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Impact of very low dose rivaroxaban in addition to dual antiplatelet therapy on endogenous fibrinolysis in acute coronary syndrome: The VaLiDate-R study

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ABSTRACT

Background: Impaired endogenous fibrinolysis is adverse cardiovascular risk factor in acute coronary syndrome (ACS) patients. Addition of very low dose rivaroxaban (VLDR) to dual antiplatelet therapy (DAPT) reduces cardiovascular events but increases bleeding.

Objective: We aimed to assess whether addition of VLDR to DAPT can enhance endogenous fibrinolysis.

Methods: In a prospective, open-label trial, we assessed endogenous fibrinolysis in whole blood, in 549 patients with ACS using the Global Thrombosis Test (GTT) and Thromboelastography (TEG). Patients (n = 180) who demonstrated impaired endogenous fibrinolysis (lysis time [LT] >2000s with the GTT) were randomised 1:1:1 to (i) clopidogrel 75 mg daily; (ii) clopidogrel 75 mg daily plus rivaroxaban 2.5 mg twice daily; or (iii) ticagrelor 90 mg twice daily, for 30 days, in addition to aspirin. Fibrinolytic status was assessed at 0, 2, 4 and 8 weeks. The primary outcome was the change in LT from admission to week 4. We also measured thrombotic occlusion time (OT) at high shear, and rivaroxaban level.

Results: There was no difference between the groups with respect to LT or clot lysis with TEG, and no change in these parameters compared to baseline during study drug allocation. In the rivaroxaban plus clopidogrel group, OT was prolonged compared to the other groups, although rivaroxaban levels were low, suggesting non-compliance.

Conclusion: Addition of rivaroxaban 2.5 mg twice daily to DAPT does not affect endogenous fibrinolysis of thrombus formed at either high or low shear. Further studies are needed to determine whether higher doses of rivaroxaban can favourably modulate fibrinolysis.

Condensed abstract: Impaired endogenous fibrinolysis is a strong risk factor in ACS. We aimed to assess whether adding very low dose rivaroxaban (VLDR) to DAPT can enhance fibrinolysis. Fibrin and clot lysis were assessed in whole blood. ACS patients with impaired fibrinolysis were randomised 1:1:1 to clopidogrel 75 mg daily; clopidogrel 75 mg plus VLDR; or ticagrelor 90 mg twice daily, in addition to aspirin. At 30-days, there was no difference in lysis time between the groups, nor change from baseline. VLDR does not improve fibrinolysis at high

Abbreviations: ACS, Acute coronary syndrome; AF, Atrial fibrillation; CLT, Clot lysis time; CI, