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Plasma factor and inhibitor composition contributes to thrombin generation dynamics in patients with acute or previous cerebrovascular events

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Abstract

Introduction—More than 80% of cerebrovascular events are ischemic and largely thromboembolic by nature. We evaluated whether plasma factor composition and thrombin generation dynamics might be a contributor to the thrombotic phenotype of ischemic cerebrovascular events.

Materials and Methods—We studied (1) 100 patients with acute ischemic stroke (n=50) or transient ischemic attack (TIA) (n=50) within the first 24 hours from symptom onset, and (2) 100 individuals 1 to 4 years following ischemic stroke (n=50) or TIA (n=50). The tissue factor pathway to thrombin generation was simulated with a mathematical model using plasma levels of clotting factors (F)II, V, VII, VIII, IX, X, antithrombin and free tissue factor pathway inhibitor (TFPI).

Results—The plasma levels of free TFPI, FII, FVIII, and FX were higher, while antithrombin was lower, in the acute patients compared to the previous event group (all $p \leq 0.02$). Thrombin generation during acute events was enhanced, with an 11% faster maximum rate, a 15% higher maximum level and a 26% larger total production (all $p < 0.01$). The increased thrombin generation in acute patients was determined by higher FII and lower antithrombin, while increased free TFPI mediated this effect. When the groups are classified by etiology, all stroke sub-types except cardioembolic have increased TFPI and decreased AT and total thrombin produced.

Conclusion—Augmented thrombin generation in acute stroke/TIA is to some extent determined by altered plasma levels of coagulation factors.

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Keywords

stroke; thrombin generation; factor composition; computational modeling

Previous studies have shown that patients with acute stroke have increased thrombin generation relative to normal controls using platelet-rich plasma [1]. Markers of thrombin generation, such as thrombin-antithrombin complex (TAT) [2;3], and the activation peptide for thrombin activatable fibrinolysis inhibitor [4] are elevated in acute stroke patients [5]. Prothrombin fragment 1.2 has been shown to be a predictor of time to ischemic events in a follow-up study of transient ischemic attack (TIA) patients [6]. Acute TIA is also associated with enhanced levels of fibrinopeptide A and TAT, both of which returned to normal after one month [7].

Several alterations in coagulation factor levels in venous blood have been demonstrated in acute stroke. He *et al.* [8] reported increased factor (F)VII and FII, accompanied by impaired anticoagulant mechanisms reflected by lower antithrombin (AT) in acute stroke patients admitted within 24 h of the symptom onset. Adams *et al.* [9] demonstrated during the first 7 days of acute cerebrovascular ischemia, unaltered levels of tissue factor (TF) antigen and increased activity of tissue factor pathway inhibitor (TFPI), while free TFPI antigen displaying anticoagulant activity *in vivo* was similar to normal. Elevated FVIII levels in acute stroke patients compared to healthy controls have also been reported [10], although these findings are inconsistent. In a population of mild to moderate ischemic stroke patients, levels of AT were significantly lower for at least three months following the event [11]. To our knowledge, no comprehensive analysis of coagulation factors and inhibitors in TIA patients has been published. Our goal is to understand whether thrombin generation based upon plasma composition show differences in acute or previous stroke.

Previously, we have utilized our mathematical model of blood coagulation [12-14] to simulate thrombin generation in the Leiden Thrombophilia Study population [15] which resulted in stratification of groups of individuals similar to their documented cases of thrombotic events. Additionally, we have identified plasma factor composition dependence on the simulated thrombin generation curves in patients with acute coronary syndrome [16] and in women undergoing *in vitro* fertilization [17]. In this study, we simulated thrombin generation using plasma factor levels from patients who had suffered a recent ischemic stroke or TIA and those with a previous cerebrovascular ischemic event.

