Blood clot properties, thrombin generation, and platelet activation in patients with dysglycemia and established atherosclerotic cardiovascular disease: The CASCARA study

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Editorial
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ABSTRACT

Background: There is a strong link between coronary artery disease (CAD), type 2 diabetes (T2D) on one hand, and altered fibrin clot properties, including increased clot density, and unfavorable fibrin clot structure on the other. T2D-related changes in fibrin clots can increase cardiovascular (CV) disease risk, including future CV events. We aimed to assess fibrin clot properties, thrombin generation, and platelet activation in CAD patients with prediabetes (PD) or T2D, compared to CAD patients without glycemic disorders.

Methods: We allocated patients to three groups: 1) Those with angiographically established CAD but without glycemic abnormalities (CAD group); 2) individuals with PD and established CAD (CAD+PD group); and 3) patients with T2D and CAD (CAD+T2D group). We conducted comparisons across these groups for thrombin generation, fibrin clot permeability, fibrin clot lysis, and platelet activation.

Results: The final analysis included 116 eligible patients: 1) CAD group (n = 31); 2) CAD+PD (n = 42); and 3) CAD+T2D (n = 43). The CAD+T2D patients enrolled had well-controlled T2D (median HbA1c level of 5.90%; IQR: 5.7%–6.3%). We found no significant differences in thrombin generation, fibrin clot properties, or platelet activation markers across the three analyzed groups (all *P*-values >0.20). However, elevated interleukin-6 (IL-6) levels were noted in both the highest and lowest glucose concentration quartiles. Additionally, a substantial increase in endogenous thrombin potential (ETP) was observed in patients in the highest glycated hemoglobin quintile.

Conclusions: Individuals with established CAD and concomitant PD or well-controlled T2D exhibited comparable fibrin clot phenotypes, thrombin generation potential, and platelet activation when compared to CAD patients without dysglycemia.

Key words: coronary artery disease, diabetes, fibrin clot, platelet activation, prediabetes, thrombin generation

INTRODUCTION

Prior studies have consistently indicated that individuals with type 2 diabetes (T2D) exhibit altered fibrin clot characteristics, including increased clot density and an unfavorable fibrin clot structure. These alterations in fibrin clot properties are associated with an elevated risk of cardiovascular (CV) disease, particularly atherosclerotic events. Altered fibrin clot properties in T2D may result from mechanisms involving inflammation, oxidative stress, and hyperlipidemia. These factors can induce modifications in fibrin(ogen), leading to changes in clot structure and function [1–3]. Studies have shown that fibrinogen from individuals with T2D generates

WHAT'S NEW?

This study aimed to examine fibrin clot properties, thrombin generation, and platelet activation in patients with high CV risk, with and without glucose metabolism disorders (dysglycemia). We included patients with 1) CAD without dysglycemia; 2) CAD and prediabetes (PD) and 3) CAD and type 2 diabetes (T2D). Finally, patients with CAD and well-controlled T2D were included and showed similar fibrin clot characteristics, thrombin generation, and platelet activation compared to those with CAD alone or CAD with PD. Only patients in the highest quintile of HbA1c concentration exhibited a significant increase in endogenous thrombin potential. Patients with both the highest and lowest glucose concentrations showed enhanced IL-6 concentration.

fibrin structures resistant to fibrinolysis due to increased α2-antiplasmin crosslinking, impaired tissue plasminogen activator (tPA) binding, and reduced plasmin generation on the fibrin clot surface [4]. Additionally, hyperglycemia and glycation alter fibrin structure, making the fibrin clot more resistant to fibrinolysis [5]. These associations likely contribute to the prothrombotic and antifibrinolytic environment characteristic of T2D, potentially increasing the risk of vascular events in this population, e.g., coronary artery disease (CAD).

Among prediabetic (PD) patients with glucose intolerance, there were elevated levels of PAI-1 and tPA antigens [6]. Among glucose-intolerant individuals, men exhibited a positive association between insulin quintiles and PAI-1, tPA antigen, and von Willebrand factor antigen levels, while factor VII antigen, fibrinogen, and plasma viscosity showed no corresponding increase [6].

The objective of this study was to assess and compare thrombin generation, fibrin clot properties, clot lysis, and platelet activation in patients diagnosed with CAD in contrast to patients with CAD accompanied by either PD or T2D.

METHODS

Study design and population

The CASCARA trial was a prospective, cohort study that aimed to compare fibrin clot characteristics in patients with a very high CV risk and dysglycemia. Patients were screened, and blood was collected at the Jagiellonian University Medical College, St. John Paul II Hospital in Kraków, Poland from January 2017 to May 2018. The investigators screened for patients with 1) established CAD without glycemia abnormalities; 2) PD diagnosed by oral glucose tolerance test (OGTT) as per the European Association for the Study of Diabetes (EASD) guidelines and concomitant CAD; 3) T2D diagnosed previously as stated in patients' medical records or diagnosis during index hospitalization by the OGTT in line with the EASD guidelines and concomitant CAD. Therefore, all recruited patients had a very high CV risk and groups 2 and 3 had dysglycemia (PD and T2D, respectively).

Exclusion criteria included pregnancy, autoimmune disorders, recent myocardial infarction (<3 months) or coronary artery bypass grafting (<1 month), acute infections, use of specific medications known to potentially influence clot properties (such as oral anticoagulants, heparins, non-steroidal anti-inflammatory drugs, and oral corticosteroids), as well as severe comorbidities such as cancer.

