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ORIGINAL RESEARCH - CLINICAL

Determinants of Endogenous Fibrinolysis in Whole Blood Under High Shear in Patients With Myocardial Infarction

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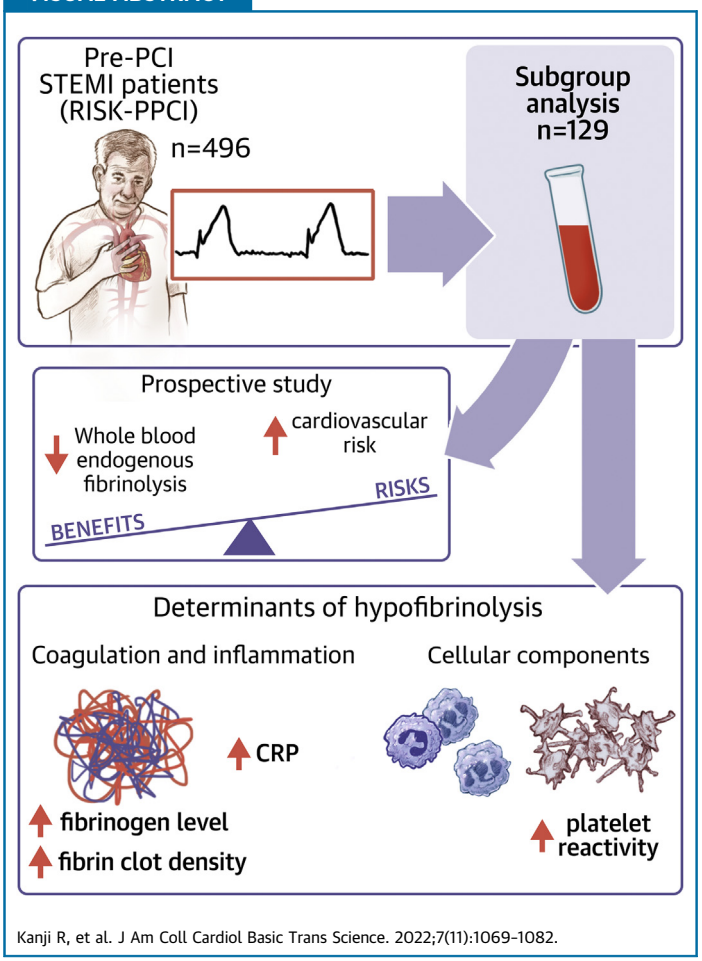
VISUAL ABSTRACT



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HIGHLIGHTS

- **Hypofibrinolysis is a recently-recognized risk factor for recurrent cardiovascular events in patients with STEMI, but its mechanistic determinants are not well understood.**
- **In patients with STEMI, we show that the effectiveness of endogenous fibrinolysis in whole blood is related to fibrinogen, hs-CRP, and shear-induced platelet reactivity, the latter related to thrombin generation.**
- **Endogenous fibrinolysis in whole blood is only weakly related to plasma clot lysis in response to t-PA, indicating an important role for cellular components in determining fibrinolytic status.**
- **These findings strengthen the evidence for bidirectional crosstalk between coagulation and inflammation and provide mechanistic insights that could help guide pharmacological strategies to treat hypofibrinolysis, a potentially modifiable cardiovascular risk factor.**

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

LT = lysis time

MACE = major adverse cardiovascular events

OT = occlusion time

PAI = plasminogen activator inhibitor

PCI = percutaneous coronary intervention

STEMI = ST-segment elevation myocardial infarction

t-PA = tissue plasminogen activator

vWF = von Willebrand factor

SUMMARY

Hypofibrinolysis is a recently-recognized risk factor for recurrent cardiovascular events in patients with ST-segment elevation myocardial infarction (STEMI), but the mechanistic determinants of this are not well understood. In patients with STEMI, we show that the effectiveness of endogenous fibrinolysis in whole blood is determined in part by fibrinogen level, high sensitivity C-reactive protein, and shear-induced platelet reactivity, the latter directly related to the speed of thrombin generation. Our findings strengthen the evidence for the role of cellular components and bidirectional crosstalk between coagulatory and inflammatory pathways as determinants of hypofibrinolysis. (J Am Coll Cardiol Basic Trans Science 2022;7:1069–1082)

Less efficient endogenous fibrinolysis, as evidenced by prolonged lysis time (LT) in vitro, is a recently recognized risk factor for recurrent thrombotic events in patients with acute coronary syndrome (ACS).[1,2] In health, an effective endogenous fibrinolytic system can counter prothrombotic drivers to prevent lasting arterial thrombotic occlusion.[3] Until recently, assessment of endogenous fibrinolysis relied on the measurement of circulating soluble biomarkers such as tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI)-1, which provided weak correlation with the occurrence of atherothrombotic events.[4] However, a more recently available global test of endogenous fibrinolysis, which employs whole blood, has been shown to be a strong predictor of residual cardiovascular risk in patients with ACS.

In earlier work from our group, in the RISK-PPCI (RIsk stratification in patients with STEMI undergoing Primary Percutaneous Coronary Intervention) study, patients with ST-segment elevation myocardial infarction (STEMI) who exhibited prolonged endogenous fibrinolysis (LT ≥2,500 seconds) in whole blood in vitro, showed a 9-fold increased risk of subsequent major adverse cardiovascular events (MACE), compared with those with shorter endogenous LT.[5] The enhanced risk of MACE occurred despite optimal contemporary treatments with primary percutaneous coronary intervention (PCI) and antithrombotic medication. In another important substudy of the PLATO (PLATelet inhibition and patient Outcomes) trial, assessment of citrated plasma from ACS patients showed that plasma clot LT, measured using a turbidimetric assay (a measure of clot density), was predictive of 1-year cardiovascular death and spontaneous myocardial infarction.[6] After adjusting for cardiovascular risk factors, each 50% increase in plasma clot LT was associated with a 1.17-fold increase in the risk of cardiovascular death or spontaneous myocardial infarction, with those patients in the highest quartile of LT showing a 1.48-fold higher rate of adverse events compared with those with more effective lysis. Current antiplatelet medications used in patients with ACS are highly effective at inhibition of platelet reactivity[7]; however, these medications do not appear to affect endogenous fibrinolysis.[5,7]

It is clear that we require a better understanding of the pathomechanism behind the prolonged endogenous fibrinolysis exhibited by some patients with ACS, which may identify potential avenues to target with pharmacotherapy. In this study, we assessed endogenous fibrinolysis in whole blood of patients with ACS and aimed to relate this to plasma clot lysis, platelet reactivity under high shear, fibrinogen level, and markers of thrombin generation and inflammation.

METHODS

We performed a prespecified subgroup analysis of the RISK-PPCI study, which was a prospective, observational, single-center study in 496 patients presenting with STEMI.[5] The study, already reported earlier,

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showed that prolonged endogenous fibrinolysis was highly predictive of recurrent MACE (HR: 9.10, 95% CI: 5.29-15.75; $P < 0.001$), driven by cardiovascular death and myocardial infarction, particularly within 30 days.[5] Delayed fibrinolysis remained strongly predictive of MACE after adjustment for conventional risk factors (HR: 8.03; 95% CI: 4.28-15.03; $P < 0.001$).

The aim of this substudy was to provide mechanistic insight into the determinants of endogenous fibrinolysis in patients with ACS. The study was approved by the National Research Ethics Service and the UK Health Research Authority (NCT02562690) and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice.

STUDY DESIGN. This was a retrospective analysis using frozen citrated plasma samples from the RISK-PPCI study. In the main study, 496 patients had endogenous whole blood LT measured at the point of hospital admission and frozen citrated plasma samples stored at −80 °C for subsequent analysis. To identify a subgroup of 129 representative patients across a range of LTs, the 496 patients in the RISK-PPCI study were divided into quartiles (Q) based on whole blood endogenous thrombolysis time (LT) at presentation: Q_1 622-1,410 seconds; Q_2 1,411-2,250 seconds; Q_3 2,251-3,768 seconds; Q_4 3,769-6,000 seconds. From each quartile, 32 patients were selected at random (total of 129 patients), and additional analyses were performed to evaluate the relationship between whole blood LT with plasma clot LT, fibrin level, thrombin generation, and shear-induced thrombotic occlusion time (OT).