Materials and Methods

We enrolled 200 subjects under the age of 70 years old who had suffered a first-ever cerebrovascular ischemic episode. Ischemic stroke was diagnosed according to WHO criteria [18]. A TIA was defined as the occurrence of a sudden, focal neurological deficit that lasts for less than 24 hours, presumed to be of vascular origin and was confined by the area of the brain or eye perfused by a specific artery. Exclusion criteria were: acute illness, cancer, hepatic or renal dysfunction, acute coronary syndrome within the preceding 6 months, treatment with oral anticoagulants, heparins or clopidogrel, and antiphospholipid syndrome. Patients over the age of 70 were also excluded to reduce a potential impact of comorbidities associated with the elderly. All patients had computed tomography (CT) or CT followed by conventional magnetic resonance imaging (MRI) performed within their hospital stay due to the acute brain ischemia. The study was approved by the Jagiellonian University Ethical Committee. All participants gave informed consent.

All subjects were classified based on the timing and severity of the thromboembolic event and separated into four categories: previous stroke (n=50), acute stroke (n=50), previous TIA

(n=50) and acute TIA (n=50). Those classified as “acute” were enrolled within 24 hours of a stroke or TIA and “previous” subjects included those who had survived a stroke or TIA in the previous one to four years; all subjects with previous stroke or TIA did not experience an additional cerebrovascular event between the index event and the time of blood collection. In 10 patients classified as TIA, CT or MRI scans showed an infarct in a relevant vascular territory, however these individuals remained in the categories formed according to the WHO criteria.

Subjects were also classified according to etiology. Diagnostic evaluation involved carotid ultrasound with Doppler imaging, echocardiography, electrocardiography and Holter monitoring, where indicated, and autoimmune work-up. The known causes of stroke in this population included large vessel disease stroke, or cardioembolic stroke due to atrial fibrillation, heart valve disease or cardiac insufficiency (left ventricular ejection fraction <40%) [19]. Subjects with a cryptogenic cause (n=68) included 16 previous stroke, 24 acute stroke, 10 previous TIA and 18 acute TIA. Subjects with carotid stenosis (n=61) included 21 previous stroke, 10 acute stroke, 16 previous TIA and 14 acute TIA. Subjects with an atrial septal defect (n=29) included 5 previous stroke, 8 acute stroke, 12 previous TIA and 4 acute TIA. Subjects with an atrial fibrillation (n=42) included 8 previous stroke, 8 acute stroke, 12 previous TIA and 14 acute TIA.

Blood Collection and Coagulation Protein Analyses

Blood cell counts, biochemical analysis, including lipid profile, albumin and glucose levels were assessed by standard automated laboratory methods. Blood was drawn into 0.1 volume of 3.2% trisodium citrate from an antecubital vein with minimal stasis, centrifuged within 15 minutes of collection and stored in aliquots at -80°C until further use. FII, FV, FVII, FVIII, FIX and FX were measured by one-stage clotting assays (Dade Behring, Liederbach, Germany) using factor-deficient plasmas. AT activity was measured using a Berichrom chromogenic assay (Dade Behring). Free TFPI was determined using an ELISA (Diagnostica Stago, Asnieres, France). C-reactive protein (CRP) was determined in serum using nephelometry (Dade Behring). Interleukin-6 (IL-6) was measured in plasma using a commercially available ELISA (R&D Systems, Abington, UK). All intraassay coefficients of variation are between 5.6% and 7.4%, and all interassay coefficients of variation are between 6.1% and 8.4%.

Mathematical Model

The current mathematical model is based upon prior publications by Jones *et al.*, [12] Hockin *et al.* [13] and Butenas *et al.* [14] and yields concentration versus time profiles for selected species when electronic mixtures of procoagulants FII, FIX, FX, FVII/FVIIa, FV, and FVIII and anticoagulants TFPI and AT are exposed to picomolar concentrations of TF.

All procoagulant and anticoagulant factor levels were determined for each individual (n=200). Factor levels expressed as a percentage were translated into molar (M) concentrations using literature values for the mean plasma concentrations [20]. Each individual's blood factor concentration was entered into the computer database, and simulated reactions were initiated with 5 pmol/L TF. The standard control simulation sets zymogen, procofactor and inhibitor concentrations at mean physiological values as previously described [21].

Simulated reactions were solved for active thrombin over 1200 s. The outputs of these active thrombin curves are evaluated by parameters that describe the initiation, propagation and termination phases of thrombin generation: maximum level of thrombin generated (MaxL), maximum rate of thrombin generated (MaxR), time to 10 nmol/L thrombin (clot time) and total thrombin generated (area under the curve, AUC) [15].

