether other risk factors influence this risk.

Methods

Study design & participants The RATIO (Risk of Arterial thrombosis in relation to oral contraceptives) study is a multicenter case-control study set up to identify risk factors for arterial thrombosis in young women, and has been described in detail earlier.[15–17] In short, young women (18-50 years) diagnosed with a form of arterial thrombosis in the 16 participating hospitals including eight academic medical centres in the Netherlands, were approached to participate. Diagnosis of myocardial infarction was based on the presence of symptoms, elevated cardiac-enzyme levels, and electrocardiographic changes, whereas ischaemic stroke was diagnosed on the basis of medical history, neurological examination, and CT or MRI scan by experienced neurologists in the participating centres. Women without a history of arterial thrombosis were approached by random digit dialling and frequency-matched with the case groups on age (in five year categories), year of event and area of residence. All participants were asked to fill out a standardised questionnaire on several topics such as demographic characteristics, medical history among which oral contraceptive use in the year prior to event or comparable time frame for healthy controls.

Measurements Citrated plasma used for these measurements was not thawed previously. Clot lysis time was assessed by measuring the changes in plasma turbidity during tissue-factor induced clot formation and subsequent lysis by exogenous t-PA.[9] In short, 50 µl of

mixture containing phospholipid vesicles (40% L-α-dioleoylphosphatidylcholine, 40% L-α-dioleoylphosphatidylethanolamine and 20% L-α-dioleoylphosphatidylserine in a final concentration of 10 µmol/l), t-PA (final concentration 56 ng/ml), tissue factor (final dilution 1/1000) and $CaCl_2$ (final concentration 17 mmol/L) diluted in HEPES buffer [25mmol/L HEPES (N-2-hydroxytethylpiperazine-N'2ethanesulfonic acid) 137mmol/L NaCl, 3,5 mmol/L KCl, 3 mmol/L $CaCl_2$, 0,1% bovine serum albumin, pH 7,4] was added to 50 µl of citrated plasma, all in a 96-well microtiter plate. After thorough mixing, the plate was placed in a Spectramax 340 kinetic microplate reader at 37º C (molecular devices corporation, Menlo Park, CA, USA). Optical density (OD) was measured every 20 seconds at 405 nm, resulting in a clot-lysis turbidity profile. Clot-lysis time is defined as the time from the midpoint of the clear to the maximal turbid transition, representing clot formation, to the midpoint of the maximum turbid to clear transition, representing clot lysis. The laboratory technician

Table 1. Characteristics of participants stratified by case and control status

	Myocardial infarction N=205	Ischaemic stroke N=175	Control N=638
Mean age[1]	42	39	39
Caucasian ethnicity	195 (95%)	167 (97%)	602 (94%)
History of [2]			
Hypertension	53 (26%)	50 (29%)	40 (6%)
Diabetes	10 (5%)	7 (4%)	10 (2%)
hypercholesterolaemia	21 (10%)	14 (8%)	19 (3%)
Oral contraceptives use [2]	81 (40%)	92 (53%)	231 (33%)
Smoking [2]	169 (82%)	105 (60%)	270 (42%)
Median BMI [3] *(Q1 - Q3) ka/m²*	24.6 (22.4-27.7)	23.3 (21.3-27.0)	22.8 (21.0-25.1)
Median triglycerides [3] *(Q1 - Q3) mmol/L*	1.68 (1.13-2.71)	NA	1.24 (0.88-1.84)
Clot-lysis time[3,] *minutes*			
Median (Q1 - Q3)	70.7 (60.8- 4.0)	60.6 (52.7-72.0)	61.9 (56.1- 69.7)
Mean (SD)	75.2 (25.0)	68.1 (36.3)	64.4 (14.0)

[1] at moment of event (cases) or index date (controls).[2] in the year prior to event (cases) or index year (controls). [3] at time of blood draw. SD = standard deviation, Q1 - Q3= 25th and 75th percentile, CLT = Clot-lysis time, NA = not applicable. Clot-lysis time measurements were unavailable or missing for 8 controls, 3 cases in the myocardial infarction group, and 10 patients in the ischaemic stroke group.