PATIENT POPULATION. For the RISK-PPCI study, consecutive eligible patients presenting with STEMI to our Heart Attack Centre with a view to primary PCI were recruited. We enrolled adults (age ≥18 years) with a presumed diagnosis of STEMI based on clinical presentation and ECG criteria. Patients receiving oral anticoagulation, those with known coagulation disorders, sepsis, platelet count <100 × 10^9/L, hemoglobin <80 g/L, active malignancy, or inability to take dual antiplatelet therapy, as well as those previously enrolled in the study, were excluded.

A delayed consent strategy was used with ethical approval. Patients who died before consent could be obtained were excluded. Surviving patients were subsequently approached for consent. All participants gave written informed consent. In addition to routine blood tests upon arrival, an extra blood sample was taken to assess baseline thrombotic status through the same blood draw. Patients received standard-of-care antiplatelet therapy and underwent emergency angiography and primary PCI as clinically indicated.

Antiplatelet therapy consisted of aspirin 300 mg and either clopidogrel 600 mg or ticagrelor 180 mg orally in the ambulance or emergency department upon diagnosis. Patients receiving clopidogrel prearrival received additional ticagrelor 180 mg loading peri-primary PCI, which was continued post-procedure. Time from antiplatelet therapy loading to assessment of endogenous fibrinolysis in whole blood was <30 minutes.

BLOOD SAMPLING. Nonfasting blood samples taken immediately upon arrival to the Heart Attack Centre, after dual antiplatelet therapy loading, before heparin or glycoprotein IIb/IIIa inhibitor administration, and before primary PCI, were taken from a 6-F radial or femoral sheath, which was flushed with non-heparinized saline before insertion. A 2-syringe technique was employed, using the first 5 mL for routine tests and the second sample for thrombotic status assessment. The sample for thrombotic status assessment was immediately introduced into the Global Thrombosis Test for point-of-care analysis, and a simultaneous citrated sample was spun at 2,300 *g* for 10 minutes to yield platelet-poor plasma and stored at −80 °C for subsequent analysis.

ASSESSMENT OF THROMBOTIC STATUS. Blood samples were analyzed immediately with the Global Thrombosis Test as a point-of-care test. In the subsequent assessment of citrated plasma, investigators were blinded to the results of the endogenous fibrinolysis in whole blood.

Global thrombosis test. The Global Thrombosis Test (Thromboquest Ltd) assesses both platelet reactivity and endogenous fibrinolysis from native, non-anticoagulated whole blood, and the principle of the technique has previously been described.[8] The instrument measures the time taken for shear-induced occlusive thrombus formation (OT), and in the second phase of the test, measures the time to achieve spontaneous lysis of thrombi created during the first phase. The instrument was positioned in the catheterization laboratory, ready to use. The native blood sample taken from the patient was immediately introduced into the GTT cartridge in the instrument within 15 seconds of withdrawal, and the automated measurement began. Once introduced into the cartridge, blood flows through a conical plastic tube, passing through small gaps adjacent to 2 sequential beads. As blood flows through the gaps adjacent to the upper bead, the resulting initial high shear stress (180 dynes/cm^2) causes platelet activation. Immediately downstream in the low shear zone between the beads, the activated platelets aggregate, thrombin is generated, and eventually, the growing microthrombi

occlude the gaps adjacent to the second bead, reducing the flow rate and finally arresting flow. The instrument measures the time (d) between consecutive blood drops at the exit of the conical part of the tube, which gradually increases as thrombi start to occlude the gaps adjacent to the second bead and at an arbitrary point (d ≥15 seconds), the instrument records and displays OT (seconds). The restart of blood flow following occlusion is caused by spontaneous thrombolysis (LT, seconds). If lysis does not occur until 6,000 seconds following OT (LT cutoff time), "no lysis" is recorded.

The intra-assay and interassay coefficients of variation (CV) for OT and LT were assessed in 10 patients with stable cardiovascular disease on repeated sampling (48 hours apart) and also running samples in parallel. The intra-assay CV was 6% for OT and 8% for LT, and the interassay CV was 7% for OT and 9% for LT.

Plasma clot lysis. Plasma clot lysis was assessed using a turbidimetric technique as previously described.[9] Briefly, thawed plasma in the presence and absence of t-PA (Genetech) was prepared in 10 mmol/L Tris pH 7.5, 140 mmol/L NaCl, 0.01% Tween 20. Aliquots were added in triplicate to a microtiter plate containing an activation mix of thrombin, Ca^{2+}, and phospholipids. The plate was incubated at 37 °C and read continuously every 1 minute for 4 hours at 405 nm. Final concentrations of reactants were as follows; plasma 30%; t-PA 300 pmol/L; thrombin 0.1 U/mL; phospholipids 16 μmol/L; $CaCl_2$ 10.6 mmol/L.

Thrombin generation. Thrombin generation in patient samples was quantified using the calibrated automated thrombogram (Thrombinoscope, Diagnostica Stago) method.[10] Plasma (80 μL) was dispensed in triplicate into round 96-well plates (Immulon 2HB, Dynex) and the plate warmed to 37 °C for 5 minutes before addition of the starting reagent (20 μL/well) containing PPP low reagent (Diagnostica Stago), 2.5 mmol/L fluorogenic substrate (Z-Gly-Gly-Arg-AMC.HCl) and 16.6 mmol/L $CalCl_2$. Measurements were taken every minute for 1 hour in a Fluoroscan Ascent fluorometer (Thermo Labsystems, Thermo Fisher Scientific). Data were analyzed using the thrombinoscope software (Synpase Bv) producing standard parameters including lag time, velocity index, peak thrombin generation, and endogenous thrombin potential.

DATA COLLECTION AND FOLLOW-UP. During the index admission, case notes and electronic records were examined to allow contemporaneous completion of study-specific case record forms. Patients were followed up at 30 days in person and at 6 and 12 months by telephone and by accessing case notes.

STUDY ENDPOINTS. The primary endpoint of the RISK-PPCI study was the occurrence of MACE, defined as the composite of cardiovascular death, nonfatal myocardial infarction including stent thrombosis (defined according to the Academic Research Consortium criteria), or stroke.[5] For all endpoints, source documents were obtained, and the diagnosis was verified by 2 independent clinicians (MS, DAG) blinded to thrombotic status results.

STATISTICAL ANALYSIS. Continuous data are presented as median (25th and 75th percentile [Q1-Q3]) and categorical data as count (percentage). Differences between dichotomous variables were assessed using the chi-square test, and differences between continuous variables using the Kruskal-Wallis and Mann-Whitney *U* tests, with the Bonferroni correction applied for multiple pairwise comparisons. Correlations between continuous variables were assessed using Spearman's rank correlation coefficient. Receiver-operator characteristic (ROC) curves and Kaplan-Meier curves were constructed, and Cox analyses were performed to determine the prognostic value of plasma clot lysis, thrombin generation, and platelet reactivity, presented as HRs with 95% CIs. These were compared with the prognostic value of endogenous whole blood fibrinolysis obtained in the RISK-PPCI study.[5] Binary logistic regression was used in both univariate and multivariable analyses to assess the relationship of clinical and laboratory variables to endogenous fibrinolysis, and is presented as OR with 95% CIs. We verified the logistic model assumption by confirming linearity, plotting the log odds against the variables of interest (hs-CRP, fibrinogen, creatinine, hemoglobin). Kaplan-Meier estimates of MACE at 1 year were calculated. Statistical analyses were performed with SPSS version 26 (IBM Corp). All statistical tests were 2-sided, with $P < 0.05$ taken to indicate statistical significance.

RESULTS

The clinical and laboratory characteristics of patients, according to the quartiles of whole blood endogenous fibrinolysis time, are shown in **Table 1**. Patients in each quartile were well-matched for established cardiovascular risk factors aside from smoking habit, which was more prevalent among patients with longer fibrinolysis times.