A systematic analyses of the contribution of the plasma factors to the thrombin generation output was conducted on the populations as previously described [15;16] by adjusting the factors to mean physiologic concentrations and reevaluating the thrombin generation profiles.

Statistics

Data are either presented as the mean $\pm$ standard deviation (SD) or as medians (interquartile range). The Kolmogorov-Smirnov test was used to assess conformity with a normal distribution. Categorical values were analyzed using the χ^2 test. The t-test was used for comparisons between two groups given a normal distribution of values; otherwise the Wilcoxon rank sum test was utilized. Analysis of variance was used for comparisons of more than two groups. Tukey's method was utilized for multiple comparisons. Variance stabilizing transformations were performed as needed. A p-value <0.05 was considered significant.

Results

Study population

Table 1 shows demographic and clinical characteristics of all patients. Subjects in the acute phase of cerebrovascular ischemia (either stroke or TIA) did not differ from those with a prior stroke/TIA in terms of most traditional cardiovascular risk factors. Most notably, acute subjects in both groups had lower levels of total cholesterol and low-density lipoprotein (LDL)-cholesterol ($p<0.01$ for all comparisons). Acute subjects had higher levels of inflammatory markers IL-6 and CRP ($p<0.01$ for all comparisons).

Plasma factor contribution

Plasma factor levels (expressed as percentage of mean physiologic values) are summarized in Table 2. No significant differences were found in any of the four groups with regard to FII, FVII or FIX. Interestingly, FV was higher ($116\pm23\%$ vs. $106\pm14\%$; $p=0.04$) for acute stroke than for previous stroke, respectively. FVIII was lower for previous TIA and previous stroke than for both of the acute groups ($p<0.05$ for all comparisons). FX and free TFPI were lower and AT was higher in both previous groups than in acute stroke ($p<0.01$ for all comparisons). FX was lower and AT was higher in previous TIA compared to acute TIA, and free TFPI was higher in acute TIA compared to previous stroke ($p<0.03$ for all comparisons).

Free TFPI was positively correlated with IL-6 in each of the four groups and with CRP in both of the acute states ($p<0.05$ for all correlations, Pearson correlation coefficient (r) from 0.27 to 0.38). FVIII was positively correlated with both CRP and IL-6 in acute stroke ($p=0.04$, $r=0.29$ for CRP; $p=0.03$, $r=0.32$ for IL-6). FX showed association with CRP ($p=0.03$, $r=0.30$). No other correlations between inflammatory markers and factor levels were found. There were no correlations between any factor level and age in any of the four groups.

Thrombin generation profiles

The effect of these differences on thrombin generation is shown in Figure 1 (panels A through D) and summarized in Table 3. No detectable differences in clot time between any of the groups were seen, but all had longer clot times than that of the mean physiologic control (Previous TIA: 232 ± 43 s; Previous stroke: 212 ± 33 s; Acute TIA: 230 ± 43 s; Acute stroke: 223 ± 37 s; Mean physiologic: 197 s; $p<0.001$ for all comparisons with mean physiologic). The MaxR was accelerated by 18% in acute stroke versus previous TIA ($p=0.004$). The MaxL was higher in acute stroke than previous stroke (by 17%) and previous TIA (by 22%). The AUC was higher in acute stroke (by 32% over previous stroke and 35% over previous TIA) and in acute TIA (by 18% over previous stroke and 20% over previous TIA) than both the previous groups

($p < 0.02$ for all comparisons). In general, the trend for thrombin generation seems to increase from previous TIA < previous stroke < acute TIA < acute stroke.

In previous stroke, previous TIA and acute TIA, the clot time was positively correlated with IL-6 (i.e. the clotting time was longer with increasing IL-6 concentrations) ($p \leq 0.02$ for all correlations, Pearson correlation coefficient ranges from 0.32 to 0.34). In acute TIA, CRP levels were also positively correlated with clot time ($p = 0.03$, $r = 0.30$).

