RESEARCH LETTER

Denser fibrin clot networks in patients at high risk of recurrent arterial thromboembolism following acute limb ischemia of unknown cause

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Introduction Acute limb ischemia (ALI) is a limb-threatening condition typically observed in older patients with cardiovascular risk factors. In approximately 15% of cases there is no established cause, and this rate is even higher among young patients.[1] ALI is characterized by an enhanced activation of hemostasis, and in 2020 we reported that in patients after ALI of unknown cause, a prothrombotic state involves formation of dense fibrin networks resistant to lysis, that is, a prothrombotic clot phenotype.[2] There is evidence that in patients with peripheral artery disease (PAD), venous thromboembolism (VTE), atrial fibrillation (AF), and coronary artery disease, prothrombotic fibrin clot properties can also be observed, and they can increase the risk of recurrent thromboembolic events not only after discontinuation of anticoagulant therapy.[3-7]

Based on our previous cohort study,[2] we investigated whether the patients with ALI of unknown cause who experienced a recurrent arterial thrombotic episode (ATE) of the limbs during the follow-up while off anticoagulation are characterized by a more prothrombotic clot phenotype than as compared with those free of such episodes.

Patients and methods We assessed 43 patients with a history of ALI of unknown origin and a reference group of 43 patients after non-lacunar, cryptogenic embolic stroke matched for age and sex, as described.[2] The inclusion and exclusion criteria and definitions of comorbidities were presented previously.[2] Briefly, the diagnosis of ALI was based on typical symptoms, signs, and abnormalities on Doppler ultrasound imaging. To exclude known causes, especially AF and intracardiac thrombi, 24-h Holter monitoring as well as transthoracic and transesophageal echocardiography were performed at baseline. Severe thrombophilia was also excluded.

Blood samples were collected after cessation of anticoagulant therapy lasting for a median (interquartile range [IQR]) of 7 (6–8) months. To characterize the plasma fibrin clot phenotype, clot permeation determined by permeability coefficient (K_s), markers of fibrinolysis (clot lysis time [CLT], maximum rate of increase in D-dimer levels in the lysis assay [D-D_{rate}], and maximum values of D-dimer released from clots, [D-D_{max}]), and turbidity measurements for fibrin polymerization (lag phase of the turbidity curve and maximum absorbance at 405 nm) were used as described previously.[2] Thrombin generation using calibrated automated thrombography determined by endogenous thrombin potential (ETP) as well as peak thrombin concentration, routine laboratory investigations, fibrinolysis inhibitors, plasminogen, and 4 genetic polymorphisms were assessed using the methods previously described.[2]

The participants were followed during visits at the center or via telephone calls performed every 3 to 6 months until April 2022. Holter monitoring and/or resting electrocardiography were repeated at least every 6 months. Documented recurrent ATEs while off anticoagulation were recorded. The data were censored, since following such events the patients were put back on anticoagulation, usually in combination with antiplatelet agents. Moreover, cardiovascular death, stroke, and myocardial infarction (MI) were recorded, along with AF defined based on the European guidelines.[8]

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The study was approved by the bioethics committee of the Jagiellonian University Medical College (KBET 1072.6120.136.2018) and all participants provided written informed consent.

Statistical analysis The analysis was performed with R 4.1.3 (R Core Team, 2022; R Foundation for Statistical Computing, Vienna, Austria) using the publicly available tidyverse, rstatix, and arsenal packages with subsidiaries. Continuous variables were summarized using median and IQR, while categorical variables were reported as counts and proportions. Comparison of continuous and categorical variables was performed using the Kruskal–Wallis test and the Fisher exact test, respectively. A *P* value below 0.05 was considered significant.

Results The median (IQR) follow-up was 40 (33.5–48.0) months. Characteristics of the patients are shown in TABLE 1. In the ALI group there were 6 ATEs (14%) of the lower limb. Moreover, there were 3 cases of MI (7%), 4 ischemic strokes (9.3%), and 4 deaths (9.3%). Importantly, 4 patients in this group (9.3%) were diagnosed with paroxysmal or persistent AF, including 2 individuals (50%) with recurrent ATE, but none had ischemic stroke.

