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Circulating activated factor XI and active tissue factor as predictors of worse prognosis in patients following ischemic cerebrovascular events

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Abstract

Background—Elevated factor (F)XI is associated with an increased risk for ischemic stroke. Activated FXI (FXIa) and tissue factor (TF) have not been studied following stroke. The aim of the current study was to evaluate circulating FXIa and TF in patients with prior cerebrovascular events.

Patients/Methods—We studied 241 patients, including 162 after ischemic stroke and 79 after transient ischemic attack (TIA), recruited 6 months to 4 years (median, 36 months) after the events. Plasma TF and FXIa activity at discharge at the time of index event were determined in clotting assays by measuring the response to inhibitory monoclonal antibodies.

Results—Active TF was detected in 25 (10.4%) of the patients, while FXIa activity (median, 37.5 [IQR 397] pM) was found in 64 (26.7%) of the patients ($p < 0.01$). The prevalence of active TF and FXIa was higher in subjects with previous stroke compared with those with a history of TIA (13 vs 5.1%, $p = 0.05$, and 34% vs 11.4%, $p < 0.0001$, respectively). Patients with circulating FXIa were younger and had higher fibrinogen and interleukin-6 compared to the remainder. Patients with detectable TF or FXIa activity had higher NIHSS score, higher modified Rankin scale and lower Barthel Index than the remaining subjects (all $p < 0.05$).

Conclusion—Circulating active TF and FXIa can occur in patients with cerebrovascular ischemic events ≥ 6 months after the events. The presence of these factors is associated with worse functional outcomes, which highlights the role of persistent hypercoagulable state in cerebrovascular disease.

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Introduction

Tissue factor (TF) is the key physiological activator of blood coagulation.¹ TF is expressed in the vessel wall and in disease states in monocytes and microparticles in circulating blood in small amounts. The TF-activated factor (F) VII complex catalyzed conversion of FX to its active form, which in turn cleaves prothrombin to generate thrombin. Both thrombin and TF-FVIIa complex can activate FXI representing the intrinsic cascade in which FXII is activated via the contact pathway activators, including collagen, polyphosphates and extracellular RNA.³ Thrombin-mediated FXI activation contributes to the impairment of fibrinolysis via enhanced activation of thrombin-activatable fibrinolysis inhibitor (TAFI).⁴

It has been demonstrated that both FXII and FXI are involved in the thrombus formation and stabilization during stroke.⁴ However, there is a paucity of data on the association between FXI or TF and ischemic cerebrovascular events.⁵ A 5-fold increased risk of ischemic stroke has been shown in patients aged less than 55 years with elevated FXI levels above the 95th percentile of the control range.⁶ A higher risk of stroke observed at elevated FXI levels (>144% of normal) has been suggested to be linked with dyslipidemia.⁷ However, all the available association studies presented FXI antigen, with the results expressed as a percentage, where 100 percent was equivalent to the mean normal FXI antigen level. To our knowledge, no data on plasma coagulant FXIa activity in post-stroke patients are available.

It has been shown that a blockade of FXI may protect against cerebral ischemia without overtly affecting hemostasis in mice. Thus, FXIa inhibition could represent a viable new approach to prevent and treat thromboembolic disorders.^{9,10} These data indicate that FXIa activity might be involved in the pathophysiology of ischemic cerebrovascular events. Therefore, the aim of this observational study was to investigate associations between the presence of active TF and FXIa in circulating blood and clinical outcome in patients with cerebrovascular events.

Patients and Methods

We enrolled white patients with ischemic cerebrovascular events aged 65 years or less, who had a history of ischemic stroke or TIA within 6 months to 4 years prior to enrollment and did not experience clinically evident ischemic episodes in the meantime. Stroke was defined according to WHO criteria and demonstrated by brain imaging. TIA was defined as a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, lasting <24 hours. All patients diagnosed with stroke and most those with TIAs had computed tomography (CT) or CT followed by conventional magnetic resonance imaging (MRI) performed during their hospital stay.