In the RISK-PPCI study, the optimal cutpoint for whole blood endogenous fibrinolysis time for the prediction of MACE was 2,500 seconds. **Table 2** shows the clinical and laboratory characteristics of patients from our cohort split by this LT cutpoint. Patients with prolonged fibrinolysis time more frequently had

TABLE 1 Summary of Clinical and Biochemical Characteristics of Patients Grouped by Quartiles According to Whole Blood Endogenous LT

	LT Q_1	LT Q_2	LT Q_3	LT Q_4	*P* Value
Median LT (range), s	1,196 (622-1,410)	1,808 (1,411-2,250)	3,217 (2,251-3,768)	5,941 (3,769-6,000)	
Age, y	66 (53-72)	67 (59-75)	73 (58-82)	62 (53-76)	0.150
Male	23 (72)	25 (78)	27 (84)	26 (79)	0.687
BMI, kg/m^2	27.0 (23.7-30.1)	25.7 (23.5-27.7)	26.5 (23.9-29.9)	25.6 (23.7-29.3)	0.710
Smoking	10 (31)	13 (41)	6 (19)	17 (52)	**0.042**
Diabetes	5 (16)	5 (16)	8 (25)	9 (27)	0.530
Hypertension	18 (56)	15 (47)	13 (40)	16 (48)	0.662
Hyperlipidemia[a]	11 (34)	12 (38)	13 (41)	17 (52)	0.522
FH premature CAD	14 (44)	14 (44)	7 (23)	10 (30)	0.207
Prior MI	3 (9)	4 (13)	5 (16)	6 (18)	0.741
Prior PCI	4 (13)	3 (9)	5 (16)	6 (18)	0.741
Prior CABG	1 (3)	0 (0)	2 (6)	1 (3)	0.406
CKD[b]	2 (7)	0 (0)	1 (3)	3 (9)	0.209
PAD	3 (9)	3 (9)	2 (6)	0 (0)	0.638
CVA	0 (0)	2 (6)	2 (6)	2 (6)	0.314
Clopidogrel loading	20 (65)	24 (80)	24 (77)	21 (72)	0.532
Ticagrelor loading	11 (35)	6 (20)	7 (23)	8 (28)	0.532
Laboratory markers					
Hemoglobin, g/L	138 (132-149)	145 (135-159)	141 (113-156)	139 (126-148)	0.263
Leukocyte count	9.8 (8.6-12.4)	11.7 (8.2-13.9)	10.2 (7.7-13.0)	13.2 (9.8-14.7)	0.635
Neutrophil count	7.6 (5.6-10.0)	9.3 (5.8-10.2)	7.1 (5.7-10.7)	9.4 (7.4-10.5)	0.909
Platelets, $\times 10^9$/L	215 (191-267)	261 (225-300)	233 (210-264)	259 (214-305)	0.813
NLR	0.77 (0.64-0.82)	0.77 (0.70-0.81)	0.76 (0.67-0.82)	0.73 (0.65-0.81)	0.746
PLR	22.5 (18.3-28.4)	23.3 (18.3-27.9)	23.7 (19.5-28.8)	21.1 (17.3-28.5)	0.856
SII	176 (152-208)	184 (146-221)	1,677 (151-210)	193 (148-233)	0.920
INR	1.0 (1.0-1.1)	1.0 (1.0-1.0)	1.0 (1.0-1.1)	1.0 (0.9-1.1)	0.430
aPTT, s	24.9 (21.7-29.4)	25.9 (22.9-30.0)	25.3 (22.4-29.5)	27.3 (23.8-29.7)	0.545
Fibrinogen, g/L	3.6 (3.1-4.4)	4.0 (3.4-4.6)	4.0 (3.1-5.0)	4.4 (3.8-6.0)	**0.033**
hs-CRP, mg/L	3 (2-10)	3 (1-5)	6 (2-28)	11 (2-81)	**0.017**
Creatinine, μmol/L	74 (66-101)	81 (62-90)	103 (84-124)	89 (79-109)	**0.008**
Total cholesterol, mmol/L	5.3 (4.0-6.3)	5.1 (4.2-6.1)	4.3 (3.8-5.6)	5.2 (4.4-6.1)	0.120
LDL, mmol/L	2.7 (2.0-4.2)	3.5 (2.7-4.1)	2.7 (2.0-3.6)	3.2 (2.5-3.8)	0.274
Shear-induced platelet reactivity					
OT, s	390 (307-512)	338 (270-424)	317 (186-534)	288 (186-420)	0.154
Plasma clot lysis					
Max absorbance at 405 nm	0.42 (0.34-0.56)	0.40 (0.32-0.50)	0.45 (0.34-0.58)	0.46 (0.35-0.66)	0.380
Fold increase in max absorbance	1.33 (0.99-1.54)	1.15 (0.92-1.46)	1.34 (1.17-1.61)	1.51 (1.09-2.14)	0.051
50% clot lysis time, min	92.0 (76.4-118.3)	87.0 (72.0-126.4)	86.0 (77.0-130.0)	103.6 (90.3-167.6)	0.217
Fold increase in 50% clot lysis time	1.16 (0.84-1.31)	1.08 (0.90-1.37)	1.05 (0.94-1.45)	1.21 (0.94-1.62)	0.613
Thrombin generation					
Lag time, min	7.0 (5.4-8.4)	6.0 (5.1-7.8)	5.9 (5.6-7.2)	8.0 (6.2-9.0)	**0.010**
Peak, nm	194 (129-231)	173 (143-255)	174 (133-274)	146 (92-208)	0.275
Velocity index, nmol/L/min	41.6 (26.6-58.1)	40.5 (20.9-81.7)	50.2 (24.4-73.1)	25.8 (18.5-46.6)	0.252
ETP, nM/min	1,524 (1,113-1,720)	1,456 (1,109-1,818)	1,442 (1,063-1,833)	1,509 (1,192-1,742)	0.996

Values are median (Q1-Q3) or n (%), unless otherwise indicated. The Kruskal-Wallis test and chi-square tests were used for statistical analysis of continuous and dichotomous variables, respectively. **Bold** values indicate statistical significance ($P < 0.05$). [a]Hyperlipidemia was defined as a total cholesterol >6.5 mmol/L on admission in the absence of lipid-lowering medication, or a documented history of hyperlipidemia in the case notes. [b]CKD was defined as glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 on admission and either present on at least 1 further measurement a minimum of 3 months earlier, or a clear documentation in the case notes.

aPTT = activated partial thromboplastin time; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CKD = chronic kidney disease; CVA = cerebrovascular accident; FH = family history of premature coronary artery disease; hs-CRP = high sensitivity C-reactive protein; ETP = endogenous thrombin potential; INR = International Normalized Ratio; LDL = low-density lipoprotein; MI = myocardial infarction; NLR = neutrophil-to-leucocyte ratio; OT = occlusion time; PAD = peripheral arterial disease; PCI = percutaneous coronary intervention; PLR = platelet-to-leucocyte ratio; Q = quartile; SII = systemic immune-inflammation index.

higher creatinine, high sensitivity C-reactive protein (hs-CRP), and fibrinogen on admission, and had lower hemoglobin (but still within the normal range) when compared with patients with a shorter whole blood fibrinolysis time. Using binary logistic regression, a univariable analysis of all of the variables listed in Table 2 was conducted to assess relationship with high LT (≥2,500 seconds). Only hemoglobin (OR: 0.98;

TABLE 2 Summary of Clinical and Biochemical Characteristics of Patients With High (≥2,500 S) and Low (<2,500 S) Whole Blood LT

