Cardiovascular Diabetology

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Good metabolic control is associated with decreased circulating factor VIIa–antithrombin complexes in type 2 diabetes: a cross-sectional study

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Abstract

Background Diabetes is associated with a prothrombotic state that contributes to cardiovascular (CV) events in type 2 diabetes (T2DM). Activated factor VII (FVIIa)– antithrombin (AT) complexes are indicative of tissue factor (TF) exposure and have been associated with thromboembolic risk in coronary artery disease. To our knowledge there have been no reports on FVIIa-AT complexes in T2DM, therefore we assessed factors that determine FVIIa-AT complexes in this disease and the impact of higher complexes on a prothrombotic state.

Methods In 108 T2DM patients (mean age 63.8 years, 52.8% men, median HbA1c of 6.9 [interquartile range 6.1–8.2] %) and 83 age- and sex-matched non-diabetic subjects, we measured FVIIa-AT complexes. Metabolic control of T2DM involved fasting glucose, glycated hemoglobin (HbA1c), albumin/creatinine ratio (ACR), and lipid levels. To characterize a prothrombotic state, we determined thrombin generation parameters, fibrinolysis markers, and plasma fibrin clot properties.

Results FVII-AT complexes in T2DM patients were similar to controls (73.6 [59.4–91.7] vs. 79.6 [59.2–97.1]pM, respectively, $p = 0.30$). The T2DM patients with FVIIa-AT in the top vs. the bottom quartile had a larger prevalence of active smoking and insulin use, along with higher fasting glucose (+ 36.4%), HbA1c (+ 27.4%), ACR (+ 72.8%), total cholesterol (+ 34.5%), and LDL-cholesterol (+ 80%). FVIIa-AT complexes showed no associations with in vitro thrombin generation potential, plasma fibrin clot properties, or fibrinolysis variables. On multivariable analysis HbA1c, ACR, and total cholesterol remained independently associated with FVIIa-AT complexes in T2DM.

Conclusions This is the first study to show that in T2DM higher FVIIa-AT complexes are associated with markers of dyslipidemia and glycemia control, indicating that TF-induced coagulation activation could be suppressed by achieving treatment targets.

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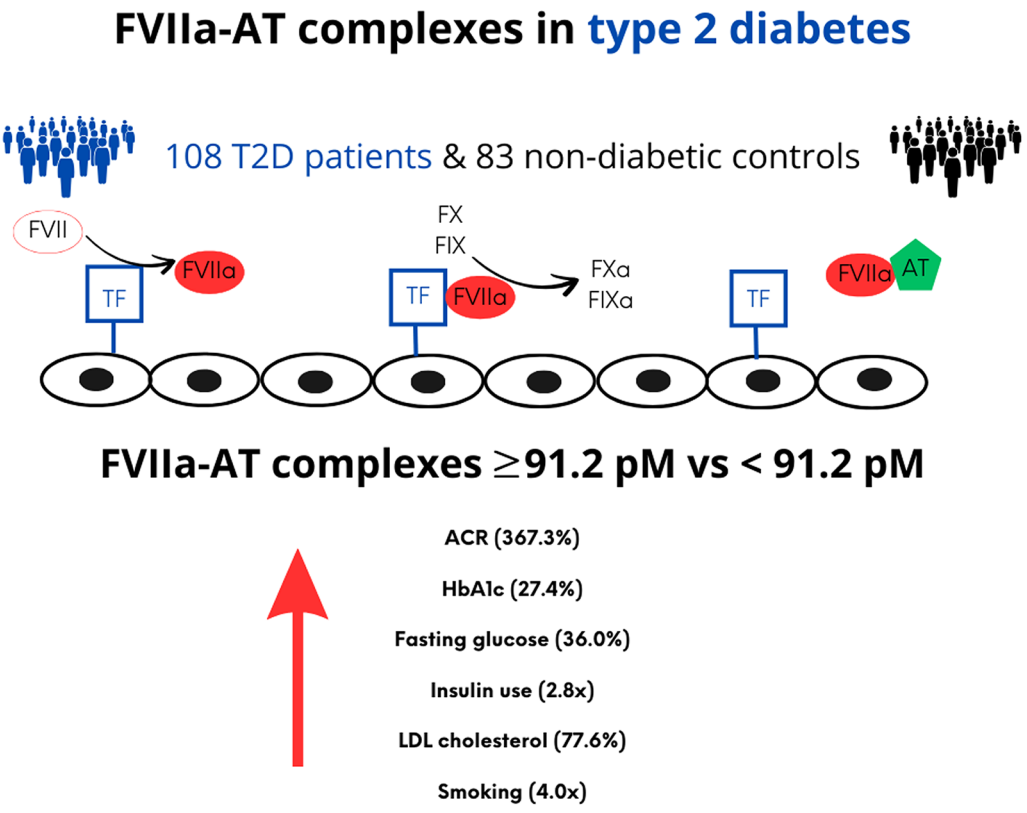
Graphical abstract



Keywords FVIIa-AT complexes, Type 2 diabetes, Glycated hemoglobin, Albumin/Creatinine ratio

Background

Type 2 diabetes (T2DM) is associated with a 2-fold increase in the risk of ischemic stroke (IS) [1] and a 2-4-times higher risk of myocardial infarction (MI) when compared to a similar non-diabetic population [2]. The incidence of adverse cardiovascular (CV) events is particularly high among patients with insufficient metabolic control [3, 4]. The Danish nationwide registry involving more than 14,000 individuals with newly diagnosed T2DM showed that good metabolic control markedly reduced the incidence of major adverse CV events (MACE) defined as MI, IS or death, however even in patients with well-controlled glycemia (HbA1c<6.5%) the 5-year risk of MACE exceeded 9% [5]. Urine albumin/creatinine ratio (ACR), a marker of diffuse microvascular dysfunction [6], is associated with poor glycemic control [7] and CV events including MI, IS, or death in T2DM [8].

DM is associated with a prothrombotic state including impaired fibrinolysis [9, 10]. Tissue factor (TF), the key initiator of blood coagulation in vivo, is mainly expressed on monocytes/macrophages, vascular smooth muscle cells, adventitial fibroblasts, and extracellular vesicles [11]. Active, circulating TF has also been detected in plasma in femtomolar amounts [12].

Chronic hyperglycemia leads to endothelial dysfunction and contributes to enhanced monocyte and subendothelial TF exposure to circulating coagulation factor (F) VII with its active form FVIIa, which results in the formation of TF-FVIIa complexes in the extrinsic coagulation pathway [13, 14]. The complexes activate FIX, which *via* FX and the prothrombinase pathway generates thrombin and eventually leads to fibrin clot formation

[15]. There is evidence for a TF-mediated procoagulant state in T2DM [16]. In vitro studies showed that monocytes exposed to advanced glycation end-products (AGEs) or glycated albumin overexpressed surface TF [13, 17]. TF-procoagulant activity measured using a two-stage clotting assay which detects cell- and microparticle-derived TF in whole blood, monocyte TF mRNA, and FVII were increased in T2DM patients as compared to controls, with further increase in TF procoagulant activity in hyperglycemia and hyperinsulinemia [18]. The TF-FVIIa complexes are inhibited foremostly by antithrombin (AT) and tissue factor pathway inhibitor (TFPI) [19, 20]. AT replaces TF in the interaction with FVIIa, forming stable FVIIa-AT complexes, which reflect intravascular TF exposure and could represent markers of TF-induced activation of blood coagulation [21]. Martinelli et al. reported median FVIIa-AT complexes of 83.9 (76.7–92.8) pM in 140 individuals including those with T2DM (8%) [22]. In patients with coronary artery disease (CAD) FVIIa–AT complexes have been associated with a significantly elevated risk of MI, IS, systemic thromboembolism, or death [22, 23]. It has been shown in CAD patients that FVIIa-AT complexes were positively correlated with peak thrombin generation and endogenous thrombin potential (ETP), also after adjustment for age, sex, and CAD presence, while high-density lipoprotein cholesterol (HDL-C) and triglycerides were independent predictors of FVIIa-AT complexes [22].