Because of similarities in both previous events and both acute events, the categories of stroke and TIA were combined into their respective acute or previous conditions. When combined, concentrations of free TFPI (Previous: $125 \pm 24\%$ and Acute: $146 \pm 26\%$; $p < 0.001$), FII (Previous: $109 \pm 13\%$ and Acute: $114 \pm 18\%$; $p = 0.02$), FV (Previous: $107 \pm 14\%$ and Acute: $114 \pm 21\%$; $p = 0.01$), FVIII (Previous: $123 \pm 25\%$ and Acute: $149 \pm 37\%$; $p < 0.001$) and FX (Previous: $110 \pm 16\%$ and Acute: $124 \pm 20\%$; $p < 0.001$) were higher in the acute group compared to the previous group, whereas AT was lower in the patients with an acute cerebrovascular event (Previous: $105 \pm 11\%$ and Acute: $96 \pm 13\%$; $p < 0.001$).

When the acute cerebrovascular events are combined and compared with previous stroke/TIA, as expected, thrombin generation was more vigorous in the acute conditions (Figure 2A). The MaxL of thrombin generation (Previous: 345 ± 66 nmol/L and Acute: 398 ± 101 nmol/L; $p < 0.001$), MaxR (Previous: 2.2 ± 0.5 nmol/L/s and Acute: 2.4 ± 0.6 nmol/L/s; $p = 0.007$) and AUC (Previous: 84 ± 18 $\mu\text{mol} \cdot \text{s/L}$ and Acute: 106 ± 36 $\mu\text{mol} \cdot \text{s/L}$; $p < 0.001$) were all higher in the acute subjects than both the previous subjects and the mean physiologic control. In addition, the MaxL and AUC were higher for both previous event groups compared to the mean physiologic values ($p < 0.01$ for both comparisons). Clot time was similar in the acute and previous conditions (Previous: 222 ± 39 s and Acute: 226 ± 40 s), but the mean physiologic value (197 s) was lower than those observed in both groups ($p < 0.001$ for both comparisons).

Etiology

Acute and previous conditions were further evaluated by etiology. Individuals were placed into subtypes of either: atrial septal defect, atrial fibrillation, carotid stenosis and cryptogenic. Our results show that when acute conditions are separated by specific etiology (Tables 4A and 5A), no factor level differences or thrombin parameter differences were seen. These comparisons were also made on all individuals with a previous cerebrovascular event (Table 4B). Our results showed that the FVIII levels of the atrial septal defect patients were higher than that of the carotid stenosis patients ($134 \pm 25\%$ vs. $115 \pm 16\%$, $p = 0.02$). The carotid stenosis patients had higher TFPI levels than the atrial septal defect patients ($131 \pm 24\%$ vs. $112 \pm 22\%$, $p = 0.04$). The FX levels of patients with a cryptogenic cause were higher than carotid stenosis patients ($115 \pm 16\%$ vs. $107 \pm 17\%$, $p = 0.04$). These changes caused differences in thrombin parameters (Table 5B) for clot time and MaxR between carotid stenosis and atrial septal defect. The clot times were shorter (201 ± 38 s vs. 235 ± 38 s, $p = 0.02$) and MaxR was faster (2.6 ± 0.6 nmol/L/s vs. 2.0 ± 0.4 nmol/L/s, $p = 0.005$) for patients with atrial septal defect.

Stroke sub-types (acute vs. previous)

When the etiology is compared between acute vs. previous conditions, our results showed no differences in either factor levels or thrombin parameters in atrial fibrillation patients. All other stroke sub-types showed that acute patients had lower AT, higher TFPI and a greater AUC. In addition, the acute cryptogenic patients had higher FV (Previous: $106 \pm 14\%$ and Acute: $116 \pm 20\%$; $p = 0.03$) than previous cryptogenic patients. Both carotid stenosis (Previous: $105 \pm 13\%$ and Acute: $128 \pm 24\%$; $p < 0.001$) and atrial septal defect patients (Previous: $107 \pm 17\%$ and Acute: $125 \pm 18\%$; $p = 0.01$) had higher FX in the acute group. Acute carotid stenosis patients also had higher FII (Previous: $108 \pm 13\%$ and Acute: $118 \pm 21\%$; $p = 0.02$), higher FVIII

(Previous: $115 \pm 16\%$ and Acute: $155 \pm 34\%$; $p < 0.001$), a faster MaxR (Previous: 2.0 ± 0.4 nmol/L/s and Acute: 2.6 ± 0.7 nmol/L/s; $p < 0.001$) and a higher MaxL (Previous: 329 ± 58 nmol/L and Acute: 427 ± 108 nmol/L; $p < 0.001$) than previous carotid stenosis patients.