Blood sampling and laboratory measurements

Fasting blood samples were obtained between 8 and 10 a.m. after overnight fasting. The samples were processed 30 to 60 minutes after blood collection and stored at –70°C until further analysis. Blood was taken from the antecubital vein, with minimal stasis at one time point. Routine blood tests, including the measurement of complete blood count, lipid profile, and levels of aspartate aminotransferase (AST), alanine transaminase (ALT), and serum creatinine, were done by automated laboratory techniques. Glycated hemoglobin (HbA1c) levels were measured using a turbidimetric inhibition immunoassay.

Thrombin generation

Plasma thrombogenic potential was assessed based on a thrombogram, analyzed with the use of the CAT (Thrombinoscope BV, Maastricht, the Netherlands), according to the protocol of the manufacturer, in a 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland) equipped with the 390/460 filter set at 37°C.

Fibrin clot lysis

Clot lysis was performed as previously described [7]. Briefly, to assess plasma clot lysis time (CLT), plasmin-mediated fibrinolysis was evaluated in the presence of a recombinant tissue plasminogen activator (Boehringer Ingelheim, Ingelheim, Germany). Lysis time was chosen as a marker of clot susceptibility to fibrinolysis. It was defined as the time needed for a 50% reduction of fibrin clot absorbance.

Fibrin clot permeation

Permeation coefficient (Ks) was determined as previously presented [8]. Briefly, calcium chloride (20 mmol/l) and human thrombin (1 U/ml) were added to 120 µl of citrated plasma to assess fibrin clot permeability. After incubation for 120 minutes, tubes with the clots were connected to a container with a buffer (10 mmol/l: 0.05 mol/l Tris-HCl; 100 mmol/l: 0.15 mol/l NaCl, pH 7.5). Its volume flowing through the gels was measured within 60 minutes. Then, a permeation coefficient was calculated, indicating the size of fibrin clot pores.

Table 1. Baseline patient anthropometric and clinical characteristics

Variable	CAD (n = 31)	CAD + PD (n = 42)	CAD + T2D (n = 43)	*P*-value
Age, years	65.55 (10.11)	66.38 (12.20)	65.95 (8.57)	0.94
Male, n (%)	27 (87.10)	35 (83.33)	32 (74.42)	0.35
Prior MI, n (%)	10 (32.26)	15 (35.71)	15 (35.71)	0.94
Hypertension, n (%)	20 (64.52)	36 (85.71)	38 (90.48)	0.01
Hypercholesterolemia, n (%)	14 (45.16)	34 (80.95)	33 (78.57)	0.001
Abdominal obesity, n (%)	11 (35.48)	16 (38.10)	24 (57.14)	0.11
Current smoking, n (%)	12 (38.71)	8 (19.05)	7 (16.67)	0.06
Family history of CAD, n (%)	5 (16.13)	10 (23.81)	10 (23.81)	0.68
Weight, kg	80.50 (68.13–87.75)	80.50 (74.25–91.50)	84.30 (75.50–93.75)	0.43
Height, m	170.00 (164.00–176.00)	170.00 (162.50–177.25)	168.50 (162.50–175.00)	0.75
BMI, kg/m^2	27.44 (3.42)	28.30 (3.20)	29.66 (3.90)	0.07
LVEF, %	55.00 (45.00–60.00)	55.00 (50.00–60.00)	55.00 (50.00–60.00)	0.84
Baseline pharmacotherapy				
ASA, n (%)	31 (100.00)	40 (95.24)	41 (97.62)	0.78
Clopidogrel, n (%)	15 (48.39)	17 (40.48)	21 (50.00)	0.65
Ticagrelor, n (%)	13 (41.94)	11 (26.19)	11 (26.19)	0.27
ß-blocker, n (%)	26 (83.87)	35 (83.33)	37 (88.10)	0.80
CCB, n (%)	15 (48.39)	22 (52.38)	13 (30.95)	0.11
ACEI, n (%)	23 (74.19)	35 (83.33)	34 (80.95)	0.61
ARB, n (%)	5 (16.13)	3 (7.14)	5 (11.90)	0.50
Statin, n (%)	30 (96.77)	39 (92.86)	42 (100.00)	0.19
Nitrate, n (%)	1 (3.23)	6 (14.29)	3 (7.14)	0.28
Fibrate, n (%)	1 (3.23)	1 (2.38)	0 (0.00)	0.74
Loop diuretic, n (%)	7 (22.58)	6 (14.29)	9 (21.43)	0.60
MRA, n (%)	9 (29.03)	11 (26.19)	15 (35.71)	0.63
Metformin, n (%)	1 (3.23)	11 (26.19)	30 (71.43)	<0.001
Sulphonylurea, n (%)	0 (0.00)	1 (2.38)	4 (9.52)	0.19
SGLT-2i, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	–
GLP-1A, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	–
Insulin, n (%)	0 (0.00)	0 (0.00)	8 (19.05)	–

Data shown as number (percentage), median and interquartile range (IQR) or mean (standard deviation [SD])

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; ASA, acetylsalicylic acid; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blockers; GLP-1A, glucagon-like peptide-1 receptor agonists; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MRA, mineralocorticoid receptor antagonists; SGLT-2i, sodium-glucose cotransporter-2 inhibitors

Platelet activation and inflammation

Commercially available immunoenzymatic assays were used to determine inflammatory markers, including human tumor necrosis factor-alpha and human interleukin-6 (both from R&D Systems, Indianapolis, IN, US), and also platelet activation markers, soluble CD40 ligand (CD40L) and platelet factor-4 (PF-4) (all from R&D Systems, Minneapolis, MN, US). All the intra-assay and inter-assay coefficients of variation for the ELISA measurements were below 7%. C-reactive protein was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

The study was designed to detect a 10% or greater difference in CLT and Ks with 90% power at a significance level of 0.05, requiring a minimum of 22 patients per group [9–11]. Continuous variables were presented as means (SD) or medians (IQR), and normality was assessed with the Shapiro–Wilk test. Categorical variables were reported as numbers and percentages. Group differences in continuous variables were assessed using analysis of variance or Kruskal–Wallis tests, followed by appropriate *post-hoc* tests (HSD or Steel-Dwass) to account for multiple comparisons. Linear regression was used to examine the relationship between blood clot properties and glycemia, adjusting for fibrinogen. A two-sided *P*-value below 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism version 8.0.1 (San Diego, CA, US) and IBM SPSS ver. 28.0 (Armonk, NY, US).

RESULTS

We consecutively enrolled 116 patients eligible for this study, and finally included: A) patients with established CAD, but without any dysglycemia (n = 31; CAD group); B) patients with established CAD and confirmed PD (n = 42; both with impaired fasting glucose and impaired glucose tolerance; group CAD+PD); and C) patients with documented both CAD and T2D (n = 43; group CAD+T2D).

All enrolled patients had at least a very high CV risk with multiple CV risk factors in addition to CAD and dysglycemia, namely: hypertension, abdominal obesity or overweight, cigarette smoking, and/or dyslipidemia (see Table 1). As presented in Table 1, baseline characteristics in all clinical parameters (except for hypercholesterolemia) and medi-

Table 2. Routine care laboratory evaluation in all three groups

Variable	CAD (n = 31)	CAD + PD (n = 42)	CAD + T2D (n = 43)	*P*-value
WBC, 10^3/µl	7.73 (6.45–10.41)	7.51 (5.81–9.20)	7.68 (6.47–9.66)	0.51
RBC, 10^6/µl	4.79 (0.62)	4.77 (0.41)	4.79 (0.55)	0.97
HGB, g/dl	14.47 (2.00)	14.37 (1.15)	14.33 (1.49)	0.94
RDW, %	12.90 (12.60–13.60)	12.90 (12.40–13.23)	12.80 (12.40–13.33)	0.55
PLT, 10^3/µl	241.00 (207.00–281.00)	234.00 (180.00-262.00)	232.00 (178.75–265.00)	0.32
Glucose, mmol/l	5.20 (4.90–5.40)	5.60 (5.13–6.00)	6.10 (5.10–7.20)	<0.001
HbA1c, %	5.50 (5.40–5.60)	5.60 (5.40–5.70)	5.90 (5.70–6.40)	<0.001
HOMA-IR, ratio	1.48 (0.91–1.97)	1.86 (0.98–2.80)	2.52 (1.68–4.66)	<0.001
Creatinine, µmol/l	83.00 (74.00–103.00)	84.00 (77.00–91.25)	88.50 (74.00–102.00)	0.68
eGFR, ml/min/ 1.73 m^2 [CKD-EPI]	78.16 (18.03)	78.86 (14.80)	73.43 (18.47)	0.30
INR	1.03 (0.97–1.08)	1.00 (0.96–1.05)	1.04 (0.99–1.08)	0.22
aPTT, s	30.40 (28.10–64.40)	28.95 (27.00–32.03)	29.75 (28.13–31.88)	0.29
hs-CRP, mg/l	4.92 (1.64–11.29)	2.48 (1.38–9.69)	3.75 (1.41–13.62)	0.50
Total cholesterol, mmol/l	4.87 (4.13–5.91)	4.37 (3.47–5.30)	3.75 (2.95–4.94)	0.006
LDL-C, mmol/l	3.26 (2.46–4.50)	2.78 (2.08–3.63)	2.47 (1.50–3.59)	0.01
HDL-C, mmol/l	1.13 (0.97–1.52)	1.21 (0.97–1.56)	1.10 (0.87–1.37)	0.42
non-HDL-C, mmol/l	3.78 (2.70–4.89)	3.17 (2.38–4.14)	2.74 (1.78–3.98)	0.02
TG, mmol/l	1.52 (1.25–1.81)	1.27 (0.99–1.68)	1.26 (1.01–1.49)	0.09

Numbers are shown as median (IQR) or mean (SD)

Abbreviations: aPTT, activated partial thromboplastin time; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HGB, hemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; INR, international normalized ratio; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non high-density lipoprotein cholesterol; PD, prediabetes; PLT, platelet count; RBC, red blood count; RDW, red blood cell distribution width; T2D, type 2 diabetes; TG, triglycerides WBC, white blood count

Table 3. Comparison of fibrinogen, thrombin generation, fibrin clot properties, platelet activation and inflammatory status in studied populations (CAD vs. CAD + PD and CAD vs. CAD + T2D)

Variable	CAD (n = 31)	CAD + PD (n = 42)	CAD + T2D (n = 43)	*P*-value
Fibrinogen, g/l	3.59 (2.95–4.25)	3.41 (2.72–4.23)	3.63 (3.13–4.74)	0.19
Lag time, min	4.58 (1.96)	4.15 (1.96)	3.64 (1.97)	0.35
ETP, nM*min	1612.64 (31.99)	1619.54 (27.47)	1662.57 (36.17)	0.87
Peak, nM	257.23 (113.31)	272.46 (113.60)	273.63 (113.99)	0.83
Time to peak, min	8.30 (3.06)	7.87 (3.07)	6.87 (3.08)	0.32
Ks, 10^{-9} cm^2	4.21 (0.92)	4.38 (0.92)	4.35 (0.93)	0.10
CLT, min[a]	106.06 (3.02)	106.78 (2.74)	110.43 (33.62)	0.83
PF-4, ng/ml	97.46 (5.51)	98.76 (5.31)	97.64 (5.43)	0.63
sCD40L, ng/ml	0.91 (0.20–3.58)	2.26 (0.78–4.57)	1.46 (0.37–4.25)	0.20
hs-CRP, mg/l	4.92 (1.64–11.29)	2.48 (1.38–9.69)	3.75 (1.41–13.62)	0.50
IL-6, pg/ml	3.55 (2.38–8.71)	3.87 (2.80–6.40)	4.65 (3.35–8.90)	0.19
TNF-alpha, pg/ml	9.95 (6.58–14.07)	10.58 (7.90–19.51)	11.10 (8.68–15.14)	0.34

Numbers are shown as median (IQR) or mean (SD)

[a]Comparison of CLT values was adjusted for the baseline fibrinogen concentration

Abbreviations: CLT, clot lysis time; ETP, endogenous thrombin potential; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; Ks, permeation coefficient; PF-4, platelet factor-4; sCD40L, soluble CD40 ligand; TNF-alpha, tumor necrosis factor-alpha

cations (excluding antidiabetic agents) were similar in the analyzed subgroups (Table 1).

As expected, glycemia, HbA1c, HOMA-IR, as well as total cholesterol, low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein levels differed significantly between the three studied groups (Table 2). However, the median levels of HbA1c, representing metabolic control in patients with T2D, were relatively low (5.90% with an IQR of 5.7 to 6.3%; Table 2). Additionally, the median levels of high-sensitivity C-reactive protein (hs-CRP) did not differ between the groups (Table 2), and the LDL-C concentration was significantly lower in the CAD+T2D group. Therefore, this group description can be understood as patients with very high CV risk but well-controlled T2D, and other CV risk factors.

Thrombin generation, fibrin clot lysis, and permeation

We found no significant differences between all analyzed groups, namely: CAD vs. CAD+PD vs. CAD+T2D in all measured coagulation parameters (Table 3). All thrombin generation assays, including ETP, peak thrombin concentration, time to peak thrombin generation, as well as Ks and CLT were similar between the groups.

Table 4. Thrombin generation, clot lysis, clot porosity, platelet activation and inflammatory status in comparison of HbA1c quintiles

Variable	Q1	Q2	Q3	Q4	Q5	*P*-value
ETP, nM*min	1426.93 (794.90–1684.72)	1644.11 (1400.05–1876.34)	1683.73 (1436.40–1939.86)	1743.31 (1570.05–2061.11)	1716.40 (1596.86–1901.88)	0.047
Lag time, min	3.62 (3.00–5.29)	3.33 (2.95–4.45)	3.28 (2.95–4.25)	3.45 (2.94–3.99)	3.67 (2.96–4.90)	0.72
Peak, nM	263.54 (50.12–309.52)	249.90 (195.01–322.72)	294.50 (232.87–307.74)	305.01 (235.48–355.40)	286.02 (215.89–323.84)	0.20
Time to peak, min	6.95 (5.67–11.00)	6.62 (5.79–8.30)	6.65 (5.38–7.66)	6.29 (5.95–8.23)	6.81 (6.30–8.32)	0.74
CLT, min	96.50 (67.75–109.00)	98.50 (90.50–108.25)	90.00 (81.00–116.00)	106.00 (92.00–146.00)	113.00 (96.00–145.75)	0.13
Ks, 10^{-9} cm^2	3.41 (3.01–5.36)	4.91 (3.62–5.33)	4.77 (3.83–5.49)	3.89 (3.32–5.31)	3.21 (2.57–4.23)	0.07
PF-4, ng/ml	97.82 (5.34)	98.05 (4.85)	98.37 (5.99)	99.18 (5.49)	99.51 (7.49)	0.96
sCD40L, ng/ml	0.96 (0.36–3.12)	1.88 (0.20–3.40)	2.65 (0.57–5.23)	3.01 (0.55–4.13)	0.77 (0.37–4.23)	0.49
hs-CRP, mg/l	1.53 (0.94–2.46)	3.23 (1.11–7.63)	3.75 (2.30–17.61)	1.12 (1.02–3.67)	2.91 (1.24–10.09)	0.20
IL–6, pg/ml	2.99 (2.25–5.84)	4.43 (2.35–6.16)	4.82 (3.71–12.75)	4.23 (3.11–9.42)	4.50 (2.80–8.81)	0.15
TNF–alpha, pg/ml	15.16 (10.25–25.68)	8.79 (5.27–12.38)	10.32 (8.35–12.38)	11.60 (10.13–15.98)	14.50 (8.80–20.64)	0.06

Numbers are shown as median (IQR) or mean (SD) with Kruskal–Wallis test or ANOVA used respectively

Abbreviations: see Table 3

Table 5. Thrombin generation, clot lysis, clot porosity, platelet activation and inflammatory status in glucose quintile comparison