Exclusion criteria were: intracerebral or subarachnoid hemorrhage, acute illness, known cancer, hepatic or renal dysfunction, acute coronary syndrome within the preceding 6 months, treatment with oral anticoagulants, heparins or clopidogrel. Patients who were treated with fibrinolytic agents or who had suffered an iatrogenic stroke due to diagnostic and/or therapeutic interventions such as catheter angiography were not included.

The study was approved by the Jagiellonian University Ethical Committee. All participants gave informed written consent.

Stroke subtyping

Stroke and TIA etiology was diagnosed according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria as large vessel disease (LVD, or atherothrombotic) stroke, small vessel disease (SVD, or lacunar) stroke, cardioembolic (CE) stroke, stroke of other

determined etiology (i.e dissection), and stroke of undetermined etiology (cryptogenic), including subjects with patent foramen ovale or two potential causes.¹¹ The diagnostic work-up included ultrasound examination of the carotid and vertebral arteries, electrocardiography, transthoracic echocardiography, and thrombophilia screening (antiphospholipid syndrome). Atherothrombotic stroke was defined as carotid and vertebral artery stenosis >50% in the arterial territory of the ischemic event. SVD was defined as a clinical lacunar syndrome with normal imaging or a compatible subcortical or brain stem lesion with a diameter <1.5 cm on CT or MRI. Cardioembolic stroke was defined on the basis of a potential cardiac source of an embolus even if the infarct was of lacunar type.¹²

Stroke scales

The severity of an ischemic cerebrovascular event was assessed at enrolment using the National Institutes of Health Stroke Scale (NIHSS) score.¹³ Functional status was recorded using the modified Rankin scale (mRS).¹⁴ The Barthel index (BI) was also used to measure basic aspects of self-care and mobility.¹⁵

Risk factors

Arterial hypertension was diagnosed if he/she met one of the following criteria: 1) a history of hypertension; 2) preadmission treatment with antihypertensives; 3) systolic or diastolic pressure ≥ 140 mm Hg or ≥ 90 mm Hg, respectively, persisting >7 days after the acute event. Previous cardiovascular events were established based on medical records. Diabetes mellitus was defined as a previous diagnosis of diabetes, or at least 2 random glucose levels of >7 mmol/l after the acute vascular event. Current smoking was defined as smoking at least 1 cigarette daily. Paroxysmal or persistent atrial fibrillation was recorded from clinical history and notes review.

Laboratory methods

Blood cell counts, biochemical analysis, including lipid profile, creatinine, glucose, were assessed by standard automated laboratory methods. Venous blood samples for clotting assays were taken into 0.13 M trisodium citrate tubes (Becton Dickinson) and centrifuged at 24 °C and 2500 g for 20 minutes within 10 min of collection. Platelet-poor plasma was immediately frozen and stored at -80 °C. Fibrinogen and C-reactive protein (CRP) were assayed by immunonephelometry (Siemens, Marburg, Germany). Commercially available immunoenzymatic assay was used to determine serum interleukin-6 (R&D Systems, Abingdon, UK). All intra-assay and inter-assay coefficients of variation were <7%.

Plasma clotting assays were performed as described previously.¹⁶ Briefly, plasma was thawed at 37 °C in the presence of corn trypsin inhibitor (CTI, an inhibitor of the contact pathway of blood coagulation by blocking FXI activation by FXIIa) and either buffer or inhibitory monoclonal anti-FXI (α FXI-2) or anti-TF (α TF-5) antibody (both produced in house) at a final 0.1 mg/ml concentration was followed by the addition of CaCl_2 at a final 15 mM concentration. Clotting was initiated by the addition of 2 μM phospholipid vesicles (PCPS) composed of 25% dioleoyl-*sn*-glycero-3-phospho-L-serine and 75% 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (both from Avanti Polar Lipids, Inc; Alabaster, AL). Clotting times were determined using the ST8 clotting instrument (Diagnostica Stago, Parsippany, NJ). FXIa and TF activity in plasma was calculated from calibration curves built by sequential dilutions of human FXIa (a gift from Dr. R. Jenny from Haematologic Technologies, Inc., Essex Junction, VT) or relipidated TF₁₋₂₄₂ (a gift from Dr. R. Lundblad from Baxter Healthcare Corp., Duarte, CA) in pooled 10-donor plasma. Laboratory personnel were “blinded” to the status of samples. Age- and sex-matched apparently healthy

control individuals (n=12) recruited from the hospital staff showed no circulating TF or FXIa.