The participants with recurrent ATE did not differ from the remaining patients with ALI in terms of demographics, clinical characteristics, results of routine laboratory investigations, and the prevalence of genetic polymorphisms (TABLE 1). The same held true for fibrinolysis and thrombin generation variables. Of note, the patients with recurrent ATE had more compact plasma fibrin clots at baseline, as reflected by 8% lower baseline K_s as compared with those without ATE, while other fibrin variables were similar (TABLE 1).

In the cryptogenic stroke group, there was only 1 ATE (2.3%), 1 MI (2.3%), 3 recurrent strokes (7%), and 4 deaths (9.3%) along with 3 cases of newly diagnosed AF (7%), including 1 patient (33.3%) who experienced ATE, and none with recurrent stroke. The patients with recurrent thromboembolic episodes (TEs) were slightly older than the other patients in this group. Apart from that, the subgroups did not differ in other variables, including fibrinolysis and thrombin generation markers (TABLE 1). Of note, the stroke patients with recurrent TE had lower baseline levels of total cholesterol and low-density lipoprotein cholesterol and an increased baseline level of fibrinogen as compared with those without TE recurrence. Importantly, the patients with cryptogenic stroke who experienced recurrent TE during the follow-up had less permeable and denser fibrin clots at baseline, as reflected by 25.4% lower K_s, and 9.5% greater D-D_{max} levels at baseline as compared with those without recurrent TEs (TABLE 1).

Discussion This study is the first to show that lower fibrin clot permeability, a key measure of plasma fibrin clot structure, is associated with the risk of recurrent ATEs in patients with prior ALI of unknown etiology. We did not observe any TE risk–related differences in either group in other prothrombotic indices, such as higher ETP or α_2-antiplasmin, or those of impaired lysis (eg, longer CLT, lower D-D_{rate}),[2] indicating that the clot permeability, even though there is no standardized method for its measurement, appears to be the best marker of future ATE risk. Even more pronounced alterations to fibrin properties were found in the patients with prior cryptogenic stroke with recurrent events, which is consistent with our previous studies on ischemic stroke patients.[5,9] The current report provides additional evidence for the impact of unfavorable fibrin clot features on the TE risk, which might have practical implications in the future.

We also found that the recurrence of ATEs in the unique group of ALI patients without concomitant PAD is a relatively common event unrelated to age or other variables determined. However, the risk was lower than in the ALI patients with PAD. Vakhitov et al[10] reported that ATE in native arteries occurs in 24.4% of typical ALI patients treated with thrombolysis over a median follow-up of 40 months.

Recurrent ATEs were observed despite the treatment with aspirin and statins, which has been shown to improve fibrin clot characteristics and prevent TEs in different vascular beds,[6,11,12] with the strongest effect on fibrin properties exerted by high-dose statins.[13] In the current study, none of the participants used high-dose statins and we did not assess the lipid profile at the time of recurrent events, but most likely aggressive statin therapy should be administered in the patients with ALI of unknown cause.

Although little is known on the role of anticoagulant treatment in the prevention of recurrent ATEs in ALI, Anand et al[14] demonstrated that when the combination of rivaroxaban 2.5 mg twice daily and low-dose aspirin was tested, a small subgroup of patients with ALI had a reduced risk of major adverse limb events, including ALI, as compared with those on aspirin alone. It remains to be established whether patients with ALI of unknown cause could benefit from such a drug combination more than from full-dose anticoagulation. Of particular importance in the clinical situation described is rigorous AF screening if the patients stop anticoagulation, given a rising prevalence of AF and its insufficient management.[15] Emboli of unknown cause could result from undiagnosed asymptomatic short AF episodes.[8] In light of the relatively high incidence of AF in this group during the follow-up, despite a low number of events, it may be concluded that patients with ALI of unknown cause are prone to experience recurrent events, and prolonged anticoagulation, if no bleeding occurs, should be considered.