To our knowledge, there have been no reports on FVIIa-AT complexes in T2DM and its metabolic control. Therefore we aimed to assess FVIIa-AT complexes, their determinants, and their potential impact on a prothrombotic state in T2DM patients.

Patients and methods

Study design

This was a cross-sectional study that included consecutive white patients aged ≥ 18 years diagnosed with T2DM [24]. Patients were recruited in Krakow, Poland, between October 2016 and July 2017, as previously described [25]. Signs of acute infection, arterial or venous thromboembolic events within six months prior to inclusion, current anticoagulant therapy, cancer, recent trauma or surgery, autoimmune diseases, chronic kidney disease grade G4/G5, and pregnancy were exclusion criteria. All subjects provided written, informed consent. The Bioethics Committee at the Jagiellonian University Medical College approved the study.

On enrolment, demographic and clinical data were collected. Arterial hypertension was diagnosed based on the following criteria: (1) history of hypertension; (2) antihypertensive treatment prior to admission; or (3) systolic or diastolic pressure ≥ 140 mmHg or ≥ 90 mmHg, respectively. CAD was diagnosed in patients who presented angina symptoms and positive results of either non-invasive testing or coronary angiography, or had a prior history of MI or percutaneous coronary intervention. Peripheral artery disease (PAD) was diagnosed based on typical symptoms with an ankle-brachial index < 0.9 or prior revascularization [26]. Chronic kidney disease was defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min measured on two separate occasions [27]. We defined treatment targets for glycemic and lipid control, at ≤ 7% HbA1c and < 2.6 mmol/L LDL-C for moderate CV risk, as per current guidelines [28].

Healthy control subjects were recruited from participants of a preventive program conducted at our center. We included individuals aged ≥ 18 years with no history of chronic disease. Exclusion criteria were as follows: acute infection, arterial or venous thromboembolic events within six months prior to inclusion, fasting glucose levels ≥ 7.0 mmol/L or known diabetes, known kidney or liver disease.

Laboratory investigations

Fasting blood samples were obtained from an antecubital vein, between 6 and 8 AM and then divided into 250 µL aliquots which were stored in -80 °C until further use. Basic laboratory parameters were measured at enrolment, while IL-6, IL-8, coagulation and fibrinolysis-associated proteins, the calibrated automated thrombogram, and fibrin clot properties were assessed at the study completion to ensure batch analysis. Samples obtained from the control group were processed in the same way as those from the study group. Routine laboratory techniques were applied for blood cell counts, fasting glucose, creatinine, and lipid profile. HbA1c was measured bliy high-performance liquid chromatography using the Variant II Turbo analyzer (Hercules, CA, US). The latex-enhanced turbidimetric immunoassay was utilized to measure high-sensitivity C-reactive protein (CRP) concentration using a Cobas 6000 analyser (Roche Diagnostics, Mannheim, Germany; Hitachi High-Technologies Corporation, Tokyo, Japan), while commercially available ELISA kits were used to measure plasma interleukin 6 (IL-6) and 8 (IL-8, R&D Systems, Minneapolis, MN, US).

The Clauss method was used to determine fibrinogen, while chromogenic assays were employed to measure levels of plasminogen and α2-antiplasmin (Siemens, Munich, Germany).

Immunoenzymatic assays were used to measure FVIIa-AT complexes (Diagnostica Stago, Gennevilliers, France, detection limit 40 pM). The FVIIa-AT complex levels were measured in 2022 in plasma aliquots thawed for the first time.

Calibrated automated thrombogram

The thrombin generation test was performed using the assay introduced by K. Mann as described [29]. Briefly, citrated plasma samples upon addition of the corn trypsin inhibitor were mixed with relipidated TF at a final concentration of 5 pM, and fluorogenic substrate, benzyloxycarbonyl-Gly-Gly-Arg-7-amido-4methyl-coumarin (Bachem, Torrance, CA, US) in a 96-well plate, followed by incubation to allow for plasma recalcification. A phospholipid vesicle solution (Avanti Polar Lipids, Inc, Alabaster, AL, US) was added to plasma samples to initiate thrombin generation. Fluorescence was recorded immediately following hydrolysis of the 7-amino-4-methylcoumarin substrate and the results were converted to thrombin concentrations using a calibration curve derived from sequential dilutions of human thrombin produced in-house [30]. The analysis was performed using the Gen5 plate reader software (BioTek, Winooski, VT, USA). The results encompassed: time following addition of the trigger until initiation of thrombin generation (lag phase, seconds), peak thrombin (nmol/L), time to reach the peak (time-to-peak, seconds), and area under the curve (ETP, nM x seconds).

Fibrin clot properties

Fibrin clot permeability was determined as described [31]. Briefly, we added 60 µL of a coagulation trigger containing 1 IU/mL human thrombin and 20 mM $CaCl_2$ to 60 µL of citrated plasma. Tubes containing the clots were connected to a reservoir of a buffer and the volume of percolating buffer within 1 h was measured. A permeation coefficient (K_s) as a measure of the average size of fibrin clot pores, was calculated using the equation: $K_s = Q \times L \times \eta / t \times A \times \Delta p$, where Q is the flow rate in time (t); L, the length of a fibrin gel; η, the viscosity of liquid (in poise); A, the cross-sectional area (in cm^2), and Δp, a differential pressure (in dynes per cm^2).

Clot lysis time (CLT) was assessed as described [32]. In short, we mixed 100 µL of citrated plasma with 15 mmol/L $CaCl_2$, 0.6 pM human TF (Innovin, Siemens), 12 µmol/L phospholipid vesicles and 60 ng/mL recombinant tPA (Boehringer Ingelheim, Ingelheim, Germany). We measured absorbance at 405 nm, 37 °C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition (clot formation), to the midpoint of the maximum-turbid-to-clear transition (clot lysis).

The inter- and intra-assay variability for the measurement of Ks and CLT were < 8%.

Statistical analysis

Statistical analyses were conducted using R Software (version 4.3.2). Clinical characteristics were described as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables. Qualitative data were presented as numbers and percentages. Normality of distribution was assessed using the Shapiro-Wilk test, and variances were compared using the F-test. To compare the means of two independent groups, either the t-test (with equal or unequal variance) or the Wilcoxon rank sum test was employed. To compare the four groups categorized by quartiles of FVIIa-AT complexes, we utilized either ANOVA or the Kruskal-Wallis test (*post hoc* analysis was conducted using pairwise t-tests with Bonferroni correction). The homogeneity of variance was assessed using the Bartlett test.