	LT <2,500 s (n = 70)	LT ≥2,500 s (n = 59)	*P* Value
Age, y	66 (55-72)	69 (56-81)	0.115
Male	54 (77)	47 (80)	0.730
BMI, kg/m^2	26.6 (23.9-29.0)	25.6 (23.7-29.4)	0.557
Smoking	23 (33)	23 (39)	0.469
Diabetes	11 (16)	16 (27)	0.113
Hypertension	35 (50)	27 (46)	0.631
Hyperlipidemia	24 (34)	29 (49)	0.087
FH premature CAD	29 (41)	16 (28)	0.103
Angina	5 (7)	9 (16)	0.131
Prior MI	8 (11)	10 (17)	0.346
Prior PCI	8 (11)	10 (17)	0.346
Prior CABG	1 (1)	3 (5)	0.226
CKD	3 (4)	3 (5)	0.873
PAD	6 (9)	2 (3)	0.224
CVA	2 (3)	4 (7)	0.282
Clopidogrel loading	48 (71)	41 (75)	0.409
Ticagrelor loading	19 (28)	13 (24)	0.588
Laboratory markers			
Hemoglobin, g/L	144 (134-156)	139 (120-150)	**0.011**
Leukocyte count	11.4 (8.9-13.6)	11.1 (9.4-13.6)	0.820
Neutrophil count	8.7 (6.1-10.3)	7.8 (6.0-10.6)	0.828
Platelets, ×10^9/L	238 (201-305)	254 (227-294)	0.669
NLR	0.77 (0.68-0.81)	0.74 (0.67-0.82)	0.841
PLR	22.7 (18.8-27.9)	21.9 (18.0-28.7)	0.975
SII	176 (149-217)	172 (154-223)	0.769
INR	1.0 (1.0-1.1)	1.0 (1.0-1.0)	0.647
APTT, s	24.9 (21.9-28.9)	26.5 (23.3-30.0)	0.314
Fibrinogen, g/L	3.7 (3.1-4.6)	4.4 (3.7-5.9)	**0.019**
hs-CRP, mg/L	2 (1-6)	8 (2-32)	**0.002**
Creatinine, μmol/L	71 (64-89)	83 (72-104)	**0.003**
Total cholesterol, mmol/L	5.1 (4.2-6.1)	4.9 (4.2-5.7)	0.341
LDL, mmol/L	2.9 (2.4-4.2)	2.9 (2.4-3.6)	0.719
Shear-induced platelet reactivity			
OT, s	334 (282-438)	312 (183-524)	0.309
Plasma clot lysis			
Max absorbance at 405 nm	0.42 (0.32-0.55)	0.44 (0.35-0.59)	0.261
Fold increase in max absorbance	1.29 (0.96-1.53)	1.37 (1.13-1.78)	0.077
50% clot lysis time, min	91.0 (73.8-117.5)	99.5 (81.0-135.3)	0.118
Fold increase in 50% clot lysis time	1.11 (0.90-1.35)	1.08 (0.94-1.56)	0.501
Thrombin generation			
Lag time, min	6.1 (5.2-7.8)	6.9 (5.8-9.0)	0.067
Peak, nm	187 (145-244)	157 (110-217)	0.086
Velocity index, nmol/L/min	41.6 (26.6-63.3)	28.0 (18.7-67.6)	0.098
ETP, nM/min	1,524 (1,113-1,814)	1,418 (1,064-1,754)	0.445

Values are median (Q1-Q3) or n (%). **Bold** values indicate statistical significance ($P < 0.05$).
ETP = endogenous thrombin potential; other abbreviations as in Table 1.

95% CI 0.96-0.99; $P = 0.011$), creatinine (OR: 1.02; 95% CI: 1.00-1.03; $P = 0.009$), fibrinogen (OR: 1.50; 95% CI: 1.11-2.02; $P = 0.008$), hs-CRP (OR: 1.04; 95% CI: 1.01-1.07; $P = 0.012$) were significantly associated. However, on multivariable analysis, only creatinine was an independent predictor of prolonged whole blood LT (OR: 1.02; 95% CI: 1.00-1.03; $P = 0.035$).

RELATIONSHIP BETWEEN WHOLE BLOOD ENDOGENOUS FIBRINOLYSIS AND PLASMA CLOT LYSIS. Whole blood endogenous fibrinolysis time was weakly correlated with 50% plasma clot LT (r = 0.195; $P = 0.032$) and with fold increase in maximal absorbance (r = 0.199; $P = 0.027$) (Figure 1). There was no relationship between whole blood endogenous fibrinolysis and plasma clot lysis measures of fold increase in 50% clot lysis or maximum absorbance (Supplemental Table 1).

RELATIONSHIP BETWEEN WHOLE BLOOD LYSIS AND THROMBIN GENERATION. There was no relationship between whole blood LT and any of the markers of thrombin generation, including lag time, peak thrombin generation, endogenous thrombin potential, or velocity index (Tables 1 and 2, Supplemental Table 1). Although when grouped by quartiles of LT, there appeared to be a difference in lag time to thrombin generation, a relationship between lag time and endogenous fibrinolysis was not observed when examining lysis as a continuous variable.

RELATIONSHIP AMONG SHEAR-INDUCED THROMBOTIC OT, WHOLE BLOOD LYSIS, AND PLASMA CLOT LYSIS. The clinical and laboratory characteristics of patients according to the quartiles of whole blood shear-induced OT are shown in Table 3. Occlusion time was weakly inversely correlated with whole blood LT (r = −0.200; $P = 0.026$) (Figure 2) but was not related to plasma clot lysis (r = 0.104; $P = 0.264$).

RELATIONSHIP AMONG SHEAR-INDUCED THROMBOTIC OT AND THROMBIN GENERATION, COAGULATION, AND INFLAMMATORY MARKERS. Shear-induced platelet reactivity (shorter OT) was associated with more rapid thrombin generation, correlating with shorter lag time (r = 0.233; $P = 0.026$), increased velocity index (r = −0.263; $P = 0.012$), and higher peak (r = −0.261; $P = 0.012$), but was not related to endogenous thrombin potential (Figures 3 and 4, Supplemental Table 2). Shear-induced platelet reactivity was not related to hematological, coagulation, or inflammatory markers (Table 3). hs-CRP correlated weakly with endogenous thrombin potential (r = 0.220; $P = 0.045$).

RELATIONSHIP AMONG MARKERS OF COAGULATION, INFLAMMATION, AND MEASURES OF FIBRINOLYSIS. Whole blood endogenous fibrinolysis time correlated with fibrinogen level (r = 0.300; $P = 0.001$) and hs-CRP (r = 0.236; $P = 0.011$), but was not related to other markers of coagulation or inflammation (Tables 1 and 2). Hs-CRP also correlated with fibrinogen level (r = 0.631; $P < 0.001$), plasma clot lysis (r = 0.269; $P = 0.005$), fibrin clot density (r = 0.367; $P = 0.001$), and weakly with endogenous thrombin potential (r = 0.220; $P = 0.045$). Fibrinogen level correlated weakly with plasma clot

LT (r = 0.194; $P = 0.041$) and moderately with fibrin clot density (r = 0.545; $P < 0.001$).