Contribution from individual factors on thrombin generation

The contribution of each individual protein was evaluated by the relative shift in the thrombin generation curve that occurs after setting that protein to the mean physiologic control. We found that AT and FII had the greatest impact on the thrombin generation curves. Adjusting both proteins to mean physiologic levels resulted in thrombin generation profiles that were very similar for previous and acute conditions (Figure 2B). Only the clot time differed between the two groups (Previous: 222 ± 34 s and Acute: 241 ± 31 s, $p < 0.001$) and with the mean physiologic (197 s; $p < 0.001$ for both comparisons).

Free TFPI levels in both groups were substantially higher (Previous: $125 \pm 24\%$ and Acute: $146 \pm 26\%$) than mean physiologic levels ($p < 0.001$ for both comparisons). Thus, an adjustment in TFPI to 100% causes the thrombin curves to become more prothrombotic. In fact, adjusting TFPI, FII and AT all to their mean physiologic levels yield average curves for both groups that are more prothrombotic than the hypothetical control curve (Acute: Figure 3A; Previous: Figure 3B). The parameters of clot time (Previous: 189 ± 7 s and Acute: 184 ± 9 s), MaxR (Previous: 2.4 ± 0.3 nmol/L/s and Acute: 2.5 ± 0.5 nmol/L/s), and MaxL (Previous: 344 ± 20 nmol/L and Acute: 353 ± 27 nmol/L) were all significantly different from both each other and the mean physiologic control when TFPI, FII and AT were all adjusted to 100% (all comparisons $p < 0.01$). Figures 3C and 3D depict the shift that occurs from the adjustment of TFPI alone for acute subjects and previous subjects, respectively. To eliminate the shift in the previous patients with regard to etiology, adjusting either FVIII or TFPI eliminates the differences between carotid stenosis and atrial septal defect patients.

Discussion

Our data show significant differences between coagulation factor levels between patients who have suffered an ischemic event in the last 24 hours and survivors of such events one to four years post-incident. These differences result in shifts in the hypothetical thrombin generation curves with the acute conditions, causing an 11% increase in the average maximum rate, a 15% increase in maximum level of thrombin and a 26% increase in the area under the curve. The current findings provide new insights into our knowledge regarding the origin and nature of hypercoagulability associated with cerebrovascular ischemic events.

Of particular interest is the fact that acute patients had no differences in any clotting factor level or thrombin parameter with regard to etiology. While a few such significant differences exist between patients who had suffered a previous cerebrovascular event (specifically showing carotid stenosis as more prothrombotic than atrial septal defect), most differences were not significant. Also of interest was the finding that all stroke sub-types except atrial fibrillation showed significant differences in AT, TFPI and AUC. The rationale for the similarities between acute and previous atrial fibrillation patients, while differences exist in the other three sub-groups (cryptogenic, carotid stenosis and atrial septal defect), requires further investigation.

Our results confirm previous studies showing enhanced thrombin formation in acute stroke/TIA using a different methodological approach [2;3]. Importantly, in this study, this prothrombotic shift is driven primarily by the increased concentration of FII and decreased concentration of AT in the acute patients compared to those with a history of previous ischemic events. The increased levels of free TFPI appear to protect the acute patients from being more severely prothrombotic. This study is the first to show that circulating clotting factors and inhibitors, mostly being within the reference ranges (i.e. not flagged as “abnormal”), can

potentially affect the kinetics of thrombin formation both in acute and previous stroke/TIA patients as seen in subjects with acute myocardial infarction versus those with stable coronary artery disease.[16]

Thrombin generation is regulated by complex zymogen to enzyme transformations and an extensive array of stoichiometric and dynamic inhibitors. Various methods utilized to profile thrombin generation have shown that individuals with thrombosis have increased thrombin generation [22]. Recently, in a longitudinal study of VTE, the maximum level of thrombin generation was found associated with VTE [23]. In this study we have shown that plasma factor based thrombin generation profiles differentiate between acute (ischemic stroke or TIA) and previous conditions (1 to 4 years following prior ischemic stroke or TIA). Thrombin parameters of maximum level, maximum rate and area under the curve were significantly elevated in acute conditions.