Statistical analyses

Data are given as mean $\pm$ SD, median (interquartile range), or percentage unless otherwise stated. Normality of a value distribution was tested using the Kolmogorov-Smirnov test. Intergroup differences for continuous variables were assessed by the Wilcoxon test for non-Gaussian distribution or Student's t-test for normal distribution. The Spearman correlation coefficient was calculated to test significant associations between variables. The Fisher exact test was used to assess intergroup difference in categorical variables. A p-value <0.05 was considered significant.

Results

Study populations

Of the 246 individuals, five patients were excluded due to incomplete clinical data. The demographic, clinical, and routine laboratory data for the 241 with a history of an ischemic cerebrovascular event are summarized in Table 1. Analysis of the study population divided into stroke and TIA patients is shown in Table 2.

Twenty-five of the 240 (10.4%) patients with previous ischemic events had circulating TF activity, including as few as 8 (3.3%) subjects with values above 0.5 pM. Sixty-four of the 240 (26.7%) patients with previous ischemic events had circulating FXIa (median, 37.5 [IQR 397] pM). The majority of the patients having active TF had also FXIa (24 [96%]). Of note, there was no difference in the time since the ischemic event to blood collection between TF or FXIa-positive and negative patients.

Active TF was detected in 21 of the 161 previous stroke patients (13%) and in 4 of the 79 previous TIA patients (5.1%; $p=0.05$). Corresponding values for FXIa were 55 of the 162 (34%) and 9 of the 79 (11.4%; $p<0.0001$), respectively. Compared with previous stroke patients, subjects diagnosed with previous TIA had more frequently diagnosed cryptogenic event and less frequently lacunar event (Table 2).

Patients with a history of cerebrovascular events and detectable TF did not differ from those without circulating TF activity with regard to demographics, clinical variables and routine laboratory tests (Table 2). Patients with circulating FXIa were younger, had lower glucose and higher fibrinogen, IL-6, HDL cholesterol compared to the remainder. Patients who experienced an ischemic event prior to the index stroke or TIA did not differ from the remaining subjects with regard to the presence of TF or FXIa (data not shown).

The etiology of ischemic events was similar in patients having circulating TF, or FXIa and those without these factors (Table 2). The only significant difference was a lower prevalence of FXIa in patients with SVD compared with the remainder ($p=0.035$). Importantly, patients following cerebrovascular ischemic event with detectable TF activity as well as those with circulating FXIa had a more severe neurological deficit reflected by higher NIHSS score at enrollment several months after the event, together with higher mRS score and lower BI as compared with those without active TF following stroke or TIA (Table 2). This also held true for patients following cerebrovascular ischemic event with detectable circulating FXIa, who had higher NIHSS and mRS scores and lower BI compared with the remainder (Table 2).

Discussion

This study shows that a proportion of patients following a median of 36 months from documented cerebrovascular ischemic events can still have active TF and FXIa. To our knowledge, this is the first study on the presence of active TF and FXIa in blood of patients with a history of cerebrovascular events. Importantly, we found that FXIa as well as TF are associated with worse prognosis and more severe neurological deficits following cerebrovascular events. This suggests that these factors could be novel thrombotic markers of worse clinical outcome in patients with ischemic cerebrovascular events.