Table 2. Hypo and hyperfibrinolysis and the risks of myocardial infarction and ischaemic stroke

		MYOCARDIAL INFARCTION					ISCHAEMIC STROKE					CONTROLS	
	CLT (min)	N	%	Odds ratio (95% confidence interval) model 1	Odds ratio (95% confidence interval) model 2	Odds ratio (95% confidence interval) model 3	N	%	Odds ratio (95% confidence interval) model 1	Odds ratio (95% confidence interval) model 2	Odds ratio (95% confidence interval) model 3	N	%
T1	0-58	36	18%	1.18 (0.69-1.95)	1.60 (0.88-2.92)	1.59 (0.87-2.90)	69	42%	2.10 (1.28-3.49)	4.07 (2.07-8.04)		207	33%
T2	58-66	37	18%	1 [REF]	1 [REF]	1 [REF]	34	21%	1 [REF]	1 [REF]	NA	209	33%
T3	66-301	129	64%	3.15 (2.04-4.86)	2.82 (1.69-4.72)	2.84 (1.69-4.76)	62	38%	2.07 (1.24-3.48)	1.50 (0.74-3.03)		214	34%

NA = not applicable, CLT = Clot-lysis time, REF = reference group, T1 = 1st tertile of clot-lysis time, or hyperfibrinolysis. T2 = 2nd tertile of clot-lysis time, or normofibrinolysis. T3 = 3rd tertile of clot-lysis time, or hypofibrinolysis. All tertiles are based on the clot-lysis time of the control group. Odds ratios are calculated with the middle tertile as reference. Regression model 1 includes stratification factors age, year of event and area of residence as covariates. Model 2 includes the covariates of model 1 plus smoking, body mass index, hypertension, diabetes and hypercholesterolaemia. Model 3 includes the covariates of model 2 plus log transformed triglycerides levels.

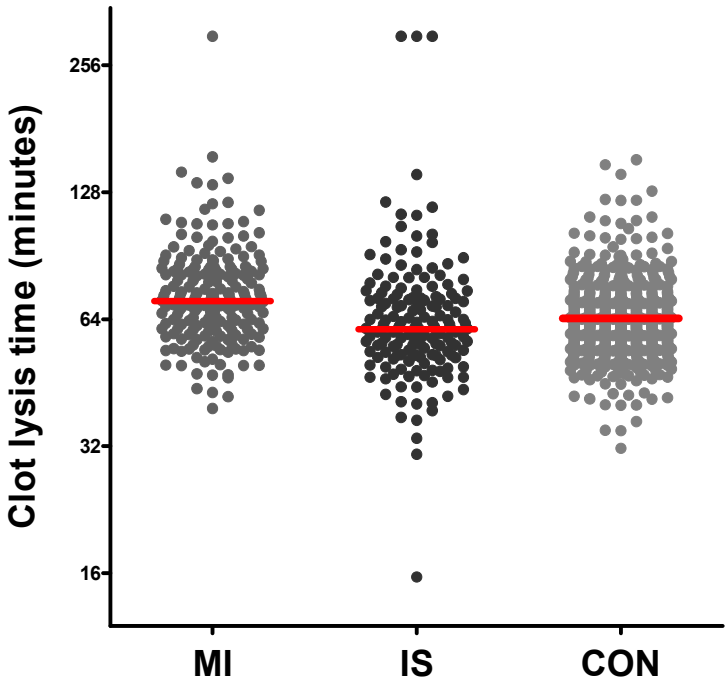
was unaware of the case-control status of the samples.