The Chi-square test or Fisher exact test were used to analyze associations for qualitative data. We fitted the univariable regression models to identify significant predictors of FVIIa-AT complexes. Based on results from univariable analysis and considering biologically sound hypotheses, we chose the following variables for multivariable analysis: smoking, insulin use, HbA1c, ACR, total and LDL-C, CRP, as well as age and sex. We selected a multivariable model through best (forward or backward) stepwise computer-assisted selection using a p value of 0.05 as the threshold for adding a variable. A p value < 0.05 was considered statistically significant.

Results

The study involved 108 patients, aged 63.8 (8.2) years, 52.8% male, with a median time since T2DM diagnosis of 7.0 (3.0–15.0) years (Table 1). Most patients (92.6%) had hypertension, while 46.3% had co-existing CAD, with 17.6% MI survivors. The median HbA1c was 6.9 (6.1–8.2)%. Microvascular complications were diagnosed in 15.8% of patients, including 9.5% neuropathy, 6.3% nephropathy, and 3.2% retinopathy.

Patients and controls were similar in terms of age, sex, and smoking status (Supplementary Table S1). As compared to controls T2DM patients had higher BMI, white blood count, creatinine, fasting glucose, CRP, and fibrinogen levels, while they had lower cholesterol levels, likely attributable to statin use (Supplementary Table S1).

The FVIIa-AT complexes in T2DM ranged from 39.7 to 118.4 pM (median 73.6 [59.4–91.7] pM) and were similar to values measured in age- and sex-matched apparently healthy control individuals (median 79.6 [59.2–97.1] pM, $p = 0.34$).

Patients with FVIIa-AT complexes in different quartiles shared similar demographic and clinical characteristics, including macrovascular and microvascular complications, with the exception of active smoking, which was four times more frequent in patients with FVIIa-AT complexes in the top quartile, as compared to the bottom one (Table 1), however current smokers ($n = 17$, 15.7%) had similar FVIIa-AT complexes as compared to non-smokers (75.3 [63.7–99.5] vs. 72.8 [58.9–88.6] pM, $p = 0.15$).

Table 1 Patient characteristics according to quartiles of plasma factor VIIa-antithrombin (FVIIa-AT) complexes

FVIIa-AT complexes [pM]						
	All patients (*n* = 108)	**Q1 (39.7–59.5)**	**Q2 (59.5–73.5)**	**Q3 (73.5–91.2)**	**Q4 (91.2–118)**	**p value**
Age, years	63.8 (8.2)	64.2 (7.1)	63.4 (9.2)	64.6 (7.7)	62.2 (8.7)	0.71
Men, n (%)	57 (52.8)	14 (51.9)	12 (44.4)	16 (59.3)	15 (55.6)	0.73
BMI, kg/m^2	32.1 (29.4; 37.2)	32 (30.4; 34.6)	30.2 (27.9; 37)	32.1 (30; 35.5)	32.7 (29.4; 38.3)	0.73
Time since diagnosis, years	7 (3; 15)	5 (2; 15)	8 (4; 18.5)	6 (3; 11)	7.5 (5.3; 15)	0.40
Current smoking, n (%)	17 (15.7)	2 (7.4)	6 (22.2)	1 (3.7)	8 (29.6)	0.03
Comorbidities, n (%)						
Hypertension	100 (92.6)	26 (96.3)	25 (92.6)	26 (96.3)	23 (85.2)	0.52
Coronary artery disease	50 (46.3)	11 (40.1)	13 (48.1)	17 (62.9)	9 (33.3)	0.16
Prior myocardial infarction	19 (17.6)	2 (7.4)	7 (25.9)	6 (22.2)	4 (14.8)	0.30
Peripheral artery disease	10 (9.3)	3 (11.1)	1 (3.7)	5 (18.5)	1 (3.7)	0.27
Chronic kidney disease	15 (13.9)	1 (3.7)	4 (14.8)	5 (18.5)	5 (18.5)	0.25
Microvascular complications*, n (%)	15 (16.5)	2 (8.0)	5 (22.7)	1 (5.0)	7 (29.2)	0.09
Pharmacotherapy, n (%)						
ASA	70 (64.8)	20 (74.1)	15 (55.6)	19 (70.4)	16 (59.3)	0.47
Clopidogrel	9 (8.3)	1 (3.7)	2 (7.4)	4 (14.8)	2 (7.4)	0.62
ACE-I	60 (55.6)	16 (59.3)	15 (55.6)	16 (59.2)	13 (48.2)	0.83
Statin	71 (65.7)	22 (81.5)	17 (63.0)	17 (63.0)	15 (55.6)	0.22
Metformin	81 (75.0)	20 (74.1)	20 (74.1)	19 (70.4)	22 (81.5)	0.82
DPP IV-I	7 (6.5)	2 (7.4)	0 (0)	2 (7.4)	3 (11.1)	0.51
SGLT2-I	5 (4.6)	1 (3.7)	2 (7.4)	1 (3.7)	1 (3.7)	1
Insulin	43 (39.8)	6 (22.2)	11 (40.7)	9 (33.3)	17 (63.0)	0.02
Basic laboratory parameters						
WBC, x 10^9 cells/L	7.4 (1.7)	7.6 (1.7)	7.5 (1.7)	7.2 (1.8)	7.4 (1.5)	0.84
Hemoglobin, g/dL	13.7 (1.3)	13.5 (1.1)	14 (1.3)	13.9 (1.0)	13.9 (1.4)	0.66
PLT, x 10^9 cells/L	232 (61.2)	246.2 (69.8)	237.7 (60.8)	217.7 (57.1)	224.7 (54.1)	0.32
Creatinine, µmol/L	76 (66; 9.8)	75 (68; 85)	79 (67.5; 93.5)	76 (67; 85)	77 (60; 96)	0.93
CRP, mg/L	2.5 (1.1; 4.8)	1.5 (0.9; 3.4)	2.9 (1.6; 5.4)	1.5 (1.1; 3.5)	3.5 (2; 5.7)	0.04**
Interleukin-6, pg/mL	3.7 (2.6; 5.9)	3.7 (2.1; 6.2)	4.0 (3.0; 5.1)	3.2 (2.7; 5.7)	3.8 (2.9; 6.3)	0.92
Interleukin-8, pg/mL	5.0 (3.9; 8.1)	5.5 (3.2; 8.2)	5.0 (4.1; 6.5)	5.2 (4; 8.1)	5.3 (3.9; 8.3)	0.95
Parameters of metabolic control						
Fasting glucose, mmol/L	7.3 (6; 8.6)	6.6 (6.1;7.5)	6.9 (5.9; 8.0)	7.1(5.8; 8.1)	9.0 (6.7; 11.0)	0.02[a]
HbA1c, %	6.9 (6.1; 8.2)	6.2 (5.8; 6.9)	6.7 (6.1; 7.9)	7.2 (6.1; 8.4)	7.9 (7.3; 9.6)	< 0.001[a, b,c]
ACR, mg/g	7.6 (4.0; 21.5)	4.9 (3.7; 7.5)	7.3 (3.7; 16.7)	7.6 (4.5; 23.3)	18.0 (7.8; 65.7)	< 0.001[a, b]
Total cholesterol, mmol/L	4.2 (3.5; 5.3)	3.6 (3.0; 4.2)	4.1 (3.9; 4.8)	4.8 (3.4; 5.4)	5.5 (4.2; 6.2)	< 0.001[a, b]
LDL-C, mmol/L	2.5 (1.8; 3.5)	2.0 (1.6; 2.6)	2.5 (2.2; 2.9)	2.5 (1.8; 3.7)	3.6 (2.6; 4.2)	< 0.001[a]
HDL-C, mmol/L	1.2 (1; 1.5)	1.1 (0.9; 1.4)	1.2 (1.1; 1.5)	1.4 (1.1; 1.6)	1.1 (1; 1.4)	0.24
Triglycerides, mmol/L	1.5 (1.2;2.0)	1.3 (1.0;1.9)	1.5 (1.2;1.9)	1.4 (1.2;1.8)	1.9 (1.5;2.2)	0.03**
Coagulation- and fibrinolysis-associated factors						
Fibrinogen, g/L	3.5 (3.2; 3.9)	3.5 (3.2; 3.8)	3.3 (3; 3.7)	3.5 (3.2; 3.9)	3.7 (3.3; 3.9)	0.26