Variable	Q1	Q2	Q3	Q4	Q5	*P*-value
ETP, nM*min	1460.52 (1276.83–1856.32)	1644.11 (1369.32–1866.21)	1574.92 (1432.19–1842.05)	1742.05 (1638.25–2017.50)	1721.62 (1585.70–1930.80)	0.07
Lag time, min	3.62 (3.00–5.33)	3.33 (3.28–4.61)	3.31 (2.95–3.99)	3.28 (2.95–4.08)	3.67 (2.94–4.96)	0.85
Peak, nM	270.49 (110.91–318.07)	247.88 (186.53–322.19)	267.06 (229.92–303.49)	310.59 (254.58–347.73)	285.80 (232.50–329.03)	0.14
Time to peak, min	6.95 (5.67–9.97)	6.67 (5.95–8.96)	6.65 (5.71–7.62)	6.29 (5.79–7.91)	6.67 (6.00–8.30)	0.74
CLT, min	98.00 (89.50–113.25)	98.00 (93.25–110.75)	94.00 (85.00–109.00)	107.50 (93.25–129.50)	113.00 (95.00–147.00)	0.07
Ks, 10^{-9} cm^2	3.53 (3.01–5.36)	4.77 (3.68–5.49)	4.60 (4.14–5.37)	3.88 (3.30–4.84)	3.56 (2.97–4.68)	0.09
PF-4, ng/ml	99.96 (3.14)	97.89 (6.69)	98.53 (5.07)	96.04 (5.26)	97.29 (6.49)	0.31
sCD40L, ng/ml	1.29 (0.20–3.38)	0.84 (0.33–3.49)	2.86 (0.91–4.71)	2.32 (0.43–4.82)	1.41 (0.26–4.62)	0.29
hs-CRP, mg/l	3.48 (1.34–7.59)	3.64 (1.54–6.55)	2.16 (1.12–11.00)	6.03 (1.35–28.78)	6.27 (2.80–24.00)	0.14
IL-6, pg/ml	4.85 (3.08–8.62)	3.49 (2.30–4.35)	4.69 (3.02–8.87)	4.01 (2.37–6.87)	4.64 (3.37–16.24)	0.047
TNF-alpha, pg/ml	9.46 (6.25–13.79)	10.22 (7.68–15.70)	10.13 (8.35–16.37)	9.91 (8.24–33.70)	11.69 (9.30–17.10)	0.35

Numbers are shown as median (IQR) or mean (SD) with Kruskal–Wallis test or ANOVA used respectively

Abbreviations: see Table 3

Platelet activation

There were no significant differences observed in both analyzed platelet activation markers, namely PF-4 and sCD40L, when comparing patients with CAD to those with CAD+PD or CAD+T2D (Table 3).

Quintile analysis

To investigate the potential impact of variations in both glycemia and HbA1c concentrations on the analyzed coagulation and platelet parameters, we systematically divided the groups into quintiles (based on glucose and HbA1c). Fasting glucose concentration was available for all studied patients, while HbA1c was only available in the CAD+PD and CAD+T2D groups.

In the analysis of HbA1c quintiles, only the ETP demonstrated a significant difference in the thrombin generation assay (Table 4). Other variables related to fibrin clot permeation and lysis were comparable between the quintiles of both HbA1c and glucose. In quintile analysis, both platelet activation markers were not significantly different across the analyzed glucose and HbA1c concentrations. Among inflammatory variables, only IL-6 exhibited a significant difference across the assessed glucose quintiles (Table 5).

Regression analysis

Regression analysis was conducted to assess the individual effects of HbA1c and glucose concentration on coagulation parameters in the CAD+PD and CAD+T2D populations. Notably, in the CAD+PD subgroup, a 1 mmol/l increase in glucose concentration resulted in the rise of the ETP by 243.16 nM × min (P = 0.02), and the prolongation of the CLT by 26.41 min (P = 0.002). Conversely, no significant impacts of glucose concentration were observed in the CAD+T2D group. Neither the CAD+T2D nor CAD+PD groups demonstrated a significant influence of HbA1c on the analyzed coagulation and platelet parameters.

All associations, including non-linear ones, are presented in detail in the Supplementary material (*Results* and *Figures S1–S3*).

DISCUSSION

This study focused on patients diagnosed with CAD and accompanying well-controlled dysglycemia (PD or T2D).

Comparative analyses among patient cohorts A) CAD vs. B) CAD+PD vs. C) CAD+T2D revealed no significant differences in thrombin generation and fibrin clot properties. Similarly, no substantial variations were demonstrated in platelet activation markers between the groups.

Despite differences in the prevalence of CV risk factors, such as hypercholesterolemia, hypertension, and higher insulin resistance, as well as elevated glucose concentration and HbA1c levels in the CAD+T2D group, no statistically significant differences were observed in thrombin generation, clot permeation, and CLT. The relatively low values of HbA1c (median of 6.10 mmol/l in the CAD+T2D group) and serum glucose (median of 5.9% in the CAD+T2D group) indicate that subjects were well-controlled, which contributed to the absence of meaningful distinctions in the analyzed blood coagulation variables. This observation is further supported by significantly lower concentrations of TC, LDL-C, and non-HDL in the CAD+T2D subgroup. Additionally, median values of fibrinogen and hs-CRP were comparable across the investigated patient groups. Notably, this study is the first to demonstrate that metabolically well-controlled patients with T2D or PD exhibit similar thrombin generation potential, as well as non-significantly higher fibrin clot porosity, CLT, and platelet activation.

Coronary artery disease

The relationship between unfavorable fibrin clot properties and enhanced thrombin generation and CAD has been known for many years [12, 13]. A large body of evidence has demonstrated that patients with higher CV risk or established CAD had higher thrombin generation, less permeable fibrin clots, and longer fibrin clot lysis [14–16]. This phenomenon was documented for both chronic [17] and acute coronary syndromes (ACS) [18, 19] and was found to be related to the subsequent risk of thrombotic events [20, 21]. It was shown that a composite of nonfatal myocardial infarction, ischemic stroke, and cardiovascular death occurred more frequently in CAD patients with enhanced clot turbidity or longer lysis [22]. Similarly, based on the PLATO substudy, both fibrin clot turbidity and CLT were found to independently predict adverse outcomes in ACS patients [23]. Hence, unfavorable fibrin clot properties may contribute to poor prognoses among CAD patients [24].

It has long been known that increased platelet activation is a predictor of CAD, and plaque stability and, concurrently, antiplatelet therapy significantly reduce the frequency of clinical events in CAD patients [25–28].