Previous studies showed acute prothrombotic changes in coagulation and fibrinolytic parameters during ischemic stroke.^{17,18} Suri et al.¹⁹ have demonstrated in a large population that elevated FXI is associated with the risk of ischemic stroke. Previously, we have shown that 76% of CAD patients with a history of MI have circulating FXIa and 6% of stable CAD patients showed TF activity in plasma.¹⁶ In the current study, the pattern of prothrombotic alterations in active TF and FXIa is similar in subjects with previous MI and those with a history of stroke or TIA. Similarly, proportions of the subjects positive for TF and FXIa were similar in patients with heart failure.²⁰

A source of TF detected in plasma in the current study remains unclear. Cell-derived microparticles could be the main carriers of circulating TF.²¹ A less likely source of TF following cerebral ischemia could be the brain tissue.²² By analogy with acute MI, TF present in the plaque may also appear in circulating blood following damage to the atherosclerotic plaque in the carotid artery. TF might be derived from monocytes activated by proinflammatory cytokines; their increased levels were reported in AIS and associated with worse prognosis.²³ Our data show that in a majority of plasma samples from patients with acute cerebrovascular event the concentration of active TF is below 1 pM. This finding is in stark contrast with the data suggesting activity of TF with a mean of 193 pg/mL in patients with AIS.²⁴ Largely due to the lack of validated and reliable commercial assays for TF antigen and activity,²⁵ there are marked discrepancies in the results of measurements of TF in blood, with high (reaching sub-nanomolar) TF concentrations reported.^{26,27} Several lines of evidence clearly indicate that such high functional TF levels would cause plasma or blood clotting within minutes (seconds).^{26,27} Since our assay for TF activity determination in citrated plasma has been validated and used in clinical settings,¹⁶ we believe that this analysis provides better quantitation of active TF concentrations in a wide spectrum of subjects with ischemic cerebrovascular events. It has been shown in a previous study using another methodology that within the first 6 months since stroke, plasma TF levels remain elevated.²⁸ It might be hypothesized that persistent presence of active TF following an ischemic cerebrovascular event is related to worse clinical outcomes. As in the acute MI patients¹⁶, we observed that detectable amounts of active FXIa are permanently present in plasma despite the abundance of numerous inhibitors for serine proteases because none of the physiologic inhibitors can effectively inhibit FXIa. As a consequence, 80 to 90% of FXIa can survive in blood for at least the 30 min required for citrate plasma preparation.¹⁶ There is no *de novo* FXIa generation observed in citrate plasma at room temperature over a period of 75 min either in the presence of contact pathway inhibition or in the absence of it.¹⁶ These data indicate that plasma FXIa in the current study does indeed reflect the *in vivo* levels of this enzyme.

Cerebrovascular ischemic events represent heterogeneous syndromes caused by several different underlying pathologies. Interestingly, lower frequency of FXIa activity in patients with previous SVD event provides additional evidence that the pathophysiology of this stroke type is probably associated with a distinct lacunar arteriopathy with a small contribution of thrombotic background.^{29,30}

In the study evaluating FXIa and TF activity in CAD patients, there was strong evidence that FXIa was generated by thrombin *via* TF pathway.¹⁶ FXIa in stroke patients is, most likely, generated by the same pathway. The enhanced inflammatory state in acute patients with FXIa in blood, reflected by elevated fibrinogen, CRP, and IL-6, suggests a possibility for blood cell (primarily monocyte) stimulation to produce TF,²⁷ which is supported by the data indicating that 96% of patients having TF activity in their plasma display FXIa as well. It has been reported that plasma TF activity is associated with microparticle-bound TF,³¹ because soluble forms of TF have little (if any) procoagulant activity.³² As a consequence, only those plasma samples which contain TF-bearing microparticles will show procoagulant TF activity. Additionally, there is no evidence suggesting a correlation between TF concentration/activity in plasma and those in the whole blood. Moreover, it is likely that in some patients following ischemic stroke the presence of circulating active TF and FXIa may reflect chronic non-vascular diseases, for example inflammatory disorders. These observations explain why only all stroke patients with FXIa activity have active TF in their plasma as well.