Study limitations should be acknowledged. The group size was relatively small and when

TABLE 1 Characteristics of the study groups (continued on the next page)

Variable	ALI with no recurrent ATE (n = 37)	ALI with recurrent ATE (n = 6)	*P* value	Stroke with no recurrent TE (n = 39)	Stroke with recurrent TE (n = 4)	*P* value
Age, y	51 (48–57)	54 (52–56.7)	0.3	55 (50–59.5)	59 (58.7–59.7)	0.01
Male sex	20 (54.1)	4 (66.7)	0.68	20 (51.3)	3 (75)	0.61
BMI, kg/m^2	27.7 (26–30.5)	24.8 (24.05–25.4)	0.13	26.5 (24.7–29.9)	24.85 (23.55–27.1)	0.29
Oral contraception	10 (27)	2 (33.3)	>0.99	7 (17.9)	0	>0.99
Cigarette smoking	26 (70.3)	4 (66.7)	>0.99	18 (46.2)	1 (25)	0.62
Family history of VTE	10 (27.0)	1 (16.7)	>0.99	1 (2.6)	1 (25)	0.18
Comorbidities						
Hypertension	28 (75.7)	3 (50)	0.33	12 (30.8)	1 (25)	>0.99
Diabetes	7 (18.9)	1 (16.7)	>0.99	2 (5.1)	0	>0.99
Medications						
ASA	37 (100)	6 (100)	>0.99	39 (100)	4 (100)	>0.99
ACEI	27 (73)	2 (33.3)	0.08	12 (30.8)	1 (25)	>0.99
β-Blocker	20 (54.1)	3 (50)	>0.99	17 (43.6)	0	0.14
Statin	29 (78.4)	6 (100)	0.57	7 (17.9)	0	>0.99
Laboratory parameters						
Hemoglobin, g/dl	14 (13–14.6)	13.4 (12.55–15.15)	0.82	13.7 (12.9–14.5)	13.95 (13.73–14.4)	0.44
White blood cells, × 10^9/l	6.46 (5.71–7.53)	7.59 (6.45–8.23)	0.19	7.07 (5.88–7.79)	7.16 (6.82–7.54)	0.72
Platelets, × 10^9/l	251 (210–305)	231 (183.25–290.75)	0.47	212 (186–264)	235.5 (192–283.75)	0.82
APTT, s	28.07 (26.2–30.6)	27.41 (27.02–27.86)	0.32	28.4 (26.35–30.6)	30.06 (28.74–30.28)	0.71
INR	1.01 (0.93–1.05)	1 (0.95–1.09)	0.56	0.99 (0.9–1.08)	0.83 (0.79–0.89)	0.08
eGFR, ml/min/1.73 m^2	96.53 (87.07–104.5)	91.6 (82.78–101.22)	0.34	94.42 (82.84–105.15)	95.96 (88.79–98.56)	0.71
Glucose, mmol/l	5 (4.6–5.5)	4.65 (4.28–4.88)	0.16	4.6 (4.32–5.08)	4.98 (4.74–5.2)	0.22
TG, mmol/l	1.13 (0.7–1.5)	1.06 (0.62–1.64)	>0.99	1.54 (0.87–2.01)	0.98 (0.69–1.32)	0.13
TC, mmol/l	5.18 (4.0–5.7)	5.55 (5.03–6.8)	0.23	5.78 (5.04–6.35)	4.66 (4.22–4.86)	0.03
HDL-C, mmol/l	1.44 (1.19–1.70)	1.56 (1.12–1.64)	0.88	1.29 (1.15–1.61)	1.61 (1.36–1.74)	0.44