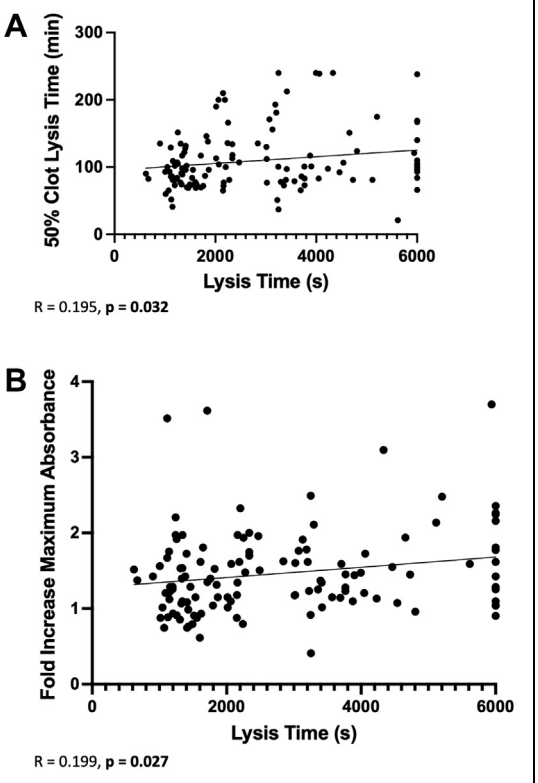
COMPARISON OF ENDOGENOUS FIBRINOLYSIS, PLASMA CLOT LYSIS ASSAY, SHEAR-INDUCED PLATELET REACTIVITY, AND THROMBIN GENERATION FOR THE PREDICTION OF RECURRENT MACE. ROC curve analysis of the subgroup of RISK-PPCI patients included here showed that endogenous fibrinolysis assessed using whole blood was the best predictor of recurrent MACE (area under the ROC curve [AUC]: 0.649), and reflected the results obtained in the main study (AUC: 0.776).[5] Specifically, in the subgroup of patients included here, for the prediction of MACE, endogenous fibrinolysis was superior to 50% plasma clot LT (AUC: 0.561), plasma clot maximal turbidity (AUC: 0.563), whole blood OT (AUC: 0.385), peak thrombin generation (AUC: 0.478), endogenous thrombin potential (AUC: 0.506), and thrombin lag time (AUC: 0.616) (Supplemental Table 3). Applying the cutpoint of 2,500 seconds for whole blood endogenous fibrinolysis time (determined by Youden's index from main study[5]) to our current cohort, revealed that patients with whole blood fibrinolysis time ≥2,500 seconds had a 3.6-fold higher risk of MACE (95% CI: 1.5-8.5; $P = 0.002$), driven predominantly by cardiovascular death, compared with patients with fibrinolysis time <2,500 seconds (**Figure 5,** Supplemental Table 4). We considered competing events, in particular non-cardiovascular death, and this occurred with similar frequency in patients with LT ≥2,500 and <2,500 seconds such that we feel this cannot account for the different MACE rate observed.

In contrast, although plasma clot LT (using a cutpoint of 78.5 minutes derived from Youden's index) could differentiate between patients with and without MACE using the log-rank test (**Figure 5**), this was not significant on Cox regression ($P = 0.057$) (Supplemental Table 4). Whole blood OT (using a cutpoint of 602 seconds derived from Youden's index) was not able to differentiate between patients with and without MACE (Supplemental Table 4), and a model assessing the combination of whole blood LT and whole blood OT for the prediction of MACE, using the cutpoints defined in the previous text, failed to provide incremental risk prediction for MACE over and above that of whole blood LT alone (Supplemental Table 5).

DISCUSSION

Our results indicate that the effectiveness of endogenous fibrinolysis in whole blood is determined in part by fibrinogen level, inflammation, and shear-induced platelet aggregation (OT). Further, plasma clot lysis in response to t-PA is only weakly correlated with endogenous fibrinolysis observed in whole blood, suggesting that the main determinants of endogenous fibrinolysis are cellular components. Although LT is not directly related to thrombin generation, shear-induced thrombotic occlusion is related to the speed and magnitude of thrombin generation.

FIGURE 1 Relationship Between Whole Blood Endogenous Fibrinolysis and Plasma Clot Lysis and Turbidity



Relationship between whole blood endogenous fibrinolysis (lysis time) and plasma clot lysis time (50% clot lysis) **(A)** and fold-increase in maximum clot absorbance as quantified by turbidity measurements **(B).**

RELATIONSHIP AMONG ENDOGENOUS FIBRINOLYSIS, FIBRINOGEN, AND HS-CRP LEVEL. Endogenous fibrinolysis in whole blood appears to correlate with elevated fibrinogen level in our study. Although the relationship between whole blood lysis and fibrinogen level is not previously described, it is supported by prior work showing that higher plasma fibrinogen levels are associated with more compact fibrin clots,[11,12] and the latter in turn have been associated with impaired lysis in response to t-PA.[13,14]

We show an inverse relationship between the effectiveness of endogenous fibrinolysis in whole

TABLE 3 Summary of Clinical and Biochemical Characteristics of Patients Grouped by Quartiles According to Whole Blood OT

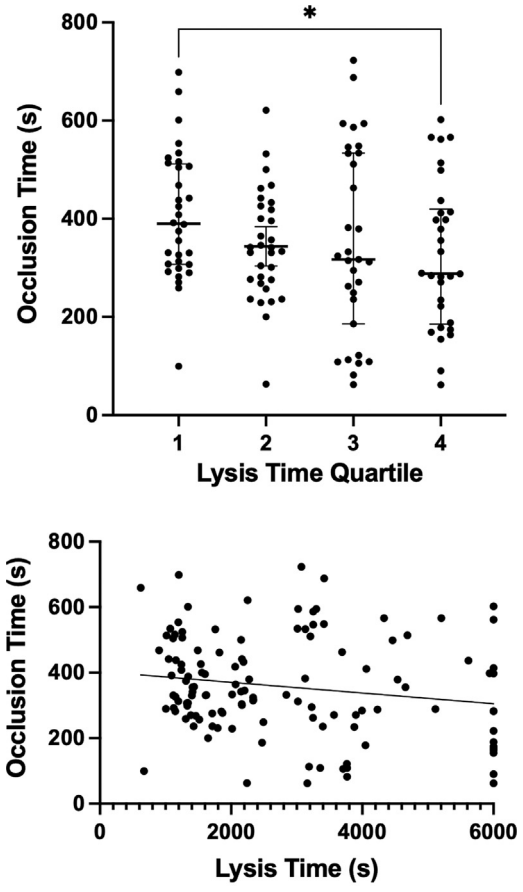
	OT Q_1	OT Q_2	OT Q_3	OT Q_4	*P* Value
Median OT (range), s	179 (62-268)	299 (270-332)	397 (333-463)	546 (468-723)	
Age, y	71 (60-80)	67 (53-77)	63 (53-71)	70 (51-82)	0.240
Male	26 (84)	26 (84)	23 (72)	22 (71)	0.425
BMI, kg/m^2	26.0 (23.7-28.7)	24.5 (22.6-27.5)	27.8 (24.3-30.3)	25.7 (23.7-29.0)	0.252
Smoking	9 (29)	9 (29)	15 (47)	12 (39)	0.384
Diabetes	8 (26)	3 (10)	8 (25)	6 (19)	0.358
Hypertension	18 (58)	15 (48)	13 (40)	14 (45)	0.561
Hyperlipidemia[a]	16 (52)	11 (35)	8 (25)	15 (48)	0.116
FH premature CAD	10 (32)	13 (42)	12 (39)	9 (29)	0.703
Prior MI	4 (13)	7 (23)	2 (6)	4 (13)	0.324
Prior PCI	6 (19)	6 (19)	2 (6)	3 (10)	0.302
Prior CABG	1 (3)	1 (3)	1 (3)	1 (3)	1.000
CKD[b]	1 (3)	0 (0)	2 (6)	3 (10)	0.209
PAD	1 (3)	4 (13)	2 (6)	1 (3)	0.394
CVA	2 (6)	1 (3)	2 (6)	1 (3)	0.870
Clopidogrel loading	22 (73)	22 (73)	25 (83)	16 (55)	0.118
Ticagrelor loading	6 (20)	8 (27)	5 (17)	13 (45)	0.070
Laboratory markers					
Hemoglobin, g/L	142 (129-154)	146 (134-162)	144 (132-153)	134 (113-152)	0.275
Leukocyte count,	10.6 (8.7-15.2)	11.2 (8.1-13.1)	9.8 (8.2-12.8)	11.7 (9.4-13.2)	0.657
Neutrophil count,	8.9 (6.3-10.8)	9.1 (6.4-10.8)	7.9 (5.4-9.5)	7.5 (6.1-9.7)	0.404
Platelets, $\times 10^9$/L	255 (216-277)	235 (200-317)	260 (215-316)	225 (191-269)	0.168
NLR	0.77 (0.72-0.82)	0.77 (0.68-0.82)	0.72 (0.63-0.79)	0.73 (0.68-0.79)	0.195
PLR	21.9 (17.8-29.2)	22.6 (17.9-27.8)	23.6 (20.4-29.0)	22.3 (19.1-28.7)	0.663
SII	183 (156-219)	191 (146-224)	1,182 (155-226)	161 (131-197)	0.271
INR	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.0)	0.918
aPTT, s	26.5 (24.5-28.1)	26.1 (23.9-29.8)	23.2 (21.7-29.4)	27.9 (22.8-30.9)	0.220
Fibrinogen, g/L	4.0 (3.5-4.4)	3.7 (3.2-4.6)	4.1 (3.0-5.1)	4.4 (3.5-6.1)	0.342
hs-CRP, mg/L	3 (1-8)	3 (1-10)	5 (1-12)	14 (2-41)	0.057
Creatinine, μmol/L	100 (71-126)	84 (71-99)	83 (72-97)	82 (62-100)	0.468
Total cholesterol, mmol/L	5.2 (4.4-6.3)	4.8 (3.9-5.9)	5.2 (4.3-5.9)	4.6 (3.9-6.1)	0.522
LDL, mmol/L	3.5 (2.7-4.1)	2.6 (1.8-3.7)	3.0 (2.2-4.1)	2.9 (2.3-3.8)	0.356
Whole blood endogenous fibrinolysis					
LT, s	3,219 (1,775-6,000)	1,850 (1,329-3,123)	1,917 (1,336-2,489)	2,200 (1,199-3,326)	**0.041**
Plasma clot lysis					
Max absorbance at 405 nm	0.40 (0.35-0.53)	0.48 (0.41-0.61)	0.42 (0.31-0.58)	0.44 (0.32-0.62)	0.622
Fold increase in max absorbance	1.28 (1.01-1.45)	1.40 (1.14-1.68)	1.18 (1.04-1.59)	1.40 (0.97-1.68)	0.609
50% clot lysis time, min	87.0 (73.6-110.0)	96.0 (74.5-120.0)	100.0 (82.5-133.3)	92.5 (73.5-143.3)	0.518
Fold increase in 50% clot lysis time	1.07 (0.89-1.32)	1.17 (0.94-1.52)	1.15 (0.96-1.37)	1.11 (0.87-1.59)	0.881
Thrombin generation					
Lag time, min	5.7 (5.2-7.2)	6.0 (5.4-7.7)	6.7 (5.8-8.1)	7.8 (5.7-9.8)	0.078
Peak, nm	226 (160-284)	171 (101-238)	185 (135-239)	140 (88-196)	**0.027**
Velocity index, nmol/L/min	66.9 (29.8-90.3)	38.2 (19.6-58.0)	38.5 (20.4-67.2)	26.2 (13.7-45.4)	**0.021**
ETP, nM/min	1,649 (1,296-1,845)	1,508 (1,046-1,788)	1,462 (1,252-1,832)	1,405 (1,030-1,759)	0.541