Previously we have shown that by utilizing mathematical modeling we can begin to understand the plasma factor composition influence on TF-initiated blood coagulation with a focus on thrombin generation in various pathologies [24]. With these models, small variations within the normal range of plasma factor composition have been shown to cause large changes in thrombin generation resulting in a prothrombotic phenotype [16;17]. Using this same approach to investigate plasma factor composition influence on acute ischemic cerebrovascular events, we identified FII and AT to be the cause of the increase in thrombin generation towards a prothrombotic phenotype, whereas increased TFPI levels in acute conditions prevent an even more prothrombotic phenotype.

Although numerous coagulation factor levels are different between subjects with an acute or previous condition, not all of them have an impact on the differences in thrombin generation that are seen. FV, FVIII and FX all differed significantly between subjects with an acute or previous condition. However, adjusting any of these (by itself or together), did not alter any thrombin generation parameter by more than 10%, except for maximum rate in the acute subjects when FVIII was adjusted.

This study has several limitations. First, evaluation of follow-up data for the acute patients was beyond the scope of the current study. Therefore, we cannot speculate on a potential impact of thrombin generation parameters on clinical endpoints, including neurological deficit or the risk of recurrent cerebrovascular events. Second, it remains unclear to what extent and how quickly acute changes in coagulation factors and inhibitors observed during the acute phase of cerebrovascular ischemic episodes subside. It might be speculated that in some patients altered levels of some factors represent a permanent feature making them predisposed to recurrent stroke or TIA. Since we excluded patients who have experienced two or more strokes or TIAs, it is not known whether thrombin generation profiles are even more prothrombotic in such patients compared to those following the first-ever event. Since all patients were under the age of 70, we cannot forecast whether the differences we observe in this study can be extrapolated to elderly stroke patients. Finally, according to the new definition proposed in 2009 [25], TIA is a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction. At present MRI, including diffusion-weighted imaging, considered the preferred brain diagnostic imaging modality in patients with TIA, has not been used. Therefore, most likely, more than 10 patients with TIA in the current study represent the stroke population according to the new tissue-based revised definition of TIA [25].

We might speculate that the monitoring of FII and AT (as well as free TFPI) might be useful as a marker of thrombotic potential in the stroke population. A large prospective study is needed to evaluate the clinical relevance of our findings.

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Abbreviation list

AT	antithrombin
AUC	area under the curve
CRP	C-reactive protein
CT	computed tomography
F	factor
IL-6	interleukin-6
LDL	low density lipoprotein
MaxL	maximum level of thrombin production
MaxR	maximum level of thrombin production
MRI	magnetic resonance imaging
TAT	thrombin-antithrombin complex
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TIA	transient ischemic attack

Figure 1. Thrombin generation curves for each group of patients with standard deviation bars at each time point. A=previous stroke; B=acute stroke; C=previous TIA; D=previous TIA.

Figure 2.

(A) Thrombin generation curves for all acute patients, all previous patients and the mean physiologic control. The acute patients are represented by the curve + SD, and the previous patients are represented by the curve - SD. (B) Thrombin generation curves for all acute patients with levels of FII and AT set to 100%, all previous patients and the mean physiologic control. The acute patients are represented by the curve + SD, and the previous patients are represented by the curve - SD.

Figure 3.

Effect of TFPI. (A and B) Thrombin generation curves for the mean physiologic control and the patients with levels of FII, AT and TFPI set to 100%. A=acute patients + SD; B=previous patients – SD. (C and D) Thrombin generation curves for the mean physiologic control and the patients with levels of TFPI set to 100%.

Table 1

Clinical characteristics of the patients with ischemic stroke or transient ischemic attack (TIA).