The study has limitations. First, the size of the subgroups is limited, and the results of such analyses should be interpreted with caution. Second, all laboratory measurements were performed on a single occasion a few months or years following the event. Therefore the study did not address what drives persistent presence of active TF and/or FXIa in patients following the event regardless of the etiology. Lack of their correlations with time elapsed from index event, however, argues for persistence of these factors in a subset of patients who survived ischemic stroke or TIA. Third, we did not assess stroke volumes or other variables based on imaging data.

In conclusion, we showed that a significant proportion of patients following ischemic cerebrovascular events exhibit circulating functionally active TF and FXIa, which is associated with worse functional outcomes. It remains to be established to what extent inhibition of these factors, in particular FXIa, may improve long-term prognosis in patients with previous cerebrovascular ischemic events.

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Table 1

Characteristics of total group of patients (n=241) and classified into 2 categories i.e. stroke and transient ischemic attack (TIA).

Variable	Total	previous stroke	previous TIA
	n=241	n=162	n=79
Age, y	37 (24-58)	36.5(25-58)	40(23-58)
Male, n(%)	96 (39.8)	65 (40.1)	31 (39.2)
BMI, kg/m ²	27.2 (24.1-29.2)	27.4(24.7-29.5)	26.6(23.8-31.2)
Smoking, n(%)	55 (22.8)	32 (19.8)	23 (29.1)
Hypertension, n(%)	144 (59.8)	111 (68.5) *	33 (41.8) *
Diabetes, n(%)	20 (8.3)	15 (9.3)	5 (6.3)
Previous MI, n(%)	21 (8.8)	15 (9.3) *	6 (7.6) *
Medication			
Statins, n(%)	64 (26.6)	41 (25.3)	23 (29.1)
β-blockers, n(%)	92 (38.2)	62 (38.3)	30 (38)
ACEIs, n(%)	82 (34)	57 (35.2)	25 (31.6)
Aspirin, n(%)	233 (96.7)	156 (96.3)	77 (97.5)
Laboratory tests			
Creatinine, μmol/l	74 (64-84)	73.7(61-83)	75(68-89)
Glucose, mmol/l	5.2 (4.7-6.2)	5.1(4.8-6.2)	5.3(4.6-6.6)
C-reactive protein, mg/l	2 (1.2-2.7)	2(1.3-2.6)	2.1(1.1)
Interleukin-6, pg/ml	2.8 (2.3-3.4)	2.8(2.3-3.7) *	2.7(2.1-3) *
Fibrinogen, g/l	3.4 (2.7-4)	3.4(2.9-4.1)	3(2.6-3.6)
Total cholesterol, mmol/l	5.2 (4.5-5.7)	5.1(1.0)	5.2(4.7-5.4)
LDL-C, mmol/l	3.1 (2.4-3.7)	3.1(0.8)	3.2(2.6-3.7)
HDL-C, mmol/l	1.4 (1.2-1.7)	1.4(0.4)	1.3(0.4)
Triglycerides, mmol/l	1.2 (0.9-1.8)	1.3(0.9-1.8)	1.3(1-1.8)
Etiology			
cryptogenic, n(%)	125 (51.8)	75 (46.3)	50 (63.3) *
atherothrombotic, n(%)	23 (9.5)	12 (7.4)	11 (13.9)
cardioembolic, n(%)	20 (8.3)	15 (9.3)	5 (6.3)
small vessel disease, n(%)	55 (22.8)	47 (29)	8 (10.1) *
other causes, n(%)	18 (7.5)	13 (8)	5 (6.3)
Scales			
NIHSS	2 (1-5)	2 (1-5)	-
Barthel index	90 (70-100)	90 (70-100)	-
Modified Rankin score	1 (1-3)	1 (1-3)	-

Values are given as median (interquartile range), or percentage.

BMI, body mass index, MI, myocardial infarction; ACEI, angiotensin-converting enzyme inhibitor; CRP, C-reactive protein; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale score obtained 6 to 48 months following the index event.