Values are median (Q1-Q3) or n (%). The Kruskal-Wallis test and chi-square tests were used for statistical analysis of continuous and dichotomous variables, respectively. **Bold** values indicate statistical significance ($P < 0.05$). [a]Hyperlipidemia was defined as a total cholesterol >6.5 mmol/L on admission in the absence of lipid-lowering medication, or a documented history of hyperlipidemia in the case notes. [b]CKD was defined as GFR <60 mL/min/1.73 m^2 on admission and either present on at least 1 further measurement a minimum of 3 months earlier, or a clear documentation in the case notes.

Abbreviations as in Table 1.

blood and hs-CRP levels, as well as between fibrinogen and hs-CRP. Our data strongly support the increasing evidence pointing to a complex bidirectional crosstalk between inflammation and coagulation pathways.[15,16] Exposure of blood to TF, either through plaque rupture or TF release from circulating monocytes, results in activation of coagulation, resulting in thrombin and ultimately fibrin generation, as well as platelet activation. These coagulation factors have additional inflammatory effects. Binding

FIGURE 2 Relationship Between Occlusion Time and Endogenous Fibrinolysis (Lysis Time) in Whole Blood



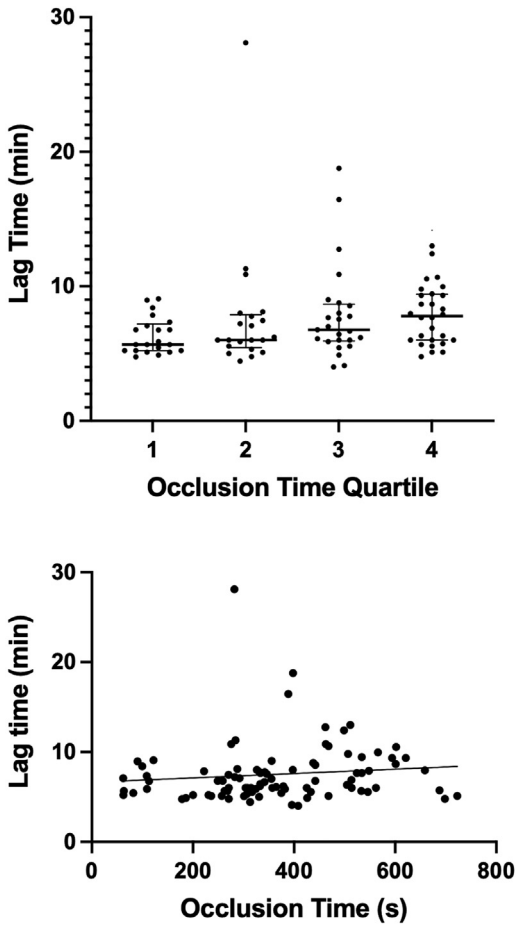
There was no significant difference between OT quartiles (Kruskal-Wallis test; $P = 0.154$). There is a relationship at the extremes of LT (ie, Q1 and Q4, Mann-Whitney *U* test $P = 0.030$) which is biologically plausible and relevant. *$P < 0.05$.

of tissue factor, thrombin, and other activated coagulation proteases to specific protease activated receptors (PARs) on inflammatory cells may induce the release of proinflammatory cytokines and chemokines, which can further modulate coagulation and fibrinolysis.[15-17] Thrombin activation of PAR-1 on endothelial cells and fibroblasts can stimulate the production of monocyte chemoattractant protein-1, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, expression of P- and E-selectin, and PAI-1. Thrombin activation of PAR-1 and -4 on platelets results in release of PAI-1. Thrombin activates the endothelium to result in t-PA and urokinase type-plasminogen activator (uPA) release, stimulating fibrinolysis. TNF-α can modulate the expression of major components of the fibrinolytic system, with its stimulatory effect on PAI-1 and down-regulation of t-PA expression in endothelial cells being most significant. IL-6, produced predominantly by macrophages and monocytes, is the chief stimulator of the production of most acute phase proteins, including inducing the hepatic synthesis of CRP. Fibrinogen can also directly stimulate expression of proinflammatory cytokines (such as TNF-α and IL-1β) on mononuclear cells and induce production of chemokines (including IL-8 and monocyte chemoattractant protein-1) by endothelial cells and fibroblasts.[17]

The main inhibitor of fibrinolysis in the circulation is PAI-1, and increased levels have been linked to myocardial infarction.[18-20] Although platelets contain the major pool of circulating PAI-1, released following platelet stimulation,[21] PAI-1 is also synthesized by other cells, predominantly endothelial cells.[22] Not only are high CRP levels associated with elevated PAI-1 levels in a number of conditions such as sepsis, inflammation, and myocardial infarction, but in experimental models, proinflammatory cytokines liberated during inflammation, including CRP, IL-6, and TNF-α, directly influence PAI-1 synthesis.[21,23-25] Incubation of human aortic endothelial cells with CRP induces a time- and dose-dependent increase in PAI-1 expression and activity[25] and reduction in t-PA activity,[26] showing a direct effect on fibrinolytic status. Furthermore, activated platelets have been shown to convert pentameric CRP to the monomeric form, which promotes platelet capture of neutrophils.[27] Our findings support a direct functional relationship between PAI-1 and CRP and are supported by earlier data showing a correlation between CRP and plasma clot LT.[6]