Statistical analyses To assess clot lysis time in relation to arterial thrombosis we calculated the mean difference and corresponding 95% confidence interval (CI) of clot lysis time in the control and case groups with the t-test for independent samples. To further study the effect of both *hypo-* and *hyperfibrinolysis* clot lysis time was divided into three categories based on the tertiles of the control group. Logistic regression models were used to obtain odds ratios and corresponding 95% CIs as measures of rate ratios with *normofibrinolysis,* defined as the middle tertile as the reference category. The covariates in these models included at least the stratification factors age (on a continuous scale), index year and area of residence. Furthermore, smoking status, body mass index, hypertension, diabetes and hypercholesterolemia were considered as confounders and included in subsequent models. Since triglyceride levels could also affect clot lysis time, we also additionally included log transformed trigyceride levels in the model.[11,13] Triglyceride data were only available for the myocardial infarction and control groups.

Results

As expected, known cardiovascular risk factors such as smoking and diabetes were more prevalent in the two case groups than in the controls (table 1). Since age was a matching variable, the three groups were similar with

Figure 1. Clot lysis time per case group



MI = Myocardial infarction, IS = Ischaemic stroke, CON = controls
The clot lysis time of all participants are shown, stratified per case group. The horizontal lines represent the mean clot lysis time for each case group.

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spect to age. Clot-lysis time was higher among myocardial infarction cases than in controls (mean difference 10.8 minutes, 95% CI 7.2 to 14.5). CLT in ischaemic stroke cases was shorter (mean difference 3.7 minutes, 95% CI -2.0 to 9.4). Figure 1 shows the clot-lysis time for each participant with the horizontal lines indicating the mean, stratified per case group.

With the middle category as reference, hypofibrinolysis (or the third tertile and longest CLT) was associated with a threefold increase in risk of myocardial infarction (OR 3.15, 95% CI 2.04 - 4.86; table 2). Adjustment for confounders, including triglyceride levels, decreased this risk slightly (OR 2.84, 95% CI 1.69 - 4.76). *Hyperfibrinolysis*, or the first tertile and shortest CLT, was not clearly associated with myocardial infarction. The effects were more pronounced in women younger than 40 years (table 3). *Hypofibrinolysis* (T3) increased the risk of ischaemic stroke about twofold (OR 2.07, 95% CI 1.24-3.48), but this risk greatly decreased after adjustment for confounders (OR 1.50, 95% CI 0.74 - 3.05). *Hyperfibrinolysis* (T1) also increased the risk of ischaemic stroke (OR 4.07, 95% CI 2.07 - 8.03) after

adjustment. The risks were most pronounced in the young (see table 3). Upon exclusion of oral anticoagulant users (16 ischaemic stroke cases and 19 myocardial infarction cases) these results were largely the same (data not shown).

Oral contraceptive use within the subgroup of participants with *normofibrinolysis* increased the risk of both myocardial infarction and ischaemic stroke (table 4) about two- to fourfold (T2/- OC vs. T2/+ OC OR 2.4, 95% CI 1.0 - 5.6 for myocardial infarction and OR 3.7, 95% CI 1.3 - 11 for ischaemic stroke). This was also true for smoking (T2/- smoking vs. T2/+ smoking OR 3.3, 95 %CI 1.4 - 7.7 for myocardial infarction and OR 1.9, 95% CI 0.7 - 5.3 for ischaemic stroke). The risk of smoking was highest in women with abnormal clot lysis time when compared with women with *normofibrinolysis* and who did not smoke (T3/+ smoking OR 14, 95% CI 6.2-31 for myocardial infarction and T1/+ smoking OR 7.2, 95% CI 2.7 - 19 for ischaemic stroke).

Discussion

Our study indicates that *hypo*fibrinolysis increases the risk of myocardial infarction in young women. Counter intuitively, we found that *hyper*fibrinolysis was associated with an increased risk of ischaemic stroke. Furthermore, the use of oral contraceptives increased the risk in all strata of CLT.