*
p<0.05,

**
<0.001 versus acute patients

Table 2

Comparisons of patients with FXIa and TF activity with those without such activity

Variable	TF=0 N=215	TF=1 N=25	FXIa=0 N=177	FXIa=1 N=64
age, y	40 (25-59)	35 (25.5-48.5)	43 (25-61)	29 (23-47.8)*
male, n(%)	84 (39.1)	11 (44)	75 (42.4)	21 (32.8)
BMI, kg/m ²	27.4 (24-30.6)	26.6 (24.3-29.1)	27.2 (23.9-30.1)	27.8 (24.5-30.3)
Smoking, n(%)	47 (21.9)	8 (32)	41 (23.2)	14 (21.9)
Hypertension, n(%)	125 (58.1)	19 (76)	100 (56.5)	44 (68.8)
Diabetes, n(%)	18 (8.4)	2 (8)	16 (9)	4 (6.3)
Previous MI, n(%)	17 (7.9)	4 (16)	16 (9)	5 (7.9)
Previous stroke, n(%)	32 (14.9)	2 (8)	28 (15.8)	6 (9.4)
medication statins, n(%)	54 (25.1)	9 (36)	47 (26.6)	17 (26.6)
β-blockers, n(%)	80 (37.2)	12 (48)	64 (36.2)	28 (43.8)
ACEIs, n(%)	72 (33.5)	10 (40)	62 (35)	20 (31.3)
aspirin, n(%)	208 (96.7)	24 (96)	171 (96.6)	62 (96.9)
Laboratory tests creatinine, μmol/l	74 (64.5-83.5)	72.2 (60.8-89.8)	74.1 (67-84.5)	72 (56-80.5)
glucose, mmol/l	5.2 (4.7-6.3)	5 (4.8-5.6)	5.3 (4.7-6.6)	5 (4.6-5.4)*
CRP, mg/l	2 (1.3-2.6)	2.3 (0.9-3.6)	2 (1.4-2.5)	1.8 (1-3.3)
interleukin-6, pg/ml	2.7 (2.2-3.3)	3.5 (2.5-4.5)*	2.7 (2.1-3.3)	2.9 (2.4-4.1)*
fibrinogen, g/l	3.3 (2.7-4)	3.4 (3-4.2)	3.2 (2.6-3.8)	3.6 (2.9-4.2)*
total cholesterol, mmol/l	5.1±1.1	5.2±1.0	5.1±1.0	5.3±1.2
LDL-C, mmol/l	3.1±0.9	3.1±0.8	3.1±0.9	3.1±0.8
HDL-C, mmol/l	1.4 (1.1-1.6)	1.4 (1.3-1.6)	1.2 (1-1.6)	1.5 (1.3-1.7)*
triglycerides, mmol/l	1.2 (0.9-1.8)	1.35 (1-1.7)	1.3 (0.9-1.9)	1.3 (0.8-1.6)
etiology cryptogenic	111 (51.7)	13 (52)	89 (50.2)	36 (56.3)
atherothrombotic	21 (9.8)	2 (8)	17 (9.6)	6 (9.4)
cardioembolic	18 (8.4)	2 (8)	17 (9.6)	3 (4.7)
small vessel disease	51 (23.7)	4 (16)	46 (26.0)	9 (14.1)*
other causes	14 (6.5)	4 (16)	8 (4.5)	10 (15.6)
scales				
NIHSS	2 (1-3)	6 (3.5-6.5)**	2 (1-3)	3 (1-6)*
Barthel index	90 (70-100)	70 (47.5-75)**	90 (75-100)	80 (56.3-95)*
Rankin score	1 (1-2)	3 (2.5-4)*	1 (1-2)	2 (1-3)*

Values are given as mean±SD, median (interquartile range), or percentage.

Abbreviations see Table 1.

*
p<0.05,

**
<0.001 versus patients not having the respective factors.