Table 1 (continued)

FVIIa-AT complexes [pM]						
	All patients (*n* = 108)	**Q1 (39.7–59.5)**	**Q2 (59.5–73.5)**	**Q3 (73.5–91.2)**	**Q4 (91.2–118)**	**p value**
α2-antiplasmin, %	104 (98; 110)	106 (102; 112)	104 (98.50 108)	103 (97; 107)	100 (95; 111.5)	0.30
Plasminogen, %	109 (100; 119)	107 (98; 117)	109 (101; 117.5)	110 (101; 119)	118 (104.5; 125.5)	0.34
Thrombin generation						
Lag Phase, s	1008 (774.0; 1326.5)	1008 (822.8; 1189.8)	1066.5 (787; 1212)	1011.5 (777.3; 1654.8)	1040.5 (754.5; 1437)	0.80
Peak thrombin, nM	117.6 (89.6; 155.7)	119.1 (98.3;150.8)	117.9 (89.3;157.6)	121.7 (95.7;152.7)	107.3 (85.6;155.6)	0.94
Time-to-peak, s	1379 (1112;1847)	1359 (1154;1733)	1405 (1125;1694)	1392 (1130;1907)	1379 (1028;1973)	0.96
ETP, nM x s	106,823 (29,686)	107,705 (20,702)	111,969 (31,497)	102,874 (36,594)	104,251 (30,474)	0.62
Fibrin clot properties						
K_s, $\times 10^{-9}$ cm^2	6.5 (0.9)	6.7 (0.7)	6.3 (0.9)	6.3 (0.9)	6.5 (0.9)	0.30
CLT, min	95.1 (19.1)	90.8 (18.7)	99.7 (20.6)	99 (20.2)	91.6 (17.6)	0.19

Note: Post hoc analyses: significant differences between: a- Q1 and Q4, b- Q2 and Q4, c- Q3 and Q4;

*microvascular complications defined as retinopathy, neuropathy, or nephropathy

**non-significant in *post hoc* analysis

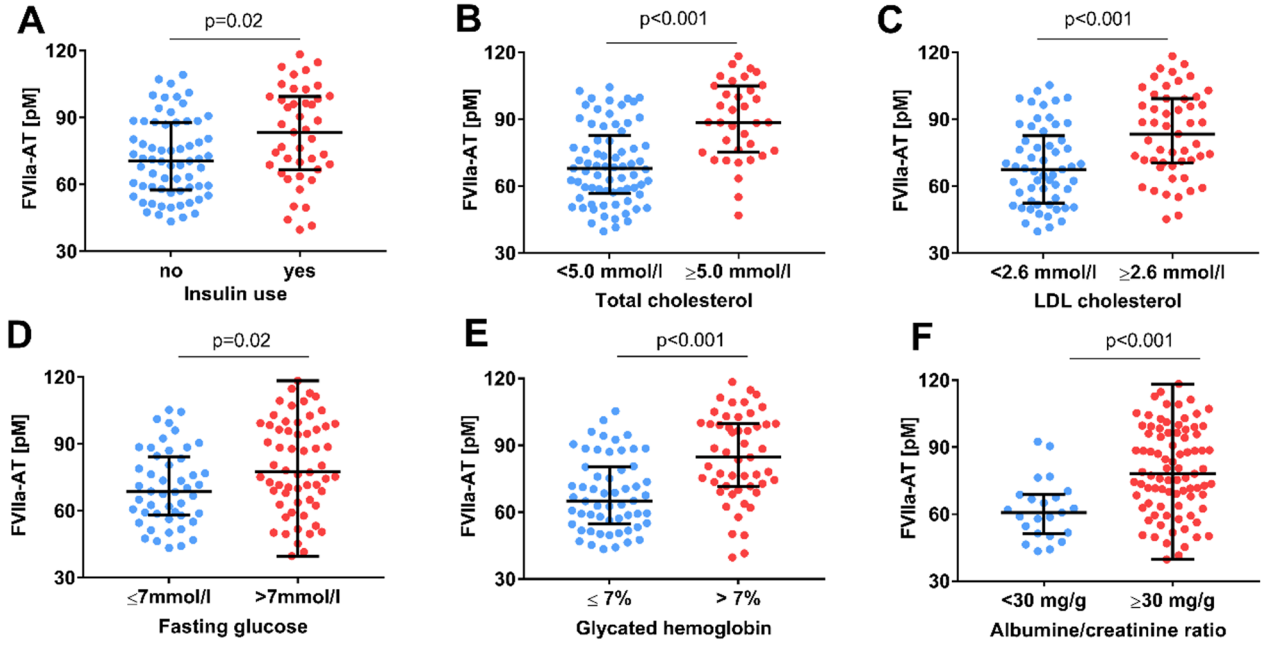


Fig. 1 FVIIa-AT complexes with regard to **A**– insulin use, **B**– total cholesterol level, **C**– LDL cholesterol level, **D**– fasting glucose, **E**– glycated hemoglobin, **F**– albumin/creatinine ratio

There were no differences in pharmacotherapy associated with FVIIa-AT complexes (Table 1) apart from insulin, which was used more commonly in patients with FVIIa-AT complexes in the top quartile as compared with the bottom one (Table 1). Patients on insulin (n=43, 39.8%) had a median level of FVIIa-AT complexes of 83.4 (66.7–99.5) pM which was 12.9% higher when compared with those using oral antidiabetic agents (n=65, 60.2%, 70.5 [57.9–87.8] pM, p=0.02, Fig. 1A). This corresponded with differences in fasting glycemia (7.8 [6.2–11.0] vs. 6.9 [5.9–7.9] mmol/L, respectively, p=0.02) and HbA1c (8.2 [7.6–9.2] vs. 6.3 [5.8-7.0]%, respectively, p<0.001).