	Previous TIA (n=50)	Previous Stroke (n=50)	Acute TIA (n=50)	Acute Stroke (n=50)
Male, %	66	64	54	48
Age, y	53 (47-60)	57 (50-61)	56 (42-61)	49 (34-61) ²
Body Mass Index, kg/m ²	26.6 (23.8-28.6)	25.7 (24.0-28.1)	26.2 (24.2-28.7)	26.6 (24.4-28.8)
Previous Myocardial Infarction, %	16	28	24	24
Carotid Stenosis, %	32	42	30	20
Current Smoking, % *	34	44	30	26
Hypertension, % *	60	56	62	44
Diabetes, %	14	8	12	14
Creatinine, μmol/L	78 (68-89)	76 (63-92)	80 (71-88)	78 (69-89)
Glucose, mmol/L	5.0 (4.4-5.5)	5.3 (4.8-6.1)	4.9 (4.5-5.6)	5.0 (4.4-5.6)
Total Cholesterol, mmol/L	5.0 (4.5-5.9)	5.4 (4.7-6.0)	4.2 (3.7-5.1) ^{1,2}	4.6 (3.4-5.2) ^{1,2}
HDL-cholesterol, mmol/L	1.30 (1.07-1.52)	1.28 (1.11-1.48)	1.19 (1.06-1.38)	1.20 (1.00-1.49)
LDL-cholesterol, mmol/L	3.09 (2.49-3.78)	3.37 (2.88-3.92)	2.44 (1.94-2.98) ^{1,2}	2.66 (1.88-3.19) ^{1,2}
Triglycerides, mmol/L	1.15 (0.84-1.77)	1.42 (1.02-1.98)	1.34 (0.92-1.86)	1.45 (0.87-1.84)
Interleukin-6, pg/mL	2.03 (1.21-3.52)	1.74 (0.92-4.34)	4.88 (2.06-6.18) ^{1,2}	4.81 (2.26-6.39) ^{1,2}
C-Reactive Protein, mg/L	2.08 (1.29-3.52)	2.26 (1.24-3.64)	3.04 (1.60-5.93) ^{1,2}	3.90 (2.74-6.25) ^{1,2}
Platelets, 10 ³ /μL	230 (199-259)	232 (206-261)	238 (208-269)	230 (203-271)
β-Blockers, %	52	52	74	58
Angiotensin-converting enzyme inhibitors, %	72	48	68	60
Aspirin, %	84	92	82	68
Statins, %	68	68	74	54

Data are expressed as median (IQR) or %.

* Current smoking was defined as smoking at least 1 cigarette daily.

Hypertension as documented history of arterial hypertension (>140/90 mmHg), regardless of treatment, immediately before enrollment.

¹ – Significant difference from Previous TIA² – Significant difference from Previous Stroke³ – Significant difference from Acute TIA

Table 2

Plasma factor and inhibitor concentrations in the study population by diagnosis.

Factor	Previous TIA (n=50)	Previous Stroke (n=50)	Acute TIA (n=50)	Acute Stroke (n=50)
FII, %	110 (14)	107 (11)	112 (18)	115 (17)
FV, %	108 (14)	106 (14)	111 (19)	116 (23) ²
FVII, %	107 (11)	109 (12)	107 (19)	113 (20)
FVIII, %	119 (21)	127 (29)	143 (35) ^{1,2}	155 (39) ^{1,2}
FIX, %	111 (16)	113 (17)	117 (24)	115 (22)
FX, %	109 (14)	110 (18)	119 (18) ¹	128 (21) ^{1,2}
AT, %	107 (10)	103 (11)	98 (13) ¹	93 (13) ^{1,2}
Free TFPI, %	130 (26)	119 (20)	143 (29) ²	148 (23) ^{1,2}

Data are expressed as mean (SD) (mean physiologic value in healthy subjects = 100%).

AT denotes antithrombin, TFPI denotes tissue factor pathway inhibitor.

¹ – Significant difference from Previous TIA

² – Significant difference from Previous Stroke

³ – Significant difference from Acute TIA

Table 3

Thrombin generation parameters by diagnosis.

