

Impaired Fibrinolysis Predicts Adverse Outcome in Acute Coronary Syndrome Patients with Diabetes: A PLATO Sub-Study

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Thromb Haemost 2020;120:412–422.

Abstract

Hypofibrinolysis is a key abnormality in diabetes but the role of impaired clot lysis in predicting vascular events and mortality in this population is yet to be determined. We aimed to investigate the relationship between fibrin clot properties and clinical outcomes in patients with diabetes and recent acute coronary syndrome (ACS). Plasma samples were collected at hospital discharge from 974 ACS patients with diabetes randomised to clopidogrel or ticagrelor in the PLATO trial. A validated turbidimetric assay was employed to study fibrin clot lysis and maximum turbidity. One-year rates of cardiovascular (CV) death, spontaneous myocardial infarction (MI) and PLATO-defined major bleeding events were assessed after sample collection. Hazard ratios (HRs) were determined using Cox proportional analysis. After adjusting for CV risk factors, each 50% increase in lysis time was associated with increased risk of CV death/MI (HR 1.21; 95% confidence interval [CI] 1.02–1.44; $p = 0.026$) and CV death alone (HR 1.38; 1.08–1.76; $p = 0.01$). Similarly, each 50% increase in maximum turbidity was associated with increased risk of CV death/MI (HR 1.25; 1.02–1.53; $p = 0.031$) and CV death alone (HR 1.49; 1.08–2.04; $p = 0.014$). The relationship between lysis time and the combined outcome of CV death and MI remained significant after adjusting for multiple prognostic vascular biomarkers ($p = 0.034$). Neither lysis time nor maximum turbidity was associated with major bleeding events. Impaired fibrin clot lysis predicts 1-year CV death and MI in diabetes patients following ACS.

Keywords

- ▶ acute coronary syndrome
- ▶ diabetes
- ▶ fibrinolysis

Clinical Trial Registration URL: <http://www.clinicaltrials.gov>. Unique identifier NCT00391872.

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received
August 28, 2019
accepted after revision
December 6, 2019

DOI <https://doi.org/10.1055/s-0039-1701011>.
ISSN 0340-6245.

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Introduction

Up to 30% of patients presenting with acute coronary syndrome (ACS) suffer from diabetes mellitus.^{1,2} These individuals have worse vascular outcomes despite contemporary therapies.^{1,3,4} A 'pro-thrombotic' state, characterised by adverse fibrin clot properties and increased platelet reactivity, has been repeatedly described in patients with diabetes.⁵⁻⁷ Despite the altered thrombotic milieu in this condition, long-term preventative anti-thrombotic treatment post-ACS remains similar compared with individuals without diabetes.^{8,9} Offering more intensive therapies may be one approach to improve outcomes, but this can be challenging in diabetes given the heterogeneity of this condition, which is characterised by variable risks of thrombosis and bleeding.¹⁰

Potent P2Y₁₂ inhibitors (ticagrelor/prasugrel) post-ACS improved outcomes in patients with diabetes without an apparent penalty of increased major bleeding compared with clopidogrel.^{4,11} Targeting the protein arm of coagulation with low-dose anti-factor Xa therapy, in addition to clopidogrel-based dual anti-platelet therapy, was shown to reduce cardiovascular (CV) events and mortality in ACS patients, regardless of diabetes status.¹² However, the observed increase in bleeding and the guideline-recommended use of dual anti-platelet therapy with ticagrelor or prasugrel, rather than clopidogrel, has limited widespread adoption of this approach.

Clinical characteristics, such as the extent of coronary artery disease, history of recurrent events or renal impairment, and elevated CV biomarkers could help guide intensity of treatment,¹³ but functional biomarkers that address thrombosis risk are lacking. Identification of such biomarkers could potentially make it possible to implement tailored anti-thrombotic therapy in this population, helping to maximise benefits and minimise risks.

Cross-sectional studies have repeatedly shown a relationship between coronary artery disease and dense fibrin networks that are resistant to lysis.¹⁴⁻¹⁶ These associations were documented in individuals with and without diabetes, although the latter group was generally found to have a more thrombotic clot phenotype.¹⁷⁻¹⁹ We have recently demonstrated that impaired fibrin clot lysis independently predicts CV death following ACS,²⁰ indicating that the fibrin network has clinical prognostic significance. Diabetes was also associated with impaired fibrin clot lysis but the magnitude of the association between prolonged fibrin clot lysis and adverse outcomes in diabetes patients was not assessed.²⁰ In this sub-analysis, we aimed to assess the association between fibrin network properties and adverse clinical outcome in ACS patients with diabetes.

Methods

Study Population and Patient Samples

The PLATElet inhibition and patient Outcomes (PLATO) trial was an international multi-centre, double-blind, randomised controlled trial of ticagrelor compared with clopidogrel in 18,624 moderate-to-high-risk ACS patients.^{21,22} Study design and results have been previously reported.^{21,22} Briefly, patients admitted with ACS were recruited within 24 hours

of symptom onset and randomised to either clopidogrel or ticagrelor. Patients were followed up at 1 to 3, 6 to 9 and 12 months. The PLATO fibrin sub-study included 4,354 patients who donated blood at hospital discharge.²⁰ This is a sub-group analysis involving all 974 patients with diabetes. Citrated plasma was derived and stored at -80°C at Uppsala Clinical Research Centre (Uppsala, Sweden) prior to transfer to the University of Sheffield (Sheffield, United Kingdom) for fibrin clot analysis.

Fibrin Clot Assessment

This was performed using a turbidimetric assay as previously described.²⁰ Briefly, plasma mixed with tissue plasminogen activator (tPA) (83 ng/mL) was re-calcified (CaCl_2 7.5 mM) and clotting was initiated with thrombin (0.03 U/mL). Fibrin clot maximum turbidity (a measure of fibrin clot density) and lysis time were determined using a Multiskan FC (Thermo scientific) plate reader in all 974 plasma samples taken at hospital discharge and 820 plasma samples taken at 1 month. All laboratory analysis was performed blinded to clinical outcomes, treatment allocation and other biomarker levels.

Statistical Methods

Biomarker levels were natural log transformed before analysis. Continuous data are presented as medians and interquartile ranges and compared using Kruskal-Wallis tests, Wilcoxon tests or multivariable linear regression models, as appropriate. Categorical data are presented as numbers and percentages and compared using chi-square tests. The primary outcome of interest was the composite of CV death and spontaneous myocardial infarction (MI). Secondary outcomes were CV death alone, MI alone, PLATO-defined major bleeding and all-cause mortality. Cox-proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). HRs are expressed per 50% fibrin variable level increase. We adjusted for clinical risk factors (model 1) and for known prognostic biomarkers. Model 1 included all clinical risk factors, including randomised treatment, age, gender, body mass index (BMI), smoking history, hypertension, dyslipidaemia, chronic kidney disease (CKD), ST-elevation ACS and previous MI, congestive heart failure, revascularisation, ischaemic stroke or peripheral artery disease. Prognostic biomarkers were added sequentially to adjustment model 1. Model 2 included adjustment for model 1, white cell count and C-reactive protein (CRP). Model 3 included adjustment for model 1 clinical risk except for CKD, white cell count, CRP and cystatin C. Model 4 included adjustment for model 3 risk factors, troponin T and N-terminal pro B-type natriuretic peptide (NT-proBNP). Model 5 included all model 4 risk factors and growth differentiation factor-15. Restricted cubic splines were used to visually assess the relationship between fibrin clot properties and clinical outcomes. Interactions between prognostic value of fibrin clot parameters and each of randomised treatment, treatment strategy and treatment with low-molecular-weight heparin (LMWH) (within 2 days of sampling) were assessed using restricted cubic splines. Mean \pm standard deviation fibrin clot properties at hospital discharge and 1 month were compared using Wilcoxon signed-rank test. p -Values < 0.05 from

two-tailed tests were considered statistically significant. *p*-Values were not adjusted for multiple testing. All statistical analyses were performed at Uppsala Clinical Research Centre using R statistics software (Version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Diabetes, Associated Conditions and Biomarkers