DIFFERENCES BETWEEN FIBRINOLYSIS ASSESSED WITH THE GLOBAL THROMBOSIS TEST AND PLASMA CLOT LYSIS. It is important to appreciate the fundamental differences between these 2 techniques in reflecting the effectiveness of fibrinolysis. In the Global Thrombosis Test, nonanticoagulated *whole blood* is used, and LT reflects the *spontaneous* restart of flow after occlusive thrombus formation, ie, endogenous fibrinolysis. On the other hand, the clot lysis assay employs citrated *plasma*, which is recalcified and clotting initiated with thrombin. Lysis is only achieved by addition of *exogenous* t-PA. Indeed, in the absence of added plasminogen activator, these clots are exceptionally stable to fibrinolytic degradation. In physiological systems, plasminogen activators including t-PA and uPA are supplied by cells. The endothelium is the main source of t-PA, but it

FIGURE 3 Relationship Between Occlusion Time in Whole Blood and Lag Time to Thrombin Generation



Individual pairwise comparisons were conducted, and after Bonferroni correction, no significant differences were detected.

circulates at low concentrations, mostly in complex with its primary inhibitor, PAI-1.[28,29] uPA is synthesized by cells of fibroblast-type morphology but also epithelial cells,[30] monocytes, and macrophages.[31,32] Therefore, in a system that is devoid of cells, such as the plasma clot lysis assay, the quiescence of the fibrinolytic system is maintained by the excess of inhibitors, primarily PAI-1 and α_2-antiplasmin. Importantly, the use of whole blood in the GTT allows a more global assessment of fibrinolysis in blood, incorporating contributions from platelets, leukocytes, and erythrocytes. It is therefore not surprising that the correlation between the result of the GTT and the clot lysis assay was relatively weak.

RELATIONSHIP AMONG ENDOGENOUS FIBRINOLYSIS, SHEAR-INDUCED THROMBOTIC OCCLUSION, AND THROMBIN GENERATION. Our results indicate that endogenous fibrinolysis is directly related to the degree of shear-induced platelet aggregation, which correlates with the speed and the magnitude of thrombin generation. More rapid thrombin generation is associated with faster thrombotic occlusion at high shear, and the latter is related to the effectiveness of endogenous fibrinolysis. The observed relationship between thrombin generation in plasma and thrombotic occlusion at high shear is not surprising, given the fundamental role of the platelet surface in the generation of thrombin[33] and, furthermore, that thrombin is the most potent platelet agonist. However, given the significant contribution of the platelet surface in the promotion and regulation of thrombin generation and fibrin formation, it may not be surprising that we did not show a relationship between thrombin generation measured in *plasma* and endogenous fibrinolysis as measured in *whole blood*. In the Global Thrombosis Test, blood is subjected to a high shear rate, which is responsible for occlusive thrombus formation. In coronary arteries with severe luminal narrowing, pathological shear rates in excess of 10,000 seconds^{-1} are observed, which create a markedly prothrombotic milieu.[34,35] Although platelet aggregation is determined by $\alpha_{IIb}\beta_3$-dependent interactions under low shear conditions, von Willebrand Factor (vWF)-dependent interactions predominate at high shear rates,[35,36] and enhanced shear-induced platelet aggregation associated with increased vWF concentration has been documented in patients with myocardial infarction.[37] Whether inhibition of high-shear induced thrombosis may also favorably impact endogenous fibrinolysis requires investigation.

POSSIBLE CONTRIBUTION OF CELLULAR COMPONENTS TO ENDOGENOUS FIBRINOLYSIS IN WHOLE BLOOD. Our findings support the concept that cellular components are the main determinants of the effectiveness of fibrinolysis. Platelets are not only structural components of arterial thrombi; the platelet surface also plays a central role in the promotion and regulation of thrombin generation, in what has been termed the cell-based model of coagulation.[38] In this model, initiation of coagulation takes place on tissue factor-bearing cells, leading to activation of platelets and cofactors (amplification), which sets the stage for large-scale thrombin generation on the platelet surface.[38] Thus, platelets control thrombin generation, enhance fibrin formation, and regulate clot retraction. Although platelets are well-recognized as powerhouses of coagulation, their regulation of

fibrinolysis is less well defined. Indeed, they are known to harbor high concentrations of PAI-1 and other serpins, such as C1-inhibitor and protease nexin 1, that can inhibit fibrinolytic activity, with platelet activation leading to PAI-1 release.[39] Platelet PAI-1 is considered to be less active than plasma PAI-1, but its abundance means that it accounts for 50% of the circulating pool. We have recently shown that thrombi formed at high shear incorporate less t-PA and plasminogen but increased PAI-1, enhancing resistance to degradation.[40] The interaction of platelets with the fibrin matrix is key to the process of clot retraction, which is mechanistically coupled to fibrinolysis.[41]

However, plasminogen binding to platelets[42] and other cells including monocytes and neutrophils[43] is also well documented and with cell-bound plasmin protected from inhibition by α_2-antiplasmin, plasminogen activation on the cell surface is more efficient at augmenting fibrinolysis.[43] Although the inverse relationship between OT at high shear and endogenous fibrinolysis in our study supports the concept that platelets make the most important cellular contribution to resistance to lysis, the importance of other cellular components should not be underestimated. Neutrophils contribute to fibrinolysis through the release of elastase and additional membrane proteolytic activity, which aid early plasmin-mediated fibrinolysis,[44] and monocytes are rich in the receptor for uPA, promoting plasminogen activation. Extracellular nucleic acids and histones present in neutrophil extracellular traps promote coagulation through the binding of platelets, factor XII, vWF, and fibrinogen[45,46] and the expression of tissue factor.[47]

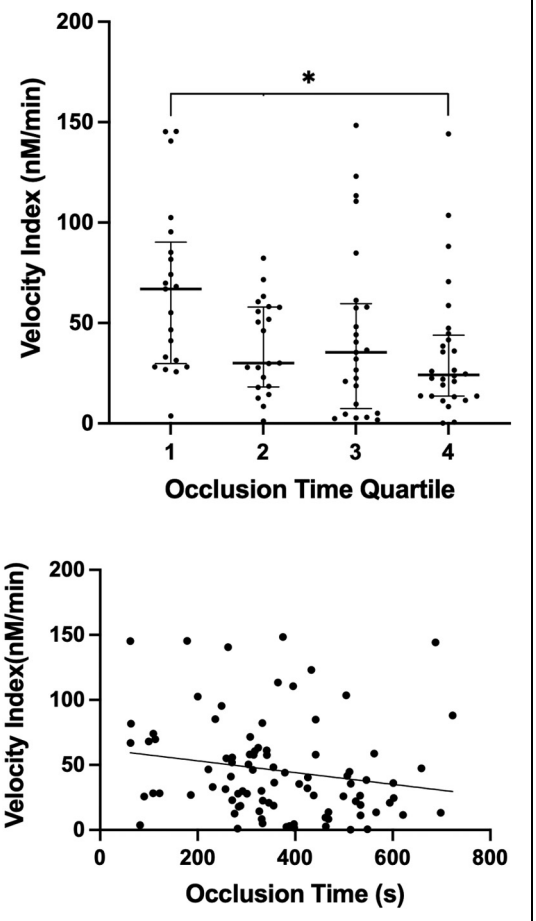
RELATIONSHIP BETWEEN WHOLE BLOOD FIBRINOLYSIS TIME AND HEMOGLOBIN. Although patients with LT ≥2,500 seconds had a slightly lower hemoglobin level than those with LT <2,500 seconds, the hemoglobin level in both groups was within the normal range. This may be a manifestation of renal impairment, reflected by the higher creatinine levels in patients with longer LT. Although the history of chronic kidney disease was not different between patients with LT ≥2,500 or <2,000 seconds, many of our patients presented for the first time with STEMI with no prior creatinine measurement available, and it is possible that the slightly lower hemoglobin and higher creatinine in patients with LT ≥2,500 seconds represents a higher prevalence of chronic kidney disease.