Type 2 diabetes

It is known that T2D doubles the risk of CAD and CV death [29], and those clinical conditions were at least partly associated with altered fibrin clot properties [30]. In a study by Konieczyńska et al., it was demonstrated that the prolonged duration of T2D was related to increased thrombin production, hypofibrinolysis, and prothrombotic fibrin clot formation [31]. Moreover, coagulation parameters were affected differently and more substantially by T2D duration than by inadequate glycemic control [31]. Those observations are consistent with the results presented in our publication, in which we provided evidence that thrombin generation, fibrin clot porosity, and lysis were not significantly different between well-controlled T2D and concomitant CAD when compared with the CAD or PD and CAD subgroups. It was demonstrated that T2D patients exhibited reduced clot permeability, shorter lag time, increased clot turbidity and fiber density, along with a higher number of fibrin branches compared to healthy controls [32]. Moreover, there has also been evidence that denser fibrin clots, which were more resistant to fibrinolysis, could predict long-term CV mortality among patients with T2D [33]. Not only hyperglycemia but also fasting hypoglycemia was associated with enhanced thrombin formation and formation of denser fibrin clots [34]. Therefore, it remains unclear what is the trigger of procoagulant fibrin clot phenotype in T2D patients. Potential candidates for that would be significant transient hyperglycemia, but also hypoglycemia; duration of T2D, or poor metabolic control of T2D.

Comparable levels of platelet markers related to activation, turnover, and leukocyte-platelet interactions were observed between T2D patients vs. matched controls [35]. Moreover, poorly controlled T2D individuals exhibited elevated baseline platelet activity [36].

Coronary artery disease and type 2 diabetes

Neergaard-Petersen et al. clearly demonstrated that patients with both CAD and T2D had significantly altered fibrin clots when compared to patients with CAD only [37]. Moreover, the authors found that maximal fibrin clot meshwork density and lysis time and lysis area were significantly correlated with inflammatory markers such as hs-CRP, complement C3, and IL-6 [37]. It was also shown that hyperglycemia in the setting of an ACS was associated with enhanced thrombin generation and unfavorably altered clot characteristics [38].

In our study, we demonstrated that despite very high CV risk in all analyzed patients, well-controlled T2D and CAD as well as PD and CAD had similar fibrin clot phenotypes and thrombin generation to patients with CAD without any type of dysglycemia.

In the highest CV-risk patients with established CAD and T2D, it was evidenced that improved glycemic control reduces platelet reactivity [39]. Nevertheless, platelet dysfunction or increased activation cannot be attributed solely to glycemia. Type 1 diabetic patients with established microvascular complications, despite achieving significant improvement in glycemic control, did not experience improvement in platelet function abnormalities [40]. Similar to our study, it was shown that chronic glycemia, whether elevated or well-controlled, may potentially contribute to increased platelet activation and increased risk of cardiovascular outcomes [41].

Limitations

We acknowledge several limitations of this study. First, the sample size in the three subgroups was relatively small although power calculations based on prior research guided our study design to detect differences in fibrin clot properties, thrombin generation, or platelet activation. Future research with larger cohorts and longitudinal designs should validate and extend these findings. Additionally, caution is advised when interpreting the results of the secondary analysis that involves the quintile comparison and regression. The small sample size may impact the reliability of the findings. Nonetheless, the analysis provides insights into the trends in coagulation and platelet variables in relation to glucose and HbA1c concentrations. Second, data collection took place shortly after CAD diagnosis, potentially impacting the analyzed thrombin generation, fibrin clot properties, and platelet activation due to the recent diagnosis. Third, the diagnosis of PD in some patients relied on the OGTT which could be influenced by improper fasting glycemia, potentially affecting the outcomes. Nonetheless, HbA1c assessments were conducted for all dysglycemic patients to enhance diagnostic accuracy and evaluate overall metabolic control.

CONCLUSIONS

In conclusion, this study demonstrated that patients with well-controlled T2D and CAD exhibit blood clot parameters (thrombin generation, fibrin clot permeability, and lysis time) that are not significantly different from those observed in patients with CAD and PD or CAD alone. Similarly, there were similar results of platelet activation in the three analyzed groups. These findings highlight the importance of managing both PD and T2D effectively, as it may potentially mitigate adverse effects on the coagulation system in atherosclerotic cardiovascular disease patients.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/polish_heart_journal.