► **Table 1** outlines the difference between patients with and without diabetes. Diabetes showed associations with CV risk factors, including increased age, increased BMI, hypertension, hyperlipidaemia, CKD and peripheral artery disease. However, smoking was less prevalent in diabetes patients. Higher proportions presented with non-ST-elevation ACS, were females and had previous MI, stroke, congestive heart failure or revascularisation compared with patients without diabetes.

After adjustment for risk factors and clinical characteristics (model 1), fibrin clot lysis time and maximum turbidity were significantly higher in patients with diabetes compared with those without. The majority of other prognostic and inflammatory biomarkers were significantly higher in patients with diabetes (► **Table 2**).

Table 1 Clinical characteristics in diabetes and non-diabetes patients

Variable	Diabetes <i>n</i> = 974	No diabetes <i>n</i> = 3,380	<i>p</i> -Value
Demographics and risk factors			
Age (y)	64 (56–72)	61 (53–70)	< 0.001
Females	342 (35.1%)	931 (27.5%)	< 0.001
Body mass index (kg/m ²)	29 (26–33)	27 (25–30)	< 0.001
Current smoker	232 (23.8%)	1363 (40.3%)	< 0.001
Hypertension	806 (82.8%)	2059 (60.9%)	< 0.001
Hyperlipidaemia	533(54.7%)	1307(38.7%)	< 0.001
Previous MI	261 (26.8%)	585 (17.3%)	< 0.001
Congestive heart failure	86 (8.8%)	163 (4.8%)	< 0.001
Previous PCI	153 (15.7%)	375 (11.1%)	< 0.001
Previous CABG	78 (8.0%)	143 (4.2%)	< 0.001
Previous stroke	48 (4.9%)	103 (3.0%)	0.005
Peripheral artery disease	82 (8.4%)	191 (5.7%)	0.002
CKD	62 (6.4%)	85 (2.5%)	< 0.001
Randomised treatment			
Ticagrelor	490 (50.3%)	1687 (49.9%)	0.83
Presentation			
STE-ACS	354 (36.3%)	1668 (49.3%)	< 0.001

Abbreviations: CABG, coronary artery bypass graft; CKD, chronic kidney disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; STE-ACS, ST-elevation acute coronary syndrome. Note: Values are medians (interquartile ranges [IQRs]) for continuous data and *n* (%) for categorical data. *p*-Values were calculated using Wilcoxon test for age and chi-square test for categorical variable.

Table 2 Biomarker levels in diabetes and non-diabetes patients

Biomarker	Diabetes <i>n</i> = 974	No diabetes <i>n</i> = 3,380	Adjusted <i>p</i> -value
Lysis time (s)	732 (594–1,002)	684 (558–864)	< 0.001
Maximum turbidity (AU)	0.51 (0.39–0.64)	0.49 (0.38–0.62)	0.004
Troponin T (ng/L)	521 (105–1,535.5)	716 (123.5–2,264)	0.2
NT-proBNP (pmol/L)	621.5 (247–1,578)	562.5 (236–1,253)	< 0.001
Cystatin C (mg/L)	1.0 (0.8–1.2)	0.9 (0.8–1.1)	0.14
GDF-15	1,931 (1422–2,935)	1,469 (1,121–2,062)	< 0.001
CRP (mg/L)	15 (5.9–35)	14 (5.3–32)	< 0.001
IL-6	5.8 (3.3–11)	5.5 (2.9–10)	0.003
WCC	9.2 (7.6–11.5)	9.4 (7.4–11.7)	< 0.001
HbA1c	7.5 (6.6–8.9)	5.9 (5.6–6.2)	< 0.001

Abbreviations: AU, arbitrary unit; CRP, C-reactive protein; GDF-15, growth differentiation factor 15; HbA1c, glycated haemoglobin; IL, interleukin; NT-proBNP, N-terminal pro B-type natriuretic peptide; WCC, white cell count.

Note: *p*-Values were calculated using multivariable linear regression analysis with adjustment to all clinical characteristics (model 1). All biomarkers were measured at hospital discharge except for WCC and HbA1c which were measured at baseline.

Fibrin Clot Properties, Clinical Characteristics and Biomarker Levels

The correlation between fibrin clot maximum turbidity and lysis time was weak ($r = 0.37$, $p < 0.001$).

► **Tables 3** and **4** summarise clinical characteristics and biomarker levels across the four quartile groups of fibrin clot properties. BMI and the proportion of females increased with increasing quartile groups of lysis time. Similarly, the prevalence of CKD was highest in the highest lysis time quartile group. Levels of prognostic and inflammatory biomarkers significantly increased with increasing quartile groups of both lysis time and maximum turbidity.

Platelet count and low-density lipoprotein (LDL) cholesterol levels increased with increasing fibrin clot lysis time but showed no relationship to maximum turbidity. There was no significant difference in the proportion of patients receiving ticagrelor across the four lysis time quartile groups.

The correlation between lysis time and the inflammatory biomarker CRP was weak ($r = 0.29$, $p < 0.001$). In contrast, the relationship between fibrin clot maximum turbidity and CRP appeared more linear ($r = 0.63$, $p < 0.001$). Both lysis time ($r = 0.09$, $p = 0.005$) and maximum turbidity ($r = 0.41$, $p < 0.001$) were significantly, though weakly, correlated with troponin T.

Glycated haemoglobin (HbA1c) also significantly increased with increasing lysis time (► **Table 2**) but no association with maximum turbidity was found (► **Table 4**). Insulin treatment did not show associations with fibrin clot properties.