Regarding laboratory investigations shown in Table 1, patients with FVIIa-AT complexes in the top quartile had higher CRP but not IL-6 or IL-8 as compared with the bottom one; the association was lost in the *post hoc*

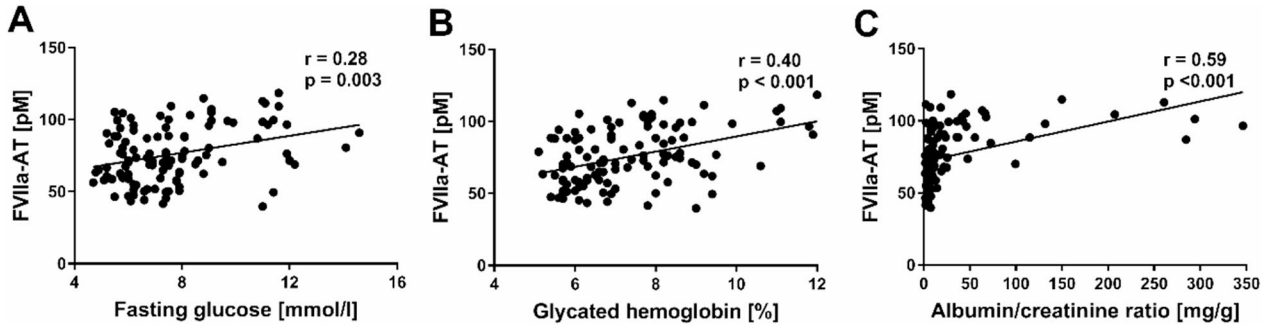


Fig. 2 Correlations between FVIIa-AT complexes and parameters of glycemic control

analysis. Total cholesterol and LDL-C were higher in individuals with FVIIa-AT complexes in the top quartile as compared to the bottom one by 52.0% and 77.6%, respectively. We observed a positive association between FVIIa-AT complexes and triglycerides, but it was lost in the *post hoc* analysis. Patients with total cholesterol≥5 mmol/L (n=35, 32.4%) had 30.3% higher FVIIa-AT complexes when compared to the remainder (n=73, 67.6%; 88.6 [75.2–105.0] vs. 68.0 [57.2–80.6] pM, respectively, p<0.001, Fig. 1B), while those with LDL-C≥2.6 mmol/L (n=51, 47.2%) had 23.6% higher FVIIa-AT complexes, as compared to the remainder (n=57, 52.8%, 83.4 [70.5–99.3] vs. 67.5 [53.3–80.6] pM, p<0.001, Fig. 1C).

FVIIa-AT complexes correlated with total cholesterol and LDL-C (r=0.46, p<0.001 and r=0.41, p<0.001, respectively). We found a similar prevalence of statin use in patients with FVIIa-AT complexes in the quartile analysis (Table 1). We found no association between lipid levels and FVIIa-AT complexes in the control group.

FVIIa-AT complexes and glycemia control

Patients with FVIIa-AT complexes in the top quartile had 36% higher fasting glucose as compared to the bottom one (Table 1). Fasting glucose correlated with FVII-AT complexes (r=0.28, p=0.003, Fig. 2A). In patients with fasting glucose>7 mmol/L (n=60, 55.6%) FVII-AT complexes were 13.3% higher than in the remainder (77.7 [64.3–98.2] vs. 68.6 [58.3–84.0] pM, p=0.02, Fig. 1D). We found no association between fasting glucose and FVIIa-AT complexes in the control group.

Patients with FVII-AT complexes in the top quartile had 27.4% higher HbA1c, when compared to those in the bottom quartile (Table 1). HbA1c showed a positive correlation with FVIIa-AT complexes (r=0.40, p<0.001, Fig. 2B). Patients with HbA1c>7% (n=51, 47.2%) presented with 30% higher FVIIa-AT complexes, when compared to the remainder (84.6 [71.6–90.7] vs. 65.1 [55.1–80.3] pM, p<0.001; Fig. 1E).

Patients with ACR≥30 mg/g (n=23, 21.3%) had 44.6% higher FVIIa-AT complexes than the remainder (99.5 [88.4–105.0] vs. 68.8 [57.8–80.3] pM, p<0.001; Fig. 1F).

Table 2 FVIIa-AT complex predictors on multivariable regression

Variable	Multivariable model 1		Multivariable model 2	
	Estimate (95% CI)	p value	Estimate (95% CI)	p value
Age, years	-		0.11 (-0.33; 0.56)	0.61
Gender	-		-1.27 (-8.25; 5.70)	0.72
HbA1c, %	3.08 (0.48; 5.69)	0.02	3.22 (0.56; 5.87)	0.02
Total cholesterol, mmol/L	5.78 (1.76; 9.81)	0.005	5.81 (1.73; 9.88)	0.01
LDL-C, mmol/L	-1.40 (-5.91; 3.10)	0.54	-1.34 (-5.91; 3.23)	0.56
ACR, per 10 mg/g	0.61 (0.02; 1.19)	0.04	0.62 (0.02; 1.22)	0.04
CRP, mg/L	0.21 (-0.38; 0.81)	0.48	0.24 (-0.38; 0.85)	0.45
Insulin use	5.10 (-3.49; 13.70)	0.24	5.06 (-3.61; 13.74)	0.25

ACR showed a potent positive correlation with FVIIa-AT complexes (r=0.59, p<0.001, Fig. 2C). In patients with FVIIa-AT complexes in the highest quartile, ACR was 3.6-fold higher than in the first quartile (Table 1).

FVIIa-AT complexes and coagulation/fibrinolysis parameteres

There were no associations of FVIIa-AT complexes with thrombin generation markers, fibrinolysis proteins, or plasma fibrin clot properties (Table 1).

Multivariable analysis

Following univariable analysis with the exclusion of significant collinearity between parameters of glycemia and lipid control, we built two multivariable models to identify independent predictors of FVIIa-AT complexes in T2DM. Model 1 included HbA1c, ACR, total cholesterol, LDL-C, CRP, and insulin therapy, while model 2 additionally contained age and sex (Table 2). On multivariable analysis HbA1c, ACR, and total cholesterol remained independently associated with FVIIa-AT complexes (Table 2).

Discussion

Our study is the first to show that T2DM patients with higher FVIIa-AT complexes are characterized by less optimal metabolic control as evidenced by higher fasting

glucose, HbA1c, ACR, and cholesterol. Of note, FVIIa-AT complexes in our group with a median HbA1c of 6.9% were similar to those observed in matched control subjects, like in patients with CAD and their controls [22]. Importantly, enhanced TF-mediated activation of blood coagulation can be observed at higher HbA1c with albuminuria and hypercholesterolemia indicating that poor metabolic control in T2DM may drive this mechanism contributing to increased CV risk. Our findings suggest that higher FVIIa-AT complexes could represent a novel coagulation marker of insufficiently controlled T2DM with the most potent association with microalbuminuria, which is not related to inflammation, plasma thrombin generation potential, or fibrin clot properties.

The current cohort represents typical T2DM patients in terms of demographic, clinical, and basic laboratory parameters, however total cholesterol and LDL-C tended to be lower when compared to an otherwise similar Polish population of patients with T2DM [33]. Better metabolic control in our study is likely attributable to the fact that our cohort involved solely patients with established T2DM treated in a specialist center. The use of insulin was relatively frequent [34], while sodium-glucose transport protein 2 (SGLT2) inhibitors or glucagon-like peptide 1 (GLP-1) agonists were prescribed uncommonly (4.6% and 0, respectively) in comparison to 8.8% and 2.1%, respectively, in the current worldwide statistics [35], however there is no evidence for a specific effect of these agents on FVIIa-AT complexes.