LDL-C, mmol/l	2.98 (2.36–3.41)	3.71 (3.02–4.59)	0.12	3.73 (3.16–4.12)	2.63 (2.25–2.87)	0.04
Fibrinogen, g/l	3.06 (2.56–3.86)	4.16 (3.15–4.31)	0.08	2.71 (2.34–3.63)	4.65 (4.08–4.92)	0.03
CRP, mg/l	2.04 (1.41–5.85)	1.51 (1.31–4.66)	0.55	1.66 (1.19–2.42)	2.86 (2.26–3.74)	0.09
D-dimer, ng/ml	289 (204–368)	340 (295–396.25)	0.29	232 (206.5–290)	295 (258.5–346.25)	0.07
Genetic polymorphisms						
Factor XIII Val34Leu	17 (45.9)	4 (66.7)	0.41	18 (46.2)	1 (25)	0.62
α-Fibrinogen Thr312Ala	23 (62.2)	4 (66.7)	>0.99	16 (41)	2 (50)	>0.99
Factor V Leiden	2 (5.4)	1 (16.7)	0.37	3 (7.7)	0	>0.99
Prothrombin 20210A mutation	1 (2.7)	0	>0.99	1 (2.6)	0	>0.99
Fibrinolysis markers						
Plasminogen, %	104 (92–112)	94 (89–100.5)	0.19	99 (93–108.5)	119 (107–126)	0.14
α2-antiplasmin, %	110 (103–120)	117.5 (111.7–121.7)	0.26	102 (91.5–114.5)	105 (92.75–117.75)	0.92
PAI-1, ng/ml	7.99 (6.91–9.6)	9.4 (7.74–10.81)	0.31	14.9 (11.65–20.2)	11.75 (9.78–14.93)	0.35
TAFI, %	97 (89–104)	97.5 (89.25–105.75)	0.79	98 (89.5–105.5)	96.5 (92–100.5)	0.68
Thrombin generation						
ETP, nM/min	1633 (1488–1854)	1498.5 (1452.5–1561)	0.25	1616 (1526–1676.5)	1646 (1625.5–1664.5)	0.53
Peak thrombin, nM	257 (211–342)	264.5 (240–288.25)	0.69	227 (209–258.5)	202 (200.75–230.25)	0.46
Time to peak thrombin concentration, s	349 (306–421)	404.5 (356–463.5)	0.38	310 (235.5–364.5)	271.5 (249.25–304.5)	0.68
Fibrin clot properties						
Lag phase, s	44 (37–48)	40.5 (35–46)	0.38	39 (36.5–41)	38.5 (35.75–42.25)	0.97
ΔAbs_{max}, 405 nm	0.83 (0.74–0.88)	0.83 (0.82–0.87)	0.75	0.79 (0.74–0.85)	0.8 (0.77–0.85)	0.4
K_s, 10^{-9} cm^2	6.5 (6.3–7.7)	6 (5.62–6.3)	0.03	6.9 (6–7.6)	5.5 (5.3–5.73)	0.008
CLT, min	90 (75–106)	87.5 (70.25–110)	0.96	89 (74.5–98)	100 (97.75–101.75)	0.07
$D\text{-}D_{max}$, mg/l	3.8 (3.60–3.98)	3.86 (3.63–3.97)	0.88	4.2 (3.71–4.39)	4.66 (4.47–4.84)	0.03
$D\text{-}D_{rate}$, mg/l/min	0.068 (0.065–0.072)	0.069 (0.067–0.071)	0.83	0.071 (0.067–0.076)	0.071 (0.068–0.074)	0.85