Table 3 Clinical characteristics and biomarkers across quartile groups of lysis time in patients with diabetes

Variables	Lysis time (s) quartile group				p-Value
	Q1 (< 594) n = 251	Q2 (594–732) n = 239	Q3 (732–1,002) n = 243	Q4 (> 1,002) n = 241	
Demographics and medical history					
Age (y)	65 (58–72)	65 (57–72)	64 (56–72)	64 (55–71)	0.25
Female	67 (26.7%)	73 (30.5%)	84 (34.6%)	118 (49.0%)	< 0.001
BMI (kg/m ²)	27.8 (25.5–31.8)	28.7 (26.4–32.5)	29.4 (26.4–32.4)	29.8 (26.3–33.9)	0.004
Hypertension	188 (74.9%)	208 (87.0%)	206 (84.8%)	204 (84.6%)	0.002
Previous MI	68 (27.1%)	61 (25.5%)	71 (29.2%)	61 (25.3%)	0.75
Previous stroke	7 (2.8%)	13 (5.4%)	13 (5.3%)	15 (6.2%)	0.31
PAD	14 (5.6%)	23 (9.6%)	23 (9.5%)	22 (9.1%)	0.31
CKD	8 (3.2%)	15 (6.3%)	15 (6.2%)	24 (10.0%)	0.02
Treatment strategy					
Invasive	159 (63.3%)	146 (61.1%)	164 (67.5%)	157 (65.1%)	0.51
Inpatient PCI	152 (60.6%)	140 (58.6%)	154 (63.4%)	146 (60.6%)	0.76
Inpatient CABG	8 (3.2%)	6 (2.5%)	10 (4.1%)	12 (5%)	0.5
Ticagrelor	118 (47%)	114 (47.7%)	130 (53.5%)	128 (53.1%)	0.32
Supine systolic BP ^a	140 (120–150)	140 (120–150)	140 (120–150)	140 (121–152)	0.64
Biomarkers					
Troponin T (ng/L)	326 (86–1,186)	371 (73–1,415)	604 (139–1,602)	703 (149–1,868)	0.01
NT-proBNP (pmol/L)	486 (238–1,052)	602 (222–1,358)	760 (307–2,118)	716 (279–2,197)	0.001
Cystatin C (mg/L)	0.92 (0.77–1.15)	0.94 (0.76–1.16)	0.94 (0.76–1.26)	1.04 (0.86–1.38)	< 0.001
GDF-15 (ng/L)	1,842 (1,435–2,651)	1,864 (1,414–2,741)	1,933 (1,392–2,891)	2,139 (1,479–3,689)	0.01
CRP (mg/L)	9 (4–21)	14 (5–27)	17 (8–52)	24 (11–58)	< 0.001
WCC ($\times 10^9/L$)	8.5 (7.1–10.6)	9.4 (7.8–11.3)	9.3 (8.0–11.8)	9.9 (7.8–12.1)	< 0.001
Platelets ($\times 10^9/L$)	218 (191–254)	235 (197–274)	235 (194–284)	251 (203–304)	< 0.001
HbA1c (%)	7.3 (6.5–8.6)	7.4 (6.4–9)	7.5 (6.6–9)	7.8 (6.8–9.1)	0.02
Glucose (mmol/L)	9.3 (7.2–12.4)	9.7 (7.3–13.8)	9.3 (7.5–13)	10.4 (7.8–13.2)	0.13
LDL (mmol/L)	2.7 (2.1–3.4)	2.8 (2.2–3.5)	3.0 (2.2–4.0)	3.0 (2.4–4.0)	0.008
HDL (mmol/L)	1.2 (1.0–1.4)	1.1 (0.9–1.4)	1.2 (1.0–1.4)	1.2 (1.0–1.3)	0.31
Pre-admission insulin treatment	56 (22.3%)	54 (22.6%)	55 (22.6%)	60 (24.9%)	0.9
Insulin treatment during admission	122 (48.6%)	115 (48.1%)	124 (51%)	139 (57.7%)	0.14

Abbreviations: BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass graft; CKD, chronic kidney disease; CRP, C-reactive protein; GDF-15, growth differentiation factor-15; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; NT-proBNP, N-terminal pro B-type natriuretic peptide; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; WCC, white cell count.

Note: Values are medians (interquartile ranges [IQRs]) for continuous data and *n* (%) for categorical data. *p*-Values calculated using chialculat test for categorical variables and Kruskal–Wallis test for continuous variables. Biomarkers measures at hospital discharge except for WCC, platelets, HbA1c, glucose and cholesterol levels which were measured at baseline.

^aBlood pressure measured at baseline.

Fibrin Clot Properties and Clinical Outcomes

During follow-up, 48 patients (4.9%) had CV death, 72 (7.4%) had MI, 67 (2.9%) had major bleeding and 21 (2.2%) had non-coronary artery bypass graft-related major bleeding.

The probability of the combined outcome of CV death and MI was higher with increasing values of lysis time (►Fig. 1A).

This was driven primarily by increased risk of CV death with increasing lysis time (►Fig. 1B). There was no clear relationship between lysis time and major bleeding events.

Similarly, the probability of the combined outcome of CV death and MI was higher with increasing maximum turbidity (►Fig. 2A). The highest quartile group, in particular, appeared

Table 4 Clinical characteristics and biomarkers across quartile groups of maximum turbidity in patients with diabetes

Variables	Maximum turbidity (AU) quartile group				p-Value
	Q1 (< 0.39) n = 244	Q2 (0.39–0.51) n = 243	Q3 (0.51–0.64) n = 244	Q4 (> 0.64) n = 243	
Demographics and medical history					
Age (y)	64 (56–72)	65 (57–72)	63 (56–71)	64 (57–71)	0.66
Female	88 (36.1%)	95 (39.1%)	86 (35.2%)	73 (30.0%)	0.21
BMI (kg/m ²)	28.4 (25.6–32.7)	29.1 (26.3–32.7)	29.1 (26.3–33.2)	29.1 (26.0–32.3)	0.71
Hypertension	203 (83.2%)	207 (85.2%)	195 (79.9%)	201 (82.7%)	0.49
Previous MI	74 (30.3%)	76 (31.3%)	62 (25.4%)	49 (20.2%)	0.02
Previous stroke	8 (3.3%)	14 (5.8%)	12 (4.9%)	14 (5.8%)	0.54
PAD	20 (8.2%)	24 (9.9%)	14 (5.7%)	24 (9.9%)	0.30
CKD	12 (4.9%)	14 (5.8%)	15 (6.1%)	21 (8.6%)	0.37
Treatment strategy					
Invasive	120 (49.2%)	156 (64.2%)	161 (66%)	189 (78%)	< 0.001
Inpatient PCI	114 (46.7%)	152 (62.6%)	153 (62.7%)	173 (71.2%)	< 0.001
Inpatient CABG	6 (2.5%)	4 (1.6%)	8 (3.3%)	18 (7.4%)	0.004
Ticagrelor	119 (48.8%)	106 (43.6%)	137 (56.1%)	128 (52.7%)	0.04
Supine systolic BP	140 (120–150)	140 (123–150)	140 (120–153)	140 (125–150)	0.95
Biomarkers					
Troponin T (ng/L)	148 (37–579)	337 (74–1,133)	645 (193–1,708)	1,223 (525–2,781)	< 0.001
NT-proBNP (pmol/L)	345 (171–797)	535 (217–1,130)	590 (299–1,565)	1,409 (552–2,934)	< 0.001
Cystatin C (mg/L)	0.93 (0.77–1.15)	0.96 (0.78–1.21)	0.94 (0.77–1.19)	1.0 (0.83–1.37)	0.02
GDF-15 (ng/L)	1,765 (1,323–2,527)	1,926 (1,441–2,766)	1,917 (1,412–2,892)	2,268 (1,576–3,759)	<.0001
CRP (mg/L)	5 (3–13)	10 (5–18)	18 (10–32)	50 (26–100)	< 0.001
WBC ($\times 10^9/L$)	8.1 (6.9–9.9)	8.8 (7.4–11.1)	9.4 (7.8–11.1)	10.7 (9–13.5)	< 0.001
Platelets ($\times 10^9/L$)	228 (201–263)	227 (192–273)	233 (198–276)	240 (194–285)	0.12
HbA1c (%)	7.3 (6.4–8.7)	7.6 (6.7–9.4)	7.6 (6.6–9)	7.5 (6.7–8.7)	0.27
Glucose (mmol/L)	9.0 (6.7–12.1)	9.5 (7.3–12.4)	9.7 (7.4–13)	10.5 (8–13.8)	0.004
LDL (mmol/L)	2.9 (2.1–3.5)	2.8 (2.2–3.6)	2.9 (2.3–3.9)	3.0 (2.3–3.7)	0.38
HDL (mmol/L)	1.1 (1–1.4)	1.2 (1–1.4)	1.2 (1–1.4)	1.2 (1–1.4)	0.41
Pre-admission insulin treatment	53 (21.7%)	57 (23.5%)	60 (24.6%)	55 (22.6%)	0.9
Insulin treatment during admission	114 (46.7%)	119 (49%)	129 (52.9%)	138 (56.8%)	0.12

Abbreviations: AU, arbitrary unit; BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass graft; CKD, chronic kidney disease; CRP, C-reactive protein; GDF-15, growth differentiation factor-15; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro B-type natriuretic peptide; MI, myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; WCC, white cell count.