Article information

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REFERENCES

1. Nwose EU, Richards RS, Kerr RG, et al. Oxidative damage indices for the assessment of subclinical diabetic macrovascular complications. Br J Biomed Sci. 2008; 65(3): 136–141, doi: 10.1080/09674845.2008.11732817, indexed in Pubmed: 18986101.
2. Natorska J, Ząbczyk M, Undas A. Neutrophil extracellular traps (NETs) in cardiovascular diseases: From molecular mechanisms to therapeutic interventions. Kardiol Pol. 2023; 81(12): 1205–1216, doi: 10.33963/v.kp.98520, indexed in Pubmed: 38189504.
3. Ząbczyk M, Ariëns RAS, Undas A. Fibrin clot properties in cardiovascular disease: from basic mechanisms to clinical practice. Cardiovasc Res. 2023; 119(1): 94–111, doi: 10.1093/cvr/cvad017, indexed in Pubmed: 36662542.
4. Dunn EJ, Philippou H, Ariëns RAS, et al. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. Diabetologia. 2006; 49(5): 1071–1080, doi: 10.1007/s00125-006-0197-4, indexed in Pubmed: 16538489.
5. Grant PJ. Diabetes mellitus as a prothrombotic condition. J Intern Med. 2007; 262(2): 157–172, doi: 10.1111/j.1365-2796.2007.01824.x, indexed in Pubmed: 17645584.
6. Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. JAMA. 2000; 283(2): 221–228, doi: 10.1001/jama.283.2.221, indexed in Pubmed: 10634338.
7. Pieters M, Philippou H, Undas A, et al. Subcommittee on Factor XIII and Fibrinogen, and the Subcommittee on Fibrinolysis. An international study on the feasibility of a standardized combined plasma clot turbidity and lysis assay: communication from the SSC of the ISTH. J Thromb Haemost. 2018; 16(5): 1007–1012, doi: 10.1111/jth.14002, indexed in Pubmed: 29658191.
8. Undas A, Zawilska K, Ciesla-Dul M, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. Blood. 2009; 114(19): 4272–4278, doi: 10.1182/blood-2009-05-222380, indexed in Pubmed: 19690336.
9. Poreba M, Rostoff P, Siniarski A, et al. Relationship between polyunsaturated fatty acid composition in serum phospholipids, systemic low-grade inflammation, and glycemic control in patients with type 2 diabetes and atherosclerotic cardiovascular disease. Cardiovasc Diabetol. 2018; 17(1): 29, doi: 10.1186/s12933-018-0672-5, indexed in Pubmed: 29452596.
10. Siniarski A, Haberka M, Mostowik M, et al. Treatment with omega-3 polyunsaturated fatty acids does not improve endothelial function in patients with type 2 diabetes and very high cardiovascular risk: A randomized, double-blind, placebo-controlled study (Omega-FMD). Atherosclerosis. 2018; 271: 148–155, doi: 10.1016/j.atherosclerosis.2018.02.030, indexed in Pubmed: 29518747.
11. Poreba M, Mostowik M, Siniarski A, et al. Treatment with high-dose n-3 PUFAs has no effect on platelet function, coagulation, metabolic status or inflammation in patients with atherosclerosis and type 2 diabetes. Cardiovasc Diabetol. 2017; 16(1): 50, doi: 10.1186/s12933-017-0523-9, indexed in Pubmed: 28410617.
12. Szczeklik A, Dropinski J, Radwan J, et al. Persistent generation of thrombin after acute myocardial infarction. Arterioscler Thromb. 1992; 12(5): 548–553, doi: 10.1161/01.atv.12.5.548, indexed in Pubmed: 1576116.
13. Carlin S, de Vries TAC, Budaj A, et al. Dual pathway inhibition for atherosclerotic cardiovascular disease: Recent advances. Kardiol Pol. 2022; 80(12): 1200–1210, doi: 10.33963/KP.a2022.0283, indexed in Pubmed: 36601884.
14. Kalz J, ten Cate H, Spronk HMH. Thrombin generation and atherosclerosis. J Thromb Thrombolysis. 2014; 37(1): 45–55, doi: 10.1007/s11239-013-1026-5, indexed in Pubmed: 24241912.
15. Ten Cate H, Hemker HC. Thrombin Generation and Atherothrombosis: What Does the Evidence Indicate? J Am Heart Assoc. 2016; 5(8), doi: 10.1161/JAHA.116.003553, indexed in Pubmed: 27503850.
16. Kietsiriroje N, Ariëns RAS, Ajjan RA. Fibrinolysis in Acute and Chronic Cardiovascular Disease. Semin Thromb Hemost. 2021; 47(5): 490–505, doi: 10.1055/s-0040-1718923, indexed in Pubmed: 33878782.
17. Rho R, Tracy RP, Bovill EG, et al. Plasma Markers of Procoagulant Activity Among Individuals with Coronary Artery Disease. J Thromb Thrombolysis. 1995; 2(3): 239–243, doi: 10.1007/BF01062716, indexed in Pubmed: 10608030.
18. Becker RC, Tracy RP, Bovill EG, et al. Surface 12-Lead Electrocardiographic Findings and Plasma Markers of Thrombin Activity and Generation in Patients with Myocardial Ischemia at Rest. J Thromb Thrombolysis. 1994; 1(1): 101–107, doi: 10.1007/BF01062003, indexed in Pubmed: 10603519.