TABLE 1 Characteristics of the study groups (continued from the previous page)

Variable	ALI with no recurrent ATE (n = 37)	ALI with recurrent ATE (n = 6)	*P* value	Stroke with no recurrent TE (n = 39)	Stroke with recurrent TE (n = 4)	*P* value
Follow-up data						
Follow-up duration, mo	40 (37–48)	26.5 (21–38)	0.04	40 (38–48)	30.5 (22–36)	0.02
Stroke	3 (8.1)	1 (16.7)	0.47	0	3 (75)	<0.001
Death	3 (8.1)	1 (16.7)	0.47	4 (10.3)	0	>0.99
Myocardial infarction	3 (8.1)	0	>0.99	1 (2.6)	0	>0.99
Atrial fibrillation	2 (5.4)	2 (33.3)	0.09	2 (5.1)	1 (25)	0.26

Data are shown as median (interquartile range) or number (percentage). *P* values refer to the Kruskal–Wallis test for continuous variables and the Fisher exact test for categorical variables.

Abbreviations: Abs_{max}, maximum absorbance on turbidimetry; ACEI, angiotensin-converting enzyme inhibitors; ALI, acute limb ischemia; APTT, activated partial thromboplastin time; ASA, acetylsalicylic acid; ATE, arterial thromboembolic episode; BMI, body mass index; CLT, clot lysis time; $D\text{-}D_{max}$, maximum D-dimer level in the lysis assay; $D\text{-}D_{rate}$, maximum rate of increase in D-dimer level in the lysis assay; eGFR, estimated glomerular filtration rate; ETP, endogenous thrombin potential; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; INR, international normalized ratio; K_s, permeability coefficient; LDL-C, low-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor 1; TAFI, thrombin activatable fibrinolysis inhibitor; TC, total cholesterol; TE, thromboembolic episode; TG, triglycerides; VTE, venous thromboembolism

analyzing the recurrence of ATEs the size of the sample decreased, causing some of the differences to be lost; therefore, the results should be interpreted with extreme caution. Our results cannot be applied to the patients with ALI not eligible for this study, such as those with malignancies, recent cardiovascular events, or severe thrombophilia. AF screening was suboptimal and longer Holter monitoring should be considered in search for this arrythmia.

In conclusion, the current study suggests that the prothrombotic fibrin clot phenotype could have a prognostic value in the patients with TEs of unknown cause, beyond that of the well-established clinical and laboratory markers. It might be postulated that, if standardized and fully automated, the measurement of plasma clot permeability could be useful in identifying high-risk patients with systemic thromboembolism who may benefit from anticoagulant therapy.

ARTICLE INFORMATION

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REFERENCES

1 Björck M, Earnshaw JJ, Acosta S, et al. European Society for Vascular Surgery (ESVS) 2020 clinical practice guidelines on the management of acute limb ischaemia. Eur J Vasc Endovasc Surg. 2020; 59: 173-218.

2 Karpińska IA, Nowakowski T, Wypasek E, et al. A prothrombotic state and denser clot formation in patients following acute limb ischemia of unknown cause. Thromb Res. 2020; 187: 32-38.

3 Undas A, Nowakowski T, Cieśla-Dul M, et al. Abnormal plasma fibrin clot characteristics are associated with worse clinical outcome in patients with peripheral arterial disease and thromboangitis obliterans. Atherosclerosis. 2011; 215: 481-486.

4 Zabczyk M, Plens K, Wojtowicz W, Undas A. Prothrombotic fibrin clot phenotype is associated with recurrent pulmonary embolism after discontinuation of anticoagulant therapy. Arterioscler Thromb Vasc Biol. 2017; 37: 365-373.

5 Janion-Sadowska A, Chrapek M, Konieczynska M, Undas A. Altered fibrin clot properties predict stroke and bleedings in patients with atrial fibrillation on rivaroxaban. Stroke. 2019; 50: 185-188.

6 Larsen JB, Hvas A-M. Fibrin clot properties in coronary artery disease: new determinants and prognostic markers. Pol Arch Intern Med. 2021; 131: 16113.

7 Matusik PT, Leśniak WJ, Heleniak Z, Undas A. Thromboembolism and bleeding in patients with atrial fibrillation and stage 4 chronic kidney disease: impact of biomarkers. Kardiol Pol. 2021; 79: 1086-1092.

8 Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. The Task Force for the management of atrial fibrillation of the European Society of Cardiology (ESC). Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J. 2016; 37: 2893-2962.

9 Drabik L, Wolkow P, Undas A. Fibrin clot permeability as a predictor of stroke and bleeding in anticoagulated patients with atrial fibrillation. Stroke. 2017; 48: 2716-2722.

10 Vakhitov D, Hakovirta H, Saarinen E, et al. Prognostic risk factors for recurrent acute lower limb ischemia in patients treated with intra-arterial thrombolysis. J Vasc Surg. 2020; 71: 1268-1275.

11 Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomized trials. Lancet. 2009; 373: 1849-1860.

12 Undas A. Statins in prevention of thromboembolic events: from seminal studies to recent advances. Pol Arch Intern Med. 2022; 132: 16208.

13 Siudut J, Zabczyk M, Wolkow P, et al. Intensive low-density lipoprotein cholesterol lowering improves fibrin clot properties: association with lipoproteins and C-reactive protein. Vascul Pharmacol. 2022; 144: 106977.

14 Anand SS, Caron F, Eikelboom JW, et al. Major adverse limb events and mortality in patients with peripheral artery disease: the COMPASS trial. J Am Coll Cardiol. 2018; 71: 2306-2315.

15 Pignatelli P, Violi F, Pastori D. Integrated care management of patients with atrial fibrillation – far from optimal. Pol Arch Intern Med. 2020; 130: 176-178.