Note: Values are medians (interquartile ranges [IQRs]) for continuous data and *n* (%) for categorical data. *p*-Values calculated using calculated test for categorical variables and Kruskal–Wallis test for continuous variables.

to be associated with greatest risk of CV death (**Fig. 2B**). In contrast, the probability of major bleeding seemed to increase with decreasing maximum turbidity.

After adjustment for clinical characteristics and CV disease risk factors (model 1), each 50% increase in lysis time was associated with increased risk of CV death/MI (HR 1.21; 95% CI 1.02–1.44) and CV death alone (HR 1.38; 95% CI 1.08–1.76). Similarly, every 50% increase in lysis time

was associated with increased risk of all-cause mortality after adjustment for clinical risk factors in model 1 (HR 1.47; 95% CI 1.16–1.85) and this remained significant after adjustment for inflammatory biomarkers (**Fig. 3**). After adjustment for prognostic biomarkers as well as clinical risk factors (model 5), the relationship between lysis time and the combined outcome of CV death and MI remained significant (*p* = 0.034).

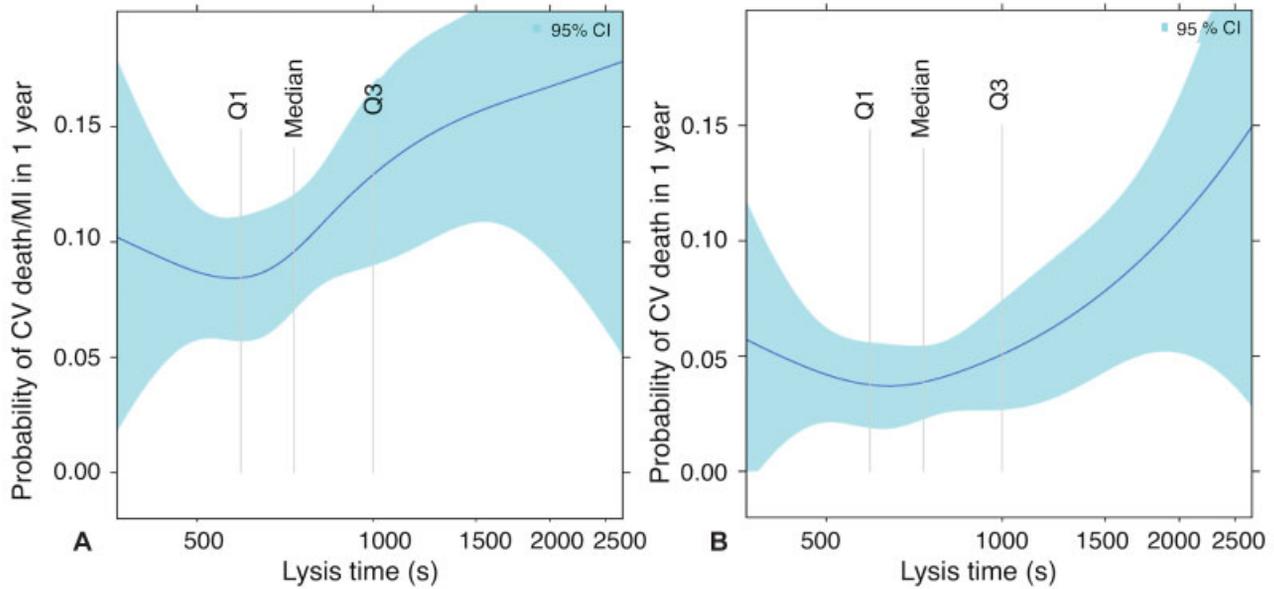


Fig. 1 Relationship between fibrin clot lysis time and 1-year clinical outcomes in patients with diabetes mellitus, 1-year rates of cardiovascular (CV) death and spontaneous myocardial infarction (MI) (A) and CV death alone (B) in relation to lysis time transformed using restricted cubic splines. Shaded areas represent 95% confidence intervals. Vertical lines indicate quartiles.

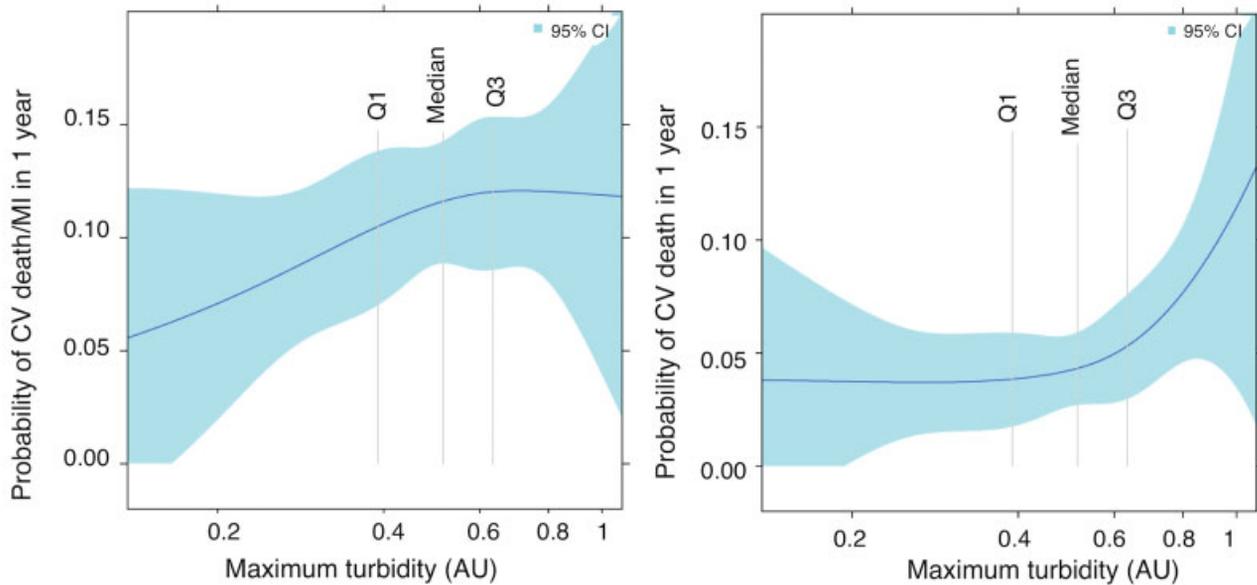


Fig. 2 Relationship between fibrin clot maximum turbidity and 1-year clinical outcomes in patients with diabetes mellitus, 1-year rates of cardiovascular (CV) death and spontaneous myocardial infarction (MI) (A) and CV death alone (B) in relation to maximum turbidity (AU, arbitrary units) transformed using restricted cubic splines. Shaded areas represent 95% confidence intervals. Vertical lines indicate quartiles.