Our key novel observation is that circulating FVIIa-AT complexes are related to glycemic control, expressed as HbA1c or ACR. Given the fact that FVIIa-AT complexes correlate with FVII and FVIIa [36], our results expand data from a few small reports showing that TF, FVII, and FVIIa may be associated with diabetic control. In 80 T2DM patients with co-existing CAD there were correlations of HbA1c levels with circulating TF ($r=0.54$, $p<0.001$) and FVIIa ($r=0.47$, $p<0.001$) [37]. In 50 patients with T2DM there was a positive correlation between monocyte TF expression and HbA1c [38]. Similar observations were made in 13 young patients with T1DM, in whom an improvement in HbA1c was associated with a decrease in FVII [39]. Kario et al. showed correlations between FVIIa and FVIIa/FVII antigen ratio with ACR in 67 T2DM patients [40]. Our study documented in a larger T2DM group that the higher HbA1c and albuminuria, the higher FVIIa-AT complexes in circulating blood. A recent analysis of more than 23,000 patients, 8.8% of whom had DM, demonstrated that increasing ACR within the normal limit of <30 mg/g was strongly associated with long-term mortality, independently of the absence or presence of diabetes [41]. Based on our observations, it might be speculated that enhanced TF-mediated coagulation contributes to higher mortality in diabetic and non-diabetic subjects with higher ACR. Taken together, one may suggest that a strict glycemic control, evidenced by low HbA1c and ACR, may reduce FVIIa-AT complexes indicative of increased TF exposure, which might have clinical consequences.

We found that cholesterol is an independent predictor of FVIIa-AT complexes in T2DM, which is consistent with data from a general population of almost 3,500 individuals aged 65 or older, showing an association between total cholesterol and FVIIa-AT complexes [36]. No such correlations were reported in CAD cohorts, where around 20% of patients had co-existing T2DM [22, 23]. This could be related to differences in patient characteristics. While in the current study men and women were similarly represented, and 15.7% were active smokers, both CAD cohorts involved predominantly men and smokers (68% current or former smokers in the study by Martinelli et al. and 38% of current smokers in the study by Paszek et al.), in whom total cholesterol and LDL-C were higher [22, 23]. The lack of correlation between FVIIa-AT and lipid levels in the control group also suggests that the effect of lipids on FVIIa-AT could depend on the population tested and its characteristics. Smoking has been associated with FVIIa-AT complexes [23, 36]. We found a high percentage of smokers in the group with the highest FVIIa-AT complexes when compared to those with the lowest, however the total number of smokers was probably too low to demonstrate a difference in FVIIa-AT complexes between smokers and non-smokers. It has been reported that statins reduce TF expression in various experimental models [42]. Martinelli et al. showed that patients with advanced CAD and high FVIIa-AT complexes were less likely to receive statins [22]. We did not observe statin-related differences in FVIIa-AT complexes in T2DM, which could be explained by the fact that only 46% in our cohort had CAD and the overall total cholesterol and LDL-C levels were significantly lower. It might be speculated that the association of statins with FVIIa-AT complexes may by modified by additional factors, such as atherosclerotic burden and baseline cholesterol concentrations. Mechanisms behind the association of blood cholesterol with circulating FVIIa-AT complexes requires further investigation.

It is known that in several clinical conditions, plasma thrombin markers such as prothrombin fragments 1+2, thrombin-antithrombin complexes, and fibrinopeptide A, show no or weak associations with the plasma thrombin generation capacity in vitro with two key parameters: ETP and peak thrombin generated [43, 44]. In diabetic patients we did not observe associations between FVIIa-AT complexes and variables describing thrombin generation such as lag time, peak thrombin levels, time-to-peak, or ETP, measured in vitro using a calibrated automated thrombogram. Campello et al. have also not reported any

correlation between FVIIa-AT and ETP, lag time, or peak thrombin levels in overweight and obese individuals [45]. This finding is in line with studies by Landgrebe et al. on patients with obesity, 30% of whom were diabetic, where no correlation was found between FVII-AT complexes or FVIIa and thrombin generation expressed as circulating F 1+2 levels and fibrin clot properties [46, 47]. Similarly, no correlation between FVIIa and F 1+2 was found in healthy volunteers [48] or patients with coronary artery disease [49]. This is in agreement with a suggestion by Pieters and de Maat [50] that FVIIa may act as a silent 'primer' for the procoagulant state, which boosts thrombin generation, albeit in the presence of additional prothrombotic factors. In line, Martinelli et al. have reported an association between higher FVIIa-AT complexes and higher ETP and peak thrombin, using a slightly different assay, but in patients with advanced CAD with higher total cholesterol (5.2 [1.1] vs. 4.2 [3.5–5.3] mmol/L) and LDL-C (3.4 [0.9] vs. 2.5 [1.8–3.5] mmol/L) and a large (68% vs. 58% of active or former smokers) prevalence of active smokers [22]. It might be speculated that in T2DM the differences related to advanced CAD are abolished, which might be confirmed by the finding that diabetic CAD patients had similar FVIIa-AT complexes as compared to the remaining T2DM patients [22, 23]. In T2DM multiple additional prothrombotic mechanisms are involved in the prothrombotic state, including platelet-derived– and complement-mediated pathways [51], but their contribution was beyond the scope of this study.

The fact that we did not observe differences in FVIIa-AT complexes between T2DM patients and controls deserves a comment. Although surprising, it is consistent with the results of a large population study, where DM did not influence FVIIa-AT complexes [36]. We hypothesize that such similar levels could be attributable to good glycemic control in our cohort, as indicated by a median HbA1c of 6.9 (6.1–8.2)%, suggesting that the quality of disease control, and not the diagnosis itself, is of greater importance for an upregulation of TF-related blood coagulation. In further support of this, El-Hagracy et al. showed significantly higher TF and FVIIa levels in T2DM patients, with a mean HbA1c of 9.9 (2.0)%, as compared to controls with a mean HbA1c of 5.2 (0.6)% [37].

Study limitations

Our study has several limitations. First, the sample size was relatively small but representative for typical T2DM patients in Europe, hence the results should be interpreted with extreme caution. We did not measure FVII or FVIIa, however associations between FVII or FVIIa and FVIIa-AT complexes have been shown in several studies [21, 22, 45, 52]. Therefore it is highly likely that FVII levels correlate with FVIIa-AT also in diabetic patients. We have decided to use FVIIa-AT complexes as it is a stable, reliable measure of intravascular TF exposure [21]. We did not measure AT but there is no association with FVIIa-AT complexes, as reported among others by Spiezia et al. [52]. Inherited AT deficiency is very rare (a prevalence of 0.02– 0.2%) in the general population, hence any significant effect of various AT levels on FVIIa-AT complexes is rather unlikely in our study. The impact of storage time might have affected the results but this probability is very low since a similar approach has been successfully used in previous seminal studies including the Verona Heart Study [22]. Unfortunately, thrombin generation was not measured in the control group, therefore associations between its parameters and FVIIa-AT complexes have not been evaluated. Finally, the study design did not include long-term follow-up to assess a potential effect of FVIIa-AT complexes on thromboembolic events in T2DM patients, which is likely given data from CAD cohorts [22, 23].

Conclusions

The current study shows a novel association between glycemic and lipid control expressed as HbA1c, ACR, and cholesterol levels with FVIIa-AT complexes, indicators of TF-induced activation of blood coagulation in T2DM. This hypothesis-generating investigation should stimulate further cohort studies to evaluate whether indeed higher FVIIa-AT complexes can predict MACE in T2DM patients.