19. Becker RC, Bovill EG, Corrao JM, et al. Dynamic Nature of Thrombin Generation, Fibrin Formation, and Platelet Activation in Unstable Angina and Non-Q-Wave Myocardial Infarction. J Thromb Thrombolysis. 1995; 2(1): 57–64, doi: 10.1007/BF01063163, indexed in Pubmed: 10639214.
20. Granger CB, Becker R, Tracy RP, et al. Thrombin generation, inhibition and clinical outcomes in patients with acute myocardial infarction treated with thrombolytic therapy and heparin: results from the GUSTO-I Trial. GUSTO-I Hemostasis Substudy Group. Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries. J Am Coll Cardiol. 1998; 31(3): 497–13505, doi: 10.1016/s0735-1097(97)00539-1, indexed in Pubmed: 9502626.
21. Grajek S, Kałużna-Oleksy M, Grajek S, et al. Non-Vitamin K Antagonist Oral Anticoagulants and Risk of Myocardial Infarction in Patients with Atrial Fibrillation with or without Percutaneous Coronary Interventions: A Meta-Analysis. J Pers Med. 2021; 11(10): 16–24, doi: 10.3390/jpm11101013, indexed in Pubmed: 34683155.
22. Neergaard-Petersen S, Larsen SB, Grove EL, et al. Imbalance between Fibrin Clot Formation and Fibrinolysis Predicts Cardiovascular Events in Patients with Stable Coronary Artery Disease. Thromb Haemost. 2020; 120(1): 75–82, doi: 10.1055/s-0039-1700873, indexed in Pubmed: 31733633.
23. Sumaya W, Wallentin L, James SK, et al. Impaired fibrinolysis predicts adverse outcome in acute coronary syndrome patients with diabetes: A PLATO sub-study. Thromb Haemost. 2020; 120(3): 412–422, doi: 10.1055/s-0039-1701011, indexed in Pubmed: 31975352.4.
24. Larsen JB, Hvas AM. Fibrin clot properties in coronary artery disease: new determinants and prognostic markers. Pol Arch Intern Med. 2021; 131(11), doi: 10.20452/pamw.16113, indexed in Pubmed: 34623063.
25. Correction to: 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes: Developed by the task force on the management of cardiovascular disease in patients with diabetes of the European Society of Cardiology (ESC). Eur Heart J. 2023; 44(48): 5060, doi: 10.1093/eurheartj/ehad774, indexed in Pubmed: 37989571.
26. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. 2020; 41(3): 407–477, doi: 10.1093/eurheartj/ehz425, indexed in Pubmed: 31504439.
27. Byrne RA, Rossello X, Coughlan JJ, et al. 2023 ESC Guidelines for the management of acute coronary syndromes: Developed by the task force on the management of acute coronary syndromes of the European Society of Cardiology (ESC). Eur Heart J. 2023; 44(38): 3720–3826, doi: 10.1093/eurheartj/ehad191, indexed in Pubmed: 37622654.
28. Linden MD, Furman MI, Frelinger AL, et al. Indices of platelet activation and the stability of coronary artery disease. J Thromb Haemost. 2007; 5(4): 761–765, doi: 10.1111/j.1538-7836.2007.02462.x, indexed in Pubmed: 17371489.
29. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2020; 41: 255–323, doi: 10.1093/eurheartj/ehz486, indexed in Pubmed: 31497854.
30. Neergaard-Petersen S, Hvas AM, Kristensen SD, et al. The influence of type 2 diabetes on fibrin clot properties in patients with coronary artery disease. Thromb Haemost. 2014; 112(6): 1142–1150, doi: 10.1160/TH14-05-0468, indexed in Pubmed: 25187394.
31. Konieczynska M, Fil K, Bazanek M, et al. Prolonged duration of type 2 diabetes is associated with increased thrombin generation, prothrombotic fibrin clot phenotype and impaired fibrinolysis. Thromb Haemost. 2014; 111(4): 685–693, doi: 10.1160/TH13-07-0566, indexed in Pubmed: 24306139.
32. Dunn EJ, Ariëns RAS, Grant PJ. The influence of type 2 diabetes on fibrin structure and function. Diabetologia. 2005; 48(6): 1198–1206, doi: 10.1007/s00125-005-1742-2, indexed in Pubmed: 15864538.
33. Bryk AH, Konieczyńska M, Polak M, et al. Plasma fibrin clot properties and cardiovascular mortality in patients with type 2 diabetes: a long-term follow-up study. Cardiovasc Diabetol. 2021; 20(1): 47, doi: 10.1186/s12933-021-01230-9, indexed in Pubmed: 33602240.
34. Gajos G, Konieczynska M, Zalewski J, et al. Low fasting glucose is associated with enhanced thrombin generation and unfavorable fibrin clot properties in type 2 diabetic patients with high cardiovascular risk. Cardiovasc Diabetol. 2015; 14: 44, doi: 10.1186/s12933-015-0207-2, indexed in Pubmed: 25928628.
35. Shlomai G, Haran-Appel T, Sella T, et al. High-risk type-2 diabetes mellitus patients, without prior ischemic events, have normal blood platelet functionality profiles: a cross-sectional study. Cardiovasc Diabetol. 2015; 14: 80, doi: 10.1186/s12933-015-0244-x, indexed in Pubmed: 26068309.
36. Lemkes BA, Bähler L, Kamphuisen PW, et al. The influence of aspirin dose and glycemic control on platelet inhibition in patients with type 2 diabetes mellitus. J Thromb Haemost. 2012; 10(4): 639–646, doi: 10.1111/j.1538-7836.2012.04632.x, indexed in Pubmed: 22252020.
37. Neergaard-Petersen S, Hvas AM, Kristensen SD, et al. The influence of type 2 diabetes on fibrin clot properties in patients with coronary artery disease. Thromb Haemost. 2014; 112(6): 1142–1150, doi: 10.1160/TH14-05-0468, indexed in Pubmed: 25187394.
38. Undas A, Wiek I, Stępień E, et al. Hyperglycemia is associated with enhanced thrombin formation, platelet activation, and fibrin clot resistance to lysis in patients with acute coronary syndrome. Diabetes Care. 2008; 31(8): 1590–1595, doi: 10.2337/dc08-0282, indexed in Pubmed: 18487475.
39. Yngen M, Norhammar A, Hjemdahl P, et al. Effects of improved metabolic control on platelet reactivity in patients with type 2 diabetes mellitus following coronary angioplasty. Diab Vasc Dis Res. 2006; 3(1): 52–56, doi: 10.3132/dvdr.2006.008, indexed in Pubmed: 16784182.
40. Roshan B, Tofler GH, Weinrauch LA, et al. Improved glycemic control and platelet function abnormalities in diabetic patients with microvascular disease. Metabolism. 2000; 49(1): 88–91, doi: 10.1016/s0026-0495(00)90813-8, indexed in Pubmed: 10647069.
41. Ferroni P, Basili S, Falco A, et al. Platelet activation in type 2 diabetes mellitus. J Thromb Haemost. 2004; 2(8): 1282–1291, doi: 10.1111/j.1538-7836.2004.00836.x, indexed in Pubmed: 15304032.