Similarly, each 50% increase in maximum turbidity was associated with increased risk of CV death/MI (HR 1.25; 95% CI 1.02–1.53) and CV death alone (HR 1.49; 95% CI 1.08–2.04). These relationships lost significance after adjustment for prognostic biomarkers. There was a numerical increase in bleeding in the lowest quartile group of maximum turbidity but this failed to reach statistical significance ($p = 0.15$).

The prognostic value of fibrin clot lysis time was consistent regardless of randomised treatment, presentation,

administration of LMWH within 2 days or invasive treatment (all interaction $p > 0.1$) (► Fig. 4).

Similarly, the prognostic value of fibrin clot lysis time was consistent regardless of diabetes status but the magnitude of relationship appears to be higher in patients with diabetes (► Fig. 5).

Fibrin Clot Properties over Time

Fibrin clot lysis time remained prolonged at 1 month (864 ± 406 seconds) compared with hospital discharge

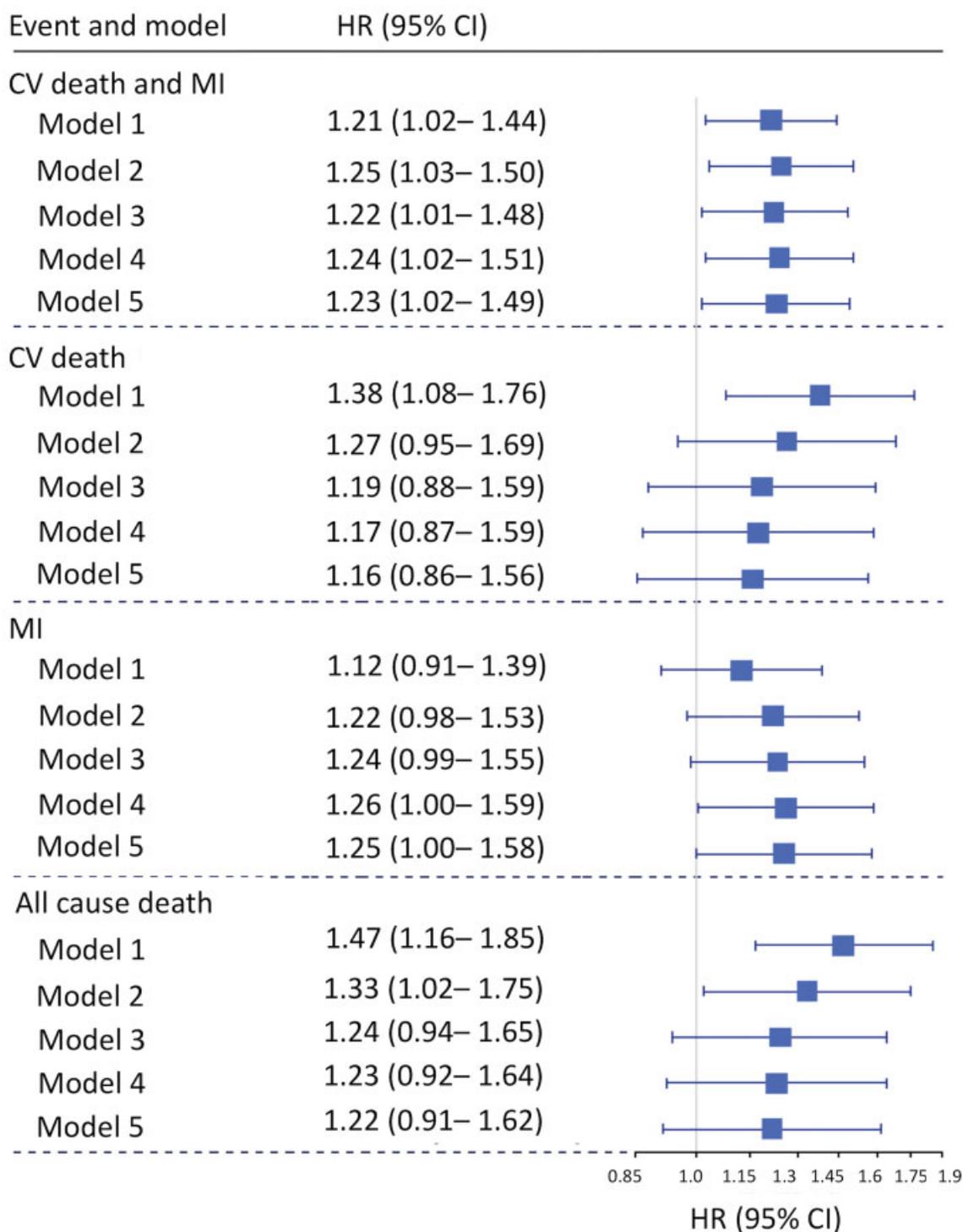


Fig. 3 Forest plot for the associations between fibrin clot lysis time and clinical outcomes following acute coronary syndrome (ACS) in patients with diabetes. Squares represent hazard ratio (HR) estimates. Horizontal lines represent 95% confidence intervals. Number of patients, 971 for model 1 and 853 for subsequent models. Model 1: Clinical characteristics including randomised treatment, age, gender, body mass index (BMI), smoking history, hypertension, dyslipidaemia, chronic kidney disease (CKD), ST-elevation ACS and previous MI, congestive heart failure, revascularisation, ischaemic stroke or peripheral artery disease; Model 2: Clinical characteristics as per model 1 + C-reactive protein (CRP) + white cell count (WCC); Model 3: All characteristics and biomarkers as per model 2 (except CKD) + cystatin C; Model 4: All characteristics and biomarkers as per model 3 + troponin + N-terminal pro B-type natriuretic peptide (NT-proBNP); Model 5: All characteristics and biomarkers as per model 4 + growth differentiation factor 15 (GDF-15).