THROMBOSIS/FIBRINOLYSIS MARKERS AS PREDICTORS OF CARDIOVASCULAR RISK. Within the constraints of the small sample size, our analysis shows that whole blood endogenous LT is superior to plasma clot lysis, high shear-induced platelet reactivity, and markers of thrombin generation in predicting outcome in ACS patients. This is supported by prior work in ACS patients showing that prolonged whole blood endogenous fibrinolysis time was associated with an HR of 4.2,[48] whereas increased plasma clot LT was associated with HR = 1.29[6] for the occurrence of recurrent cardiovascular events.

The relationship between prolonged endogenous fibrinolysis time on admission and the occurrence of MACE may relate not only to hypofibrinolysis, but also to inflammation.

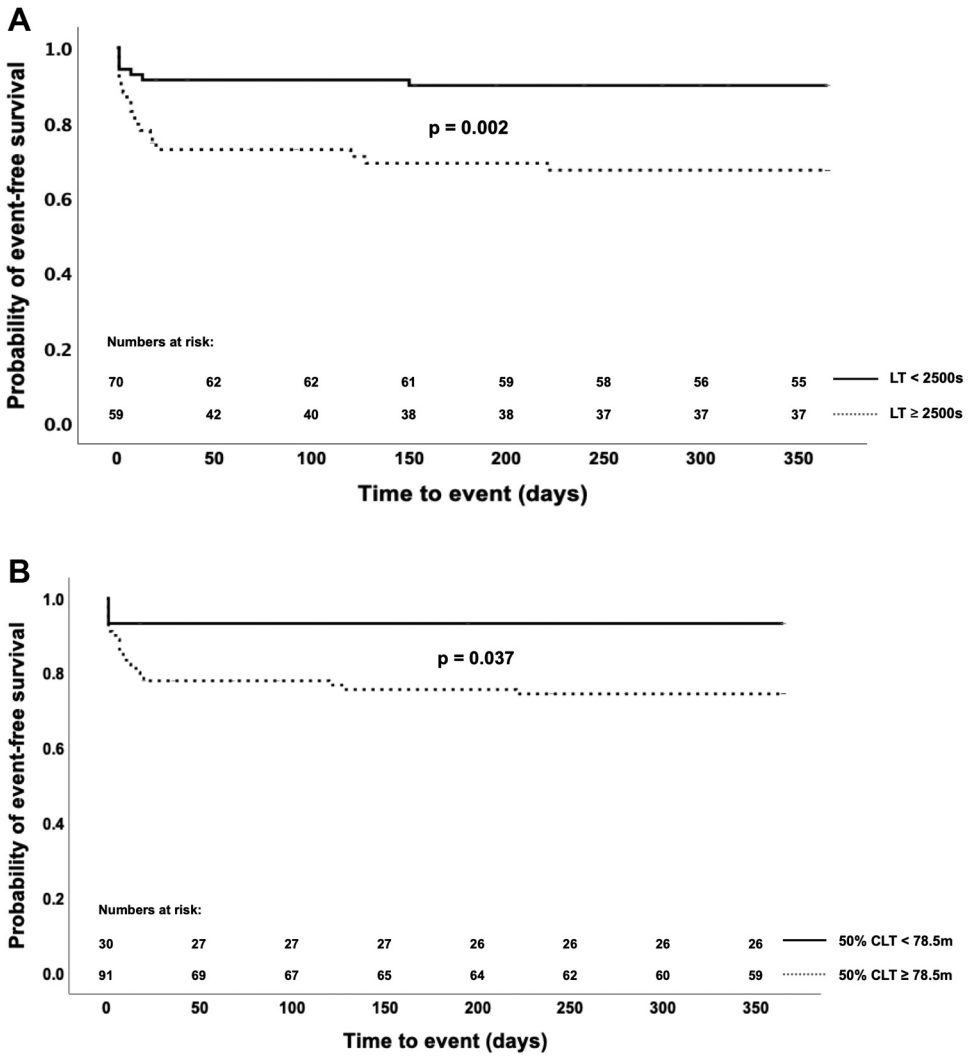
The correlation between hs-CRP and endogenous fibrinolysis and the close correlation between hs-CRP and fibrinogen levels reflect the close relationship

FIGURE 4 Relationship Between Occlusion Time in Whole Blood and Velocity Index of Thrombin Generation



Individual pairwise comparisons were conducted, and after Bonferroni correction, only the difference between Q1 and Q4 was significant. *$P < 0.05$.

FIGURE 5 Kaplan-Meier Curves Showing Event-Free Survival for Patients Based on Whole Blood Endogenous Fibrinolysis and Plasma Clot Lysis



Kaplan-Meier curves showing event-free survival for patients based on **(A)** whole blood endogenous fibrinolysis (measured in 129 patients): lysis time <2,500 and ≥2,500 seconds (log rank $P = 0.001$; HR: 3.6 [95% CI: 1.5-8.5; $P = 0.002$]) and **(B)** 50% plasma clot lysis time (measured in 121 patients [in 8 patients, stored plasma was insufficient for analysis]) <78.5 and ≥78.5 minutes (log rank $P = 0.037$; HR: 4.1 [95% CI: 0.96-17.2; $P = 0.057$]).

between inflammation and coagulation/fibrinolysis pathways.

STUDY LIMITATIONS. The sample size of our cohort is relatively small, but is sufficient to provide indications of relationships among markers of lysis, thrombin generation, and shear-induced thrombus formation in vitro. Importantly, we have taken only peripheral blood samples, and the markers measured in peripheral blood may not reflect the true pathophysiology of microenvironment at the site of the culprit coronary lesion in these STEMI patients. Furthermore, we cannot truly separate out the measures of fibrinolysis, including fibrinogen level, from inflammatory processes reflected by hs-CRP, and the crosstalk between these pathways is further supported by our findings. Although patients in the quartiles of LT were generally well-matched, smokers were over-represented among those with the longest LT, which may be causative or a confounder. Our study is too small to draw conclusions about the relationship between LT and clinical variables because of the small sample size, but this has been

evaluated in a larger cohort previously.[5] Although our study is not powered to assess the sensitivity of fibrinolysis markers to predict cardiovascular events, the finding of a strong relationship between endogenous fibrinolysis and MACE and a weaker relationship between plasma clot lysis and MACE mirrors earlier results from larger cohorts.[5,28]

CONCLUSIONS

The effectiveness of endogenous fibrinolysis in whole blood is related to fibrinogen and hs-CRP levels and shear-induced platelet reactivity, the latter being directly related to thrombin generation. Further, plasma clot lysis in response to tissue-plasminogen activator is only weakly related to endogenous fibrinolysis observed in whole blood, indicating an important role for cellular components in determining fibrinolytic status. Our data strengthen the evidence for a strong bidirectional crosstalk between coagulatory and inflammatory pathways. These mechanistic insights could help guide pharmacological strategies to treat hypofibrinolysis.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The clinical outcome of an arterial thrombotic stimulus is determined by the relative balance between prothrombotic drivers on the one hand, and the effectiveness of the natural thrombolytic (fibrinolytic) processes on the other hand. Impaired endogenous fibrinolysis is a recently recognized independent risk factor for adverse cardiovascular events in patients with acute coronary syndromes, but its mechanistic determinants are not well understood. In this study, we show that the effectiveness of endogenous fibrinolysis in whole blood is related in part to fibrinogen levels, inflammation, and shear-induced platelet reactivity. Furthermore, we show that plasma clot lysis is only weakly related to fibrinolysis in whole blood, suggesting an important role for cellular components in determining fibrinolytic status.

TRANSLATIONAL OUTLOOK: Because impaired fibrinolysis is a strong cardiovascular risk factor, improving fibrinolytic status is highly desirable, but we do not know how to modulate it pharmacologically. Our findings provide evidence for bidirectional crosstalk between coagulation and inflammation, and also demonstrate the importance of cellular components, especially high shear-induced platelet reactivity, as a determinant of fibrinolysis. This indicates the need for research to investigate novel ways to improve hypofibrinolysis, considering anti-inflammatory and antiplatelet approaches. Identification of individuals with hypofibrinolysis and subsequent favorable modification of this could reduce residual cardiovascular risk.

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KEY WORDS fibrinolysis, myocardial infarction, platelet function, thrombosis

APPENDIX For supplemental tables, please see the online version of this paper.