A decreased fibrinolytic capacity is a plausible causal risk factor for thrombosic events and the results for myocardial infarction are in correspondence with current knowledge and the results in earlier studies.[13,14] Our results, unexpectedly, indicate also that *hyperfibrinolysis* increases the risk of ischaemic stroke. This finding could be due to chance, but also raises the question whether the observed association reflects a causal mechanism in which an altered fibrinolytic capacity plays a role in the aetiology of ischaemic stroke or whether it can be explained by other mechanisms. For instance, *hyperfibrinolysis* in patients suffering from atrial fibrillation could increase the risk of cardioembolic stroke as theoretically an increased potential to dissolve blood clots can lead to instable clots and subsequent embolisation. However, the RATIO study excluded patients with an an overt cardiac source and therefore we feel that this mechanism cannot explain our results.

Our counter-intuitive findings on hyperfibrinolysis and ischaemic stroke are not necessarily in contrast with previous findings. The 4G/5G polymorphism of the PAI-1 promotor, which is associated with low levels of circulating PAI-1 (with carriers of the 5G allele having lower

Table 3. Risks of myocardial infarction and ischaemic stroke; younger vs older

	MYOCARDIAL INFARCTION		ISCHAEMIC STROKE	
	Odds ratio (95% confidence interval) Model 2		Odds ratio (95% confidence interval) Model 2	
	<40 years	≥ 40 years	<40 years	≥ 40 years
T1	3.8 (1.2 - 12)	1.0 (0.5 - 2.4)	7.7 (2.4 - 24)	2.8 (1.1 - 7.2)
T2	1 [REF]	1 [REF]	1 [REF]	1 [REF]
T3	7.8 (2.5 - 24)	2.3 (1.2 - 4.2)	2.8 (0.8 - 10)	1.0 (0.4 - 2.5)

T1 = 1st tertile of clot-lysis time, or hyperfibrinolysis. T2 = 2nd tertile of clot-lysis time, or normofibrinolysis. T3 = 3rd tertile of clot-lysis time, or hypofibrinolysis. Tertiles are based on clot-lysis time in control group. Odds ratios and corresponding confidence intervals are calculated with the middle category as reference and with smoking, body mass index, hypertension, diabetes and hypercholesterolaemia as covariables ('model 2').

Table 4. Interaction analyses; oral contraceptive use and smoking behaviour

	TERTILE	OC USE	CONTROLS	MYOCARDIAL INFARCTION		ISCHAEMIC STROKE	
				#	OR (95%CI)	#	OR (95%CI)
ORAL CONTRACEPTIVE	**T1**	-	119	15	1.5 (0.6 - 3.5)	31	6.2 (2.4 - 16)
	T1	+	88	21	3.8 (1.6 - 8.9)	38	9.1 (3.4 - 25)
	T2	-	127	19	1 [REF]	17	1 [REF]
	T2	+	82	18	2.4 (1.0 - 5.6)	17	3.7 (1.3 - 11)
	T3	-	174	88	3.1 (1.6 - 6.1)	31	1.5 (0.6 - 3.8)
	T3	+	40	41	7.4 (3.3 - 17)	31	7.0 (2.4 - 21)
		SMOKING					
SMOKING	**T1**	-	129	7	0.7 (0.2 - 1.9)	34	3.0 (1.2 - 7.8)
	T1	+	78	29	6.5 (2.7 - 16)	35	7.2 (2.7 - 19)
	T2	-	110	9	1 [REF]	13	1 [REF]
	T2	+	99	28	3.3 (1.4 - 7.7)	21	1.9 (0.7 - 5.3)
	T3	-	122	20	1.3 (0.5 - 3.3)	22	0.8 (0.3 - 2.3)
	T3	+	92	109	14 (6.2- 31)	40	4.8 (1.8 - 13)

See table 3 for description. All presented ORs are from model 2;smoking was only included in the oral contraceptive use interaction analyses.