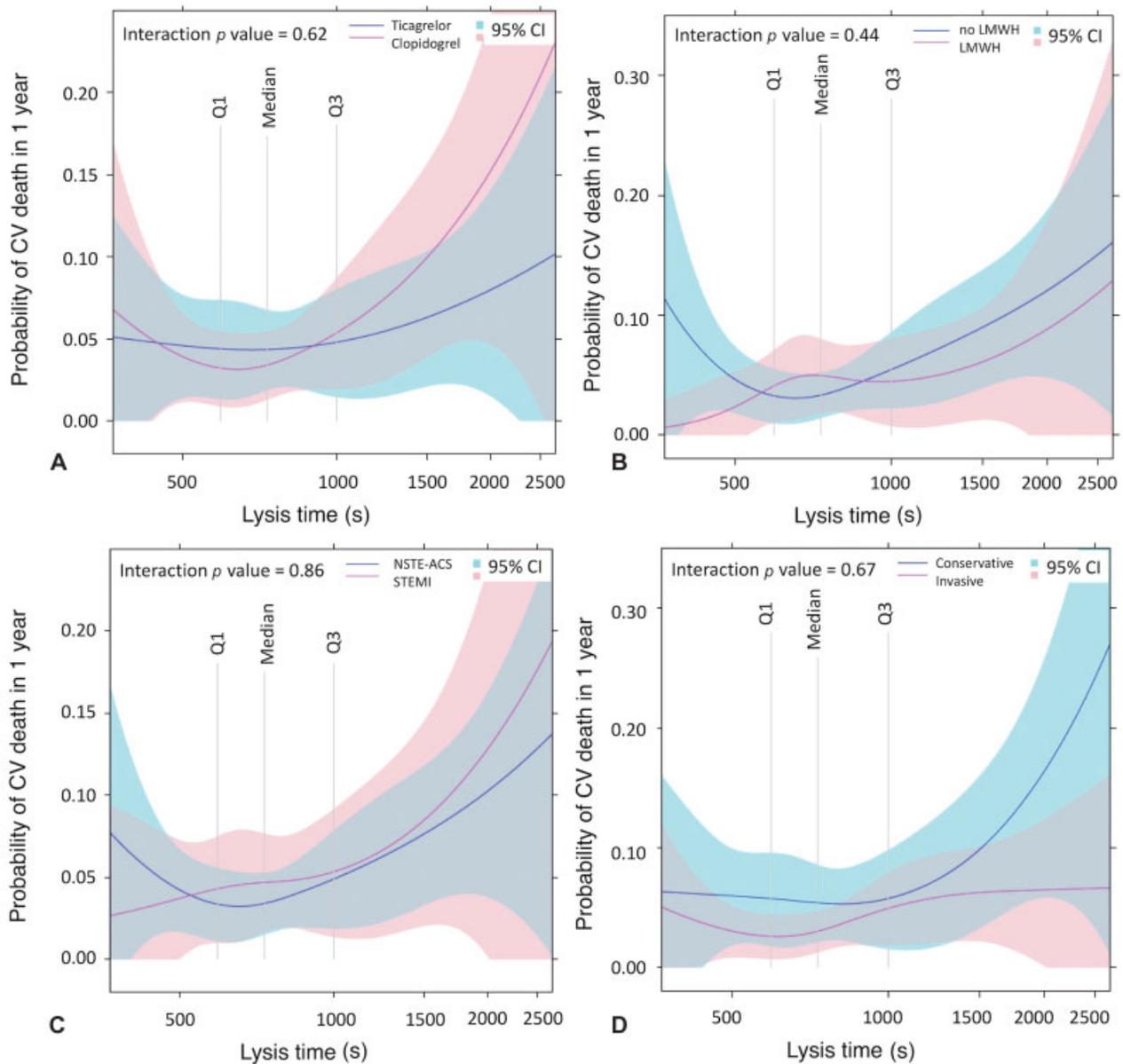


Fig. 4 Relationship between fibrin clot lysis time and cardiovascular death according to randomised treatment (A), low-molecular-weight heparin treatment (B), presentation (C) and treatment strategy (D). One-year rates of cardiovascular death in relation to lysis time, transformed using restricted cubic splines. Shaded areas represent 95% confidence intervals, vertical lines indicate quartiles. LMWH, low-molecular-weight heparin; NSTEMI-ACS, non-ST-elevation acute coronary syndrome; STEMI, ST-elevation myocardial infarction.

(863 ± 445 seconds) ($p = 0.64$) despite a drop in overall maximum turbidity (0.44 ± 0.14 vs. 0.53 ± 0.2 ; $p < 0.001$).

Discussion

For the first time, in a large cohort of ACS patients with diabetes, we have shown prolonged fibrin clot lysis to predict CV death and MI after adjustment for clinical risk predictors as well as traditional and novel prognostic biomarkers. We have also shown fibrin clot lysis time to remain prolonged at 1 month following ACS, despite a drop in fibrin clot turbidity. Being a functional assay, our methodology takes into account quantitative and qualitative changes in various clotting factors, thus giving an overall assessment of fibrin-related

thrombosis risk. The independent association between impaired lysis and worse ischaemic outcomes may indicate that some patients may benefit from further optimisation of anti-thrombotic therapy, for example, by dropping dual anti-platelet therapy and replacing it with a combination of a single anti-platelet agent and an anticoagulant, since anticoagulant therapy potentiates fibrinolysis.^{23–25} However, the safety and efficacy of such an approach requires further research.

Despite the large number of patients included in our study, the study lacks sufficient power to detect an independent relationship between individual components of the primary endpoint and fibrin clot properties. However, the trend of association with both CV death and MI is consistent.

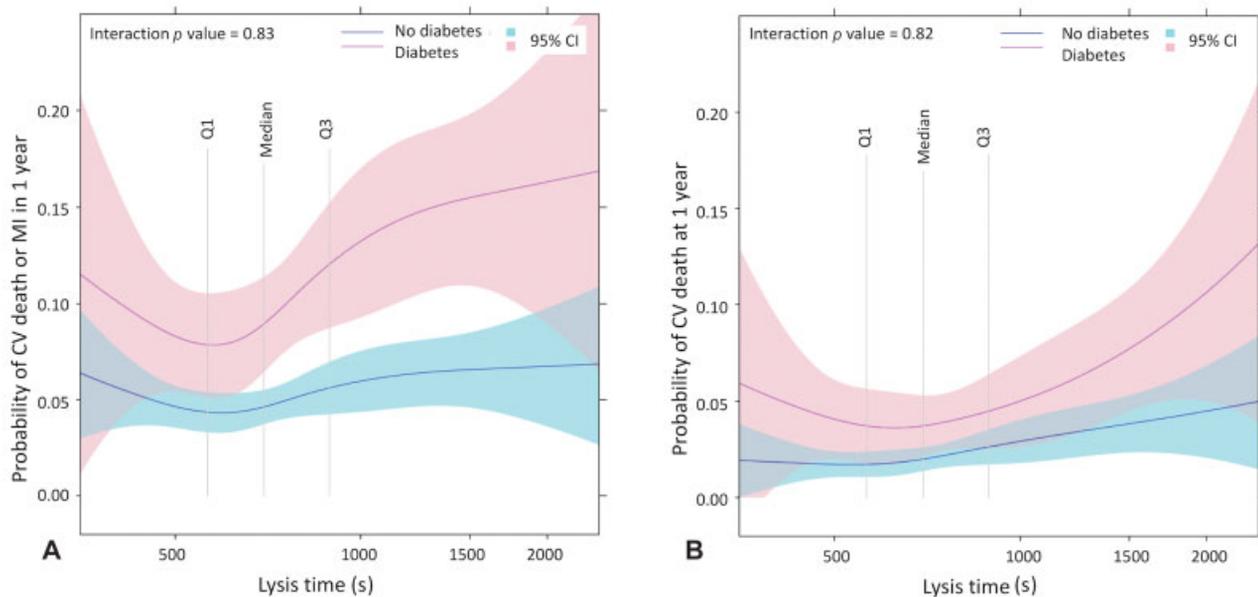


Fig. 5 Relationship between fibrin clot lysis time and clinical outcomes according to diabetes status. One-year rates of cardiovascular (CV) death or spontaneous myocardial infarction (MI) (A) and CV death alone (B) in relation to lysis time, transformed using restricted cubic splines, according to diabetes status.