Abbreviations

ACR	Albumin/creatinine ratio
ACE-I	Angiotensin converting enzyme inhibitor
ASA	Acetylsalicylic acid
AUC	Area under the curve
BMI	Body mass index
CLT	Clot lysis time
CRP	C-reactive protein
DPP IV-I	Dipeptidyl peptidase-4 inhibitor
ETP	Endogenous thrombin generation
HbA1c	Glycated hemoglobin
HDL-C	High-density lipoprotein cholesterol
Ks	Fibrin clot permeability
LDL-C	Low-density lipoprotein cholesterol
PLT	Platelet count
SGLT2-I-	Sodium-glucose Cotransporter-2 Inhibitors
WBC	white blood cell count

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

JG analyzed and interpreted the data, and drafted the manuscript and EP analyzed and interpreted the data, prepared graphs, and drafted the manuscript. ABW collected and interpreted the data, and revised the

manuscript. BM provided statistical analysis. AU designed the study, collected the data, and revised the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committee. Study participants provided informed written consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SRK, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet Lond Engl. 2010;375:2215–22. https://doi.org/10.1016/S0140-6736(10)60484-9.
2. Jain V, Qamar A, Matsushita K, Vaduganathan M, Ashley KE, Khan MS, et al. Impact of diabetes on outcomes in patients hospitalized with acute myocardial infarction: insights from the atherosclerosis risk in communities study community surveillance. J Am Heart Assoc. 2023;12:e028923. https://doi.org/10.1161/JAHA.122.028923.
3. Jankowski P, Topór-Mądry R, Gąsior M, Cegłowska U, Gierlotka M, Kubica J, et al. Management and predictors of clinical events in 75 686 patients with acute myocardial infarction. Kardiologia Pol Pol Heart J. 2022;80:468–75. https://doi.org/10.33963/KP.a2022.0058.
4. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. Heart disease and stroke statistics-2023 update: a report from the American Heart Association. Circulation. 2023;147:e93–621. https://doi.org/10.1161/CIR.0000000000001123.
5. Falkentoft AC, Gerds TA, Zareini B, Knop FK, Køber L, Torp-Pedersen C, et al. Risk of first-time major cardiovascular event among individuals with newly diagnosed type 2 diabetes: data from Danish registers. Diabetologia. 2023;66:2017–29. https://doi.org/10.1007/s00125-023-05977-6.
6. Barzilay JI, Farag YMK, Durthaler J. Albuminuria: an underappreciated risk factor for cardiovascular disease. J Am Heart Assoc. 2024;13:e030131. https://doi.org/10.1161/JAHA.123.030131.
7. Shah HS, McGill JB, Hirsch IB, Wu C, Galecki A, de Boer IH, et al. Poor glycemic control is Associated with more Rapid kidney function decline after the Onset of Diabetic kidney disease. J Clin Endocrinol Metab. 2024;dgae044. https://doi.org/10.1210/clinem/dgae044.
8. Scirica BM, Mosenzon O, Bhatt DL, Udell JA, Steg PG, McGuire DK, et al. Cardiovascular outcomes according to urinary albumin and kidney disease in patients with type 2 diabetes at high cardiovascular risk: observations from the SAVOR-TIMI 53 Trial. JAMA Cardiol. 2018;3:155–63. https://doi.org/10.1001/jamacardio.2017.4228.
9. Bryk-Wiązania AH, Undas A. Hypofibrinolysis in type 2 diabetes and its clinical implications: from mechanisms to pharmacological modulation. Cardiovasc Diabetol. 2021;20:191. https://doi.org/10.1186/s12933-021-01372-w.
10. Camm AJ, Sabbour H, Schnell O, Summaria F, Verma A. Managing thrombotic risk in patients with diabetes. Cardiovasc Diabetol. 2022;21:160. https://doi.org/10.1186/s12933-022-01581-x.
11. Grover SP, Mackman N. Tissue factor in atherosclerosis and atherothrombosis. Atherosclerosis. 2020;307:80–6. https://doi.org/10.1016/j.atherosclerosis.2020.06.003.
12. Butenas S, Orfeo T, Mann KG. Tissue factor in coagulation: which? Where? When? Arterioscler Thromb Vasc Biol. 2009;29:1989–96. https://doi.org/10.1161/ATVBAHA.108.177402.
13. Ichikawa K, Yoshinari M, Iwase M, Wakisaka M, Doi Y, Iino K, et al. Advanced glycosylation end products induced tissue factor expression in human monocyte-like U937 cells and increased tissue factor expression in monocytes from diabetic patients. Atherosclerosis. 1998;136:281–7. https://doi.org/10.1016/s0021-9150(97)00221-9.
14. Steffel J, Lüscher TF, Tanner FC. Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. Circulation. 2006;113:722–31. https://doi.org/10.1161/CIRCULATIONAHA.105.567297.
15. Grover SP, Mackman N. Tissue factor. Arterioscler Thromb Vasc Biol. 2018;38:709–25. https://doi.org/10.1161/ATVBAHA.117.309846.
16. Wang J, Ciaraldi TP, Samad F. Tissue factor expression in obese type 2 diabetic subjects and its regulation by antidiabetic agents. J Obes. 2015;2015:291209. https://doi.org/10.1155/2015/291209.
17. Khechai F, Ollivier V, Bridey F, Amar M, Hakim J, de Prost D. Effect of advanced glycation end product-modified albumin on tissue factor expression by monocytes. Role of oxidant stress and protein tyrosine kinase activation. Arterioscler Thromb Vasc Biol. 1997;17:2885–90. https://doi.org/10.1161/01.atv.17.11.2885.
18. Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. J Clin Endocrinol Metab. 2007;92:4352–8. https://doi.org/10.1210/jc.2007-0933.
19. Winckers K, ten Cate H, Hackeng TM. The role of tissue factor pathway inhibitor in atherosclerosis and arterial thrombosis. Blood Rev. 2013;27:119–32. https://doi.org/10.1016/j.blre.2013.03.001.
20. Rezaie AR, Giri H. Anticoagulant and signaling functions of antithrombin. J Thromb Haemost JTH. 2020;18:3142–53. https://doi.org/10.1111/jth.15052.
21. Spiezia L, Campello E, Valle FD, Woodhams B, Simioni P. Factor VIIa-antithrombin complex: a possible new biomarker for activated coagulation. Clin Chem Lab Med CCLM. 2017;55. https://doi.org/10.1515/cclm-2016-0399.
22. Martinelli N, Girelli D, Baroni M, Guarini P, Sandri M, Lunghi B, et al. Activated factor VII–antithrombin complex predicts mortality in patients with stable coronary artery disease: a cohort study. J Thromb Haemost. 2016;14:655–66. https://doi.org/10.1111/jth.13274.
23. Paszek E, Pociask E, Ząbczyk M, Butenas S, Undas A. Activated factor XI is associated with increased factor VIIa - antithrombin complexes in stable coronary artery disease: impact on cardiovascular outcomes. Eur J Clin Invest. 2022;e13857. https://doi.org/10.1111/eci.13857.
24. Definition. and Diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva, Switzerland: World Health Organization; 2006.
25. Bryk AH, Prior SM, Plens K, Konieczynska M, Hohendorff J, Malecki MT, et al. Predictors of neutrophil extracellular traps markers in type 2 diabetes mellitus: associations with a prothrombotic state and hypofibrinolysis. Cardiovasc Diabetol. 2019;18:49. https://doi.org/10.1186/s12933-019-0850-0.