Parameter	Previous TIA	Previous Stroke	Acute TIA	Acute Stroke	Mean Physiologic
Maximum Level (nmol/L)	336 (74)	353 (58) [†]	383 (100) [†]	413 (101) ^{†,2}	325
Maximum Rate (nmol/L/s)	2.1 (0.6)	2.3 (0.4) [†]	2.4 (0.7) [†]	2.5 (0.6) [†]	2.1
Area Under Curve (μmol*s/L)	83 (17)	85 (19)	100 (34) ^{†,2}	112 (37) ^{†,2}	78
Time to 10 nM (s)	232 (43) [†]	212 (33) [†]	230 (43) [†]	223 (37) [†]	197

Data are shown as mean (SD)

¹ – Significant difference from Previous TIA

² – Significant difference from Previous Stroke

³ – Significant difference from Acute TIA

[†] – Significant difference from Mean Physiologic

Table 4**Table 4A. Plasma factor and inhibitor concentrations for acute patients by etiology.**

Factor	Cryptogenic (n=42)	Carotid Stenosis (n=24)	Atrial Septal Defect (n=12)	Atrial Fibrillation (n=22)
FII, %	111 (16)	118 (21)	120 (15)	111 (16)
FV, %	116 (20)	108 (24)	118 (23)	113 (17)
FVII, %	113 (25)	106 (13)	106 (11)	112 (17)
FVIII, %	149 (44)	155 (35)	149 (30)	143 (29)
FIX, %	117 (26)	118 (20)	119 (21)	111 (23)
FX, %	123 (18)	128 (24)	125 (18)	118 (20)
AT, %	97 (15)	93 (10)	91 (10)	98 (13)
Free TFPI, %	146 (29)	146 (26)	156 (15)	138 (25)

Data are shown as mean (SD). No significant differences were found amongst the groups.

Table 4B. Plasma factor and inhibitor concentrations for previous patients by etiology.

Factor	Cryptogenic (n=26)	Carotid Stenosis (n=37)	Atrial Septal Defect (n=17)	Atrial Fibrillation (n=20)
FII, %	107 (12)	108 (13)	115 (16)	107 (10)
FV, %	106 (14)	105 (11)	108 (17)	109 (15)
FVII, %	111 (13)	105 (10)	113 (13)	107 (11)
FVIII, %	130 (34)	115 (16)	134 (25) ²	118 (19)
FIX, %	114 (17)	110 (15)	116 (20)	110 (15)
FX, %	115 (16)	105 (13) ¹	107 (17)	114 (17)
AT, %	105 (10)	106 (11)	108 (10)	101 (10)
Free TFPI, %	122 (10)	131 (24)	112 (22) ²	126 (25)

Data are shown as mean (SD).

¹ – Significant difference from Cryptogenic

² – Significant difference from Carotid Stenosis

Table 5**Table 5A. Thrombin generation parameters for acute patients by etiology.**

Parameter	Cryptogenic	Carotid Stenosis	Atrial Septal Defect	Atrial Fibrillation
Maximum Level (nmol/L)	382 (103)	427 (108)	432 (87)	379 (93)
Maximum Rate (nmol/L/s)	2.3 (0.6)	2.6 (0.7)	2.5 (0.5)	2.3 (0.6)
Area Under Curve (μmol*s/L)	102 (38)	114 (36)	120 (35)	99 (30)
Time to 10 nM (s)	229 (43)	223 (41)	227 (35)	223 (38)

Data are shown as mean (SD). No significant differences were found amongst the groups.

Table 5B. Thrombin generation parameters for previous patients by etiology.

Parameter	Cryptogenic	Carotid Stenosis	Atrial Septal Defect	Atrial Fibrillation
Maximum Level (nmol/L)	345 (71)	329 (58)	380 (75)	343 (61)
Maximum Rate (nmol/L/s)	2.2 (0.5)	2.0 (0.4)	2.6 (0.6) ²	2.1 (0.6)
Area Under Curve (μmol*s/L)	84 (23)	82 (15)	87 (19)	86 (14)
Time to 10 nM (s)	218 (36)	235 (38)	201 (38) ²	221 (40)

Data are shown as mean (SD).

² – Significant difference from Carotid Stenosis