Diabetes and prolonged lysis are both associated with many high-risk patient characteristics and prognostic biomarkers. We have adjusted for many potential confounders and although the association between prolonged fibrin clot lysis and CV death/spontaneous MI remained significant, including many confounders in adjustment models is a limitation. Reassuringly, the results are very similar with the many different models we tested for.

Another limitation of our methodology is that we studied fibrinolysis in plasma samples rather than whole blood. This may exclude the cellular effects on the fibrinolytic pathway. However, our results provide a 'proof-of-concept' of the importance of intrinsic fibrinolysis in predicting outcomes.

The very weak correlation between fibrin clot lysis potential and troponin levels indicates a possible pathophysiological link with worse outcomes that is independent of the size of MI. Fibrin clot maximum turbidity had a stronger relationship with CRP as well as troponin and NT-proBNP. This is to be expected as turbidity reflects fibrin clot density, which is more closely related to fibrinogen levels, and fibrinogen levels are well known to increase with inflammation, such as may be provoked by large MIs.²⁰

Although fibrin clot turbidity is only marginally higher in the diabetes population compared with individuals without diabetes, lysis time is significantly longer in the former group, consistent with previous studies.^{7,26} There are several diabetes-specific mechanisms that lead to impaired clot lysis, including glycation of fibrinogen, which results in more compact clots that resist fibrinolysis,²⁷ increased incorporation of anti-fibrinolytic proteins into the clot²⁸ and glycation of plasminogen, which inhibits plasmin generation and modulates enzyme activity, thus further impairing the fibrinolytic process.²⁹ These previous findings may explain our results demonstrating a relationship between HbA1c and clot lysis

time. Improving glycaemic control can modulate outcomes post-ACS and a reduction in fibrin clot lysis time may be one of the mechanisms involved.³⁰ Similarly, approaches to ameliorate inflammation may be successful at improving lysis potential. In studies involving human aortic endothelial cells, CRP resulted in increased plasminogen activator inhibitor-1 expression and reduction in tPA activity, which may explain the observed relationship between increased inflammation and pro-thrombotic changes in fibrin parameters.^{31,32} Although diabetes was associated with increased maximum turbidity, there was no clear association between this fibrin parameter and glycaemic control. This suggests that the relationship between glycaemic control and lysis time is related to alteration in the fibrinolytic proteins rather than changes in fibrin clot structure in the cohort studied. However, it should be noted that over-treatment of high glucose levels and precipitation of hypoglycaemia can also impair fibrin clot lysis,³³ potentially explaining the inconsistent relationship between improved glycaemic control and outcome observed in clinical studies.³⁴⁻³⁶

LDL cholesterol levels and platelet count increased with increasing lysis time. These observations are intriguing and support previously reported effects of cholesterol and increased platelet reactivity on fibrinolysis.³⁷ However, randomisation to ticagrelor was not associated with fibrinolysis potential and this suggests that platelet reactivity has limited impact on fibrin clot lysis in plasma. Statins and fenofibrates were previously shown to improve fibrinolysis.³⁸ We are unable to confirm this in our study as >90% of patients were receiving statins but the relationship between increased lysis and increased LDL levels suggests a role for cholesterol-lowering therapy in modulating fibrinolysis.

Increased incorporation of anti-fibrinolytic proteins into the fibrin clot represents another mechanism for impaired

fibrinolysis in diabetes.³⁹ Developing therapies against one or more of these proteins may represent a targeted approach that helps to improve prognosis in ACS patients with diabetes, while minimising bleeding risk.

The relationship between impaired fibrin clot lysis and female gender is consistent with previously observed results in other cohorts of high-risk vascular patients with type 2 diabetes.^{26,40} However, another study, in a younger cohort, found no difference in lysis potential between males and females.⁴¹ The latter study was much smaller than the other two, including a limited number of younger subjects who had type 1 rather than type 2 diabetes, and these differences in study populations are likely to explain the discrepancies. Prolonged lysis may be one mechanism for the reduction in CV protection in women with diabetes and further work is needed in this area to understand the exact pathways involved.

Conclusion

Adverse fibrin clots that resist lysis predict CV death and MI in ACS patients with diabetes despite contemporary therapies. The relationship between high-risk vascular conditions and impaired lysis provide potential mechanistic insights into recurrent events. The weak correlation between fibrin clot lysis potential and troponin indicates that the association with worse outcomes is relatively independent of the magnitude of MI. Developing strategies to improve lysis tendency may help improve prognosis in high-risk ACS patients and future research in this area is warranted.

What is known about this topic?

- Diabetes is associated with worse outcomes following ACS.
- The relationship between fibrin clot properties and clinical outcomes in diabetes patients presenting with ACS remains unknown.

What does this paper add?

- Prolonged fibrin clot lysis predicts cardiovascular death and spontaneous myocardial infarction in diabetes patients presenting with ACS.
- Further research is needed to establish the suitability of targeting reduced fibrinolysis to aid improve outcomes in diabetes patients with ACS.

Funding

The present analysis was funded by the British Heart Foundation (FS/15/82/31824 clinical research training fellowship for W.S.). The PLATO trial, including plasma sample storage, was supported by AstraZeneca.

Conflict of Interest

W.S.: Speaker fee from Bayer. L.W.: Institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline, Roche Diagnostics, Merck & Co; consulting fees from Abbott; holds two patents involving GDF-15 licensed to Roche Diagnostics. S.K.J.: Institutional research grant, honoraria and consultant/advisory board fee from AstraZeneca; institutional research grant and consultant/advisory board fee from Medtronic; institutional research grants and honoraria from The Medicines Company; consultant/advisory board fees from Janssen, Bayer. A.S.: Institutional research grants from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline, Roche Diagnostics; consultancy fees from Olink Proteomics. K.G.: Institutional research grants from AstraZeneca. A.H.: Employed by AstraZeneca. R.A.A.: Research/educational support and consultancy fees from Abbott Diabetes Care, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Merck Sharp & Dohme, NovoNordisk, Roche and Takeda. R.F.S.: Institutional research grants, consultancy fees, and honoraria from AstraZeneca; consultancy fees and honoraria from Bayer and Bristol Myers Squibb/Pfizer; consultancy fees from Amgen, GlyCardial Diagnostics, Haemonetics, Novartis, Thromboserin and Idorsia.

References

- 1 Jernberg T, Hasvold P, Henriksson M, Hjelm H, Thuresson M, Janzon M. Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective. *Eur Heart J* 2015;36(19):1163–1170
- 2 Sprafka JM, Burke GL, Folsom AR, McGovern PG, Hahn LP. Trends in prevalence of diabetes mellitus in patients with myocardial infarction and effect of diabetes on survival. *The Minnesota Heart Survey. Diabetes Care* 1991;14(07):537–543
- 3 McGuire DK, Emanuelsson H, Granger CB, et al. Influence of diabetes mellitus on clinical outcomes across the spectrum of acute coronary syndromes. Findings from the GUSTO-IIb study. *GUSTO IIb Investigators. Eur Heart J* 2000;21(21):1750–1758
- 4 James S, Angiolillo DJ, Cornel JH, et al; PLATO Study Group. Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATElet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J* 2010;31(24):3006–3016
- 5 Colwell JA, Halushka PV, Sarji K, Levine J, Sagel J, Nair RM. Altered platelet function in diabetes mellitus. *Diabetes* 1976;25(2 Suppl):826–831
- 6 Sagel J, Colwell JA, Crook L, Laimins M. Increased platelet aggregation in early diabetes mellitus. *Ann Intern Med* 1975;82(06):733–738
- 7 Alzahrani SH, Ajjan RA. Coagulation and fibrinolysis in diabetes. *Diab Vasc Dis Res* 2010;7(04):260–273
- 8 Ibanez B, James S, Agewall S, et al; ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018;39(02):119–177
- 9 Valgimigli M, Bueno H, Byrne RA, et al; ESC Scientific Document Group; ESC Committee for Practice Guidelines (CPG); ESC National Cardiac Societies. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with