26. Aboyans V, Ricco J-B, Bartelink M-LEL, Björck M, Brodmann M, Cohnert T, et al. 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). Eur Heart J. 2018;39:763–816. https://doi.org/10.1093/eurheartj/ehx095.
27. Stevens PE, Ahmed SB, Carrero JJ, Foster B, Francis A, Hall RK, et al. KDIGO 2024 Clinical Practice Guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2024;105:S117–314. https://doi.org/10.1016/j.kint.2023.10.018.
28. Marx N, Federici M, Schütt K, Müller-Wieland D, Ajjan RA, Antunes MJ, et al. Wytyczne ESC 2023 dotyczące leczenia chorób sercowo-naczyniowych u pacjentów z cukrzycą. Pol Heart J Kardiologia Pol. 2023;81:103–200.
29. Mann KG, Whelihan MF, Butenas S, Orfeo T. Citrate anticoagulation and the dynamics of thrombin generation. J Thromb Haemost. 2007;5:2055–61. https://doi.org/10.1111/j.1538-7836.2007.02710.x.
30. Lundblad RL, Kingdon HS, Mann KG. [14] Thrombin. Methods Enzymol., vol. 45, Elsevier; 1976, pp. 156–76. https://doi.org/10.1016/S0076-6879(76)45017-6
31. Konieczynska M, Fil K, Bazanek M, Undas A. Prolonged duration of type 2 diabetes is associated with increased thrombin generation, prothrombotic fibrin clot phenotype and impaired fibrinolysis. Thromb Haemost. 2014;111:685–93. https://doi.org/10.1160/TH13-07-0566.
32. Cieslik J, Mrozinska S, Broniatowska E, Undas A. Altered plasma clot properties increase the risk of recurrent deep vein thrombosis: a cohort study. Blood. 2018;131:797–807. https://doi.org/10.1182/blood-2017-07-798306.
33. Zieleniewska NA, Szum-Jakubowska A, Chlabicz M, Jamiołkowski J, Kowalska I, Kamiński KA. The prevalence of diabetes and prediabetes: a population-based study. Pol Arch Intern Med. 2023;133:16407. https://doi.org/10.20452/pamw.16407.
34. Basu S, Yudkin JS, Kehlenbrink S, Davies JI, Wild SH, Lipska KJ, et al. Estimation of global insulin use for type 2 diabetes, 2018-30: a microsimulation analysis. Lancet Diabetes Endocrinol. 2019;7:25–33. https://doi.org/10.1016/S2213-8587(18)30303-6.
35. Arnold SV, Tang F, Cooper A, Chen H, Gomes MB, Rathmann W, et al. Global use of SGLT2 inhibitors and GLP-1 receptor agonists in type 2 diabetes. Results from DISCOVER. BMC Endocr Disord. 2022;22:111. https://doi.org/10.1186/s12902-022-01026-2.
36. Olson NC, Raffield LM, Lange LA, Lange EM, Longstreth WT, Chauhan G, et al. Associations of activated coagulation factor VII and factor VIIa-antithrombin levels with genome-wide polymorphisms and cardiovascular disease risk. J Thromb Haemost JTH. 2018;16:19–30. https://doi.org/10.1111/jth.13899.
37. El-Hagracy RS, Kamal GM, Sabry IM, Saad AA, Abou El Ezz NF, Nasr HAR. Tissue factor, tissue factor pathway inhibitor and factor vii activity in cardiovascular complicated type 2 diabetes mellitus. Oman Med J. 2010;25:173–8. https://doi.org/10.5001/omj.2010.52.
38. El-Ghoroury EA, El-Din HG, Abdel-Kader M, Ragab S. Study of factor VII, tissue factor pathway inhibitor and monocyte tissue factor in noninsulin-dependent diabetes mellitus. Blood Coagul Fibrinolysis Int J Haemost Thromb. 2008;19:7–13. https://doi.org/10.1097/01.mbc.0000304148.26525.da.
39. D'Elia JA, Weinrauch LA, Gleason RE, Lipinska I, Keough J, Pendse S, et al. Fibrinogen and factor VII levels improve with glycemic control in patients with type 1 diabetes mellitus who have microvascular complications. Arch Intern Med. 2001;161:98. https://doi.org/10.1001/archinte.161.1.98.
40. Kario K, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. Arterioscler Thromb Vasc Biol. 1995;15:1114–20. https://doi.org/10.1161/01.atv.15.8.1114.
41. Mahemuti N, Zou J, Liu C, Xiao Z, Liang F, Yang X. Urinary albumin-to-creatinine ratio in normal range, cardiovascular health, and all-cause mortality. JAMA Netw Open. 2023;6:e2348333. https://doi.org/10.1001/jamanetworkopen.2023.48333.
42. Undas A. Statins in prevention of thromboembolic events: from seminal studies to recent advances. Pol Arch Intern Med. 2022;132:16208. https://doi.org/10.20452/pamw.16208.
43. Kristiansen J, Grove EL, Sjúrðarson T, Mohr M, Kristensen SD, Hvas A-M. Acute and subacute effects of strenuous exercise on platelet aggregation, coagulation and fibrinolysis in patients with stable coronary artery disease. Thromb Res. 2024;236:220–7. https://doi.org/10.1016/j.thromres.2024.03.007.
44. Blasi A, von Meijenfeldt FA, Adelmeijer J, Calvo A, Ibañez C, Perdomo J, et al. In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation. J Thromb Haemost. 2020;18:2646–53. https://doi.org/10.1111/jth.15043.
45. Campello E, Zabeo E, Radu CM, Spiezia L, Gavasso S, Fadin M, et al. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. Thromb Haemost. 2015;113:85–96. https://doi.org/10.1160/TH14-02-0156.
46. Landgrebe LE, Juhl CB, Andersen V, Moitinho-Silva L, Bang C, Bladbjerg EM. Postprandial factor VII activation does not increase plasma concentrations of prothrombin fragment 1 + 2 in patients with morbid obesity. Thromb Res. 2020;196:260–7. https://doi.org/10.1016/j.thromres.2020.08.044.
47. Landgrebe LE, Andersen V, Bang C, Moitinho-Silva L, Schwarz H, Juhl CB, et al. High-fat meals do not affect thrombin formation and fibrin clot lysis in individuals with obesity during intentional weight loss. Nutr Res N Y N. 2022;97:1–10. https://doi.org/10.1016/j.nutres.2021.11.002.
48. Kapur R, Hoffman CJ, Bhushan V, Haltin MB. Postprandial elevation of activated factor VII in young adults. Arterioscler Thromb Vasc Biol. 1996;16:1327–32. https://doi.org/10.1161/01.atv.16.11.1327.
49. Bladbjerg EM, Münster AM, Marckmann P, Keller N, Jespersen J. Dietary factor VII activation does not increase plasma concentrations of prothrombin fragment 1 + 2 in patients with stable angina pectoris and coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2000;20:2494–9. https://doi.org/10.1161/01.atv.20.11.2494.
50. Pieters M, de Maat MPM. Diet and haemostasis - a comprehensive overview. Blood Rev. 2015;29:231–41. https://doi.org/10.1016/j.blre.2014.12.005.
51. Valencia I, Lumpuy-Castillo J, Magalhaes G, Sánchez-Ferrer CF, Lorenzo Ó, Peiró C. Mechanisms of endothelial activation, hypercoagulation and thrombosis in COVID-19: a link with diabetes mellitus. Cardiovasc Diabetol. 2024;23:75. https://doi.org/10.1186/s12933-023-02097-8.
52. Spiezia L, Rossetto V, Campello E, Gavasso S, Woodhams B, Tormene D, et al. Factor VIIa-antithrombin complexes in patients with arterial and venous thrombosis. Thromb Haemost. 2010;103:1188–92. https://doi.org/10.1160/TH09-08-0606.

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