levels), has been suggested to be associated with an increased risk of ischaemic stroke.[18,19] PAI-1 is one of the main determinants of CLT and is known to have functions, other than decreasing fibrinolytic potential, which could explain our findings.[10] One of these functions is the so-called tPA-serpin axis which is involved in neuronal damage after cerebral ischaemia.[20] Animal studies suggest that endogenous tPA or rtPA used as treatment after ischaemic stroke could lead to an N-Methyl-D-aspartic acid-mediated Ca2+ influx which enhances neuronal damage. PAI-1, a serpin which inhibits tPA, could counteract this mechanism. Therefore, low levels of PAI-1, reflected by hyperfibrinolysis in our study, could lead to neuronal damage. In addition, high PAI-1 has been shown to be related to a decreased tendency of plaque rupture.[20] Although atherosclerosis is not abundantly present in these young women, low levels of PAI-1 could be related to increased tendency of plaque rupture and subsequent thrombus formation and then increase the risk of ischaemic stroke of atherosclerotic origin. So, the observed association between low CLT and increased risk of ischaemic stroke in our study does not necessarily reflect a causal role of increased fibrinolytic tendency but can also indicate other causal mechanisms that include low levels of PAI-1. Given this uncertainty, we can still conclude that our results suggest a clear difference in the aetiology of myocardial infarction and ischaemic stroke.

Our study has some limitations and strengths: we cannot stratify our results according to the subtype of stroke (e.g. TOAST criteria) which hampers the interpretation of the results.[21] It is possible that our results reflect the causal mechanism of only one stroke subtype, which would imply that the effect for that subtype would even be stronger. Furthermore, due to the case-control design of this study it cannot be ruled out that the differences in clot-lysis time between the case groups are a consequence of the disease or disease treatment, instead of a cause (i.e. 'reverse causation'). A major source of this bias is the use of blood samples taken during the acute phase of the disease, directly after the event. However, since blood was drawn after the acute phase (mean 82 months, at least 23 months after the event), it is unlikely that the results are explained by this phenomenon. Other sources of this bias, such as changes in cardiovascular risk factors after the event, would have led to an underestimation of the true effect and are not likely to exert a different effect between patients suffering from myocardial infarction and ischaemic stroke. Also, treatment initiated after the event could have introduced a bias: since antihypertensive drugs and statins might increase clot lysis and hence decrease clot lysis time, the effect of clot lysis time on myocardial infarction might be underestimated

whereas the effect on ischaemic stroke might be overestimated.[22] Because treatment strategies largely overlap for these two diseases it is not likely that our main finding, the difference in effect of clot lysis time between myocardial infarction cases and ischaemic stroke cases, is to be explained by this potential source of bias. Our study only includes patients that survived a first event which may affect the results. Therefore our results only apply to those who survived their first event. If the effect of clot lysis time leads to a more severe forms of disease, our results are an underestimation of the true effect.

The secondary analyses regarding oral contraceptive and smoking yield six strata and this results in small numbers of participants per stratum, as is reflected by the wide confidence intervals. Therefore, these analyses provide some idea into the associations with the combination of risk factors, but do not allow strong conclusions on the presence of interactions. Increased levels of triglycerides could confound the relation between clot-lysis time and arterial thrombosis.[13] Unfortunately, triglyceride levels were not available for the ischaemic stroke analyses. However, adjustment for triglyceride levels in the myocardial infarction analyses only minimally changed the point estimates. Therefore, it is unlikely that effects observed in the ischaemic stroke analyses can be attributed entirely to triglycerides levels.

Conclusion We found that in young women hypofibrinolysis increased the risk of myocardial infarction whereas hyperfibrinolysis increased the risk of ischaemic stroke. Although the results obtained from the ischaemic stroke analyses are not easily interpreted and causal conclusions on the mechanism by which hyperfibrinolysis increases the risk of ischaemic stroke cannot be drawn, these results indicate that myocardial infarction and ischaemic stroke may have different causal mechanisms.

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