- EACTS: the Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J* 2018;39(03):213–260
- 10 Subherwal S, Bach RG, Chen AY, et al. Baseline risk of major bleeding in non-ST-segment-elevation myocardial infarction: the CRUSADE (Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines) Bleeding Score. *Circulation* 2009;119(14):1873–1882
 - 11 Wiviott SD, Braunwald E, Angiolillo DJ, et al; TRITON-TIMI 38 Investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation* 2008;118(16):1626–1636
 - 12 Mega JL, Braunwald E, Wiviott SD, et al; ATLAS ACS 2–TIMI 51 Investigators. Rivaroxaban in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;366(01):9–19
 - 13 Wallentin L, Lindholm D, Siegbahn A, et al; PLATO study group. Biomarkers in relation to the effects of ticagrelor in comparison with clopidogrel in non-ST-elevation acute coronary syndrome patients managed with or without in-hospital revascularization: a substudy from the Prospective Randomized Platelet Inhibition and Patient Outcomes (PLATO) trial. *Circulation* 2014;129(03):293–303
 - 14 Collet JP, Allali Y, Lesty C, et al. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;26(11):2567–2573
 - 15 Fatah K, Silveira A, Tornvall P, Karpe F, Blombäck M, Hamsten A. Proneness to formation of tight and rigid fibrin gel structures in men with myocardial infarction at a young age. *Thromb Haemost* 1996;76(04):535–540
 - 16 Undas A, Plicner D, Stepień E, Drwiła R, Sadowski J. Altered fibrin clot structure in patients with advanced coronary artery disease: a role of C-reactive protein, lipoprotein(a) and homocysteine. *J Thromb Haemost* 2007;5(09):1988–1990
 - 17 Neergaard-Petersen S, Ajjan R, Hvas AM, et al. Fibrin clot structure and platelet aggregation in patients with aspirin treatment failure. *PLoS One* 2013;8(08):e71150
 - 18 Leander K, Blombäck M, Wallén H, He S. Impaired fibrinolytic capacity and increased fibrin formation associate with myocardial infarction. *Thromb Haemost* 2012;107(06):1092–1099
 - 19 Undas A, Zalewski J, Krochin M, et al. Altered plasma fibrin clot properties are associated with in-stent thrombosis. *Arterioscler Thromb Vasc Biol* 2010;30(02):276–282
 - 20 Sumaya W, Wallentin L, James SK, et al. Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: a PLATO substudy. *Eur Heart J* 2018;39(13):1078–1085
 - 21 Wallentin L, Becker RC, Budaj A, et al; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009;361(11):1045–1057
 - 22 James S, Akerblom A, Cannon CP, et al. Comparison of ticagrelor, the first reversible oral P2Y₁₂ receptor antagonist, with clopidogrel in patients with acute coronary syndromes: rationale, design, and baseline characteristics of the PLATElet inhibition and patient Outcomes (PLATO) trial. *Am Heart J* 2009;157(04):599–605
 - 23 Sumaya W, Parker WAE, Fretwell R, et al. Pharmacodynamic effects of a 6-hour regimen of enoxaparin in patients undergoing primary percutaneous coronary intervention (PENNY PCI Study). *Thromb Haemost* 2018;118(07):1250–1256
 - 24 Varin R, Mirshahi S, Mirshahi P, et al. Clot structure modification by fondaparinux and consequence on fibrinolysis: a new mechanism of antithrombotic activity. *Thromb Haemost* 2007;97(01):27–31
 - 25 Varin R, Mirshahi S, Mirshahi P, et al. Whole blood clots are more resistant to lysis than plasma clots—greater efficacy of rivaroxaban. *Thromb Res* 2013;131(03):e100–e109
 - 26 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(06):1142–1150
 - 27 Pieters M, van Zyl DG, Rheeder P, et al. Glycation of fibrinogen in uncontrolled diabetic patients and the effects of glycaemic control on fibrinogen glycation. *Thromb Res* 2007;120(03):439–446
 - 28 Hess K, Alzahrani SH, Mathai M, et al. A novel mechanism for hypofibrinolysis in diabetes: the role of complement C3. *Diabetologia* 2012;55(04):1103–1113
 - 29 Dunn EJ, Philippou H, Ariëns RA, Grant PJ. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. *Diabetologia* 2006;49(05):1071–1080
 - 30 Bouida W, Beltaief K, Msolli MA, et al; GREAT Network. One-year outcome of intensive insulin therapy combined to glucose-insulin-potassium in acute coronary syndrome: a randomized controlled study. *J Am Heart Assoc* 2017;6(11):6
 - 31 Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 2003;107(03):398–404
 - 32 Singh U, Devaraj S, Jialal I. C-reactive protein decreases tissue plasminogen activator activity in human aortic endothelial cells: evidence that C-reactive protein is a procoagulant. *Arterioscler Thromb Vasc Biol* 2005;25(10):2216–2221
 - 33 Chow E, Iqbal A, Walkinshaw E, et al. Prolonged prothrombotic effects of antecedent hypoglycemia in individuals with type 2 diabetes. *Diabetes Care* 2018;41(12):2625–2633
 - 34 Malmberg K, Rydén L, Efendic S, et al. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. *J Am Coll Cardiol* 1995;26(01):57–65
 - 35 Malmberg K, Rydén L, Wedel H, et al; DIGAMI 2 Investigators. Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity. *Eur Heart J* 2005;26(07):650–661
 - 36 Patel A, MacMahon S, Chalmers J, et al; ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358(24):2560–2572
 - 37 Panes O, Padilla O, Matus V, et al. Clot lysis time in platelet-rich plasma: method assessment, comparison with assays in platelet-free and platelet-poor plasmas, and response to tranexamic acid. *Platelets* 2012;23(01):36–44
 - 38 Undas A, Celinska-Löwenhoff M, Löwenhoff T, Szczeklik A. Statins, fenofibrate, and quinapril increase clot permeability and enhance fibrinolysis in patients with coronary artery disease. *J Thromb Haemost* 2006;4(05):1029–1036
 - 39 Kearney K, Tomlinson D, Smith K, Ajjan R. Hypofibrinolysis in diabetes: a therapeutic target for the reduction of cardiovascular risk. *Cardiovasc Diabetol* 2017;16(01):34
 - 40 Alzahrani SH, Hess K, Price JF, et al. Gender-specific alterations in fibrin structure function in type 2 diabetes: associations with cardiometabolic and vascular markers. *J Clin Endocrinol Metab* 2012;97(12):E2282–E2287
 - 41 Tehrani S, Jörneskog G, Ågren A, Lins PE, Wallén H, Antovic A. Fibrin clot properties and haemostatic function in men and women with type 1 diabetes. *Thromb Haemost* 2015;113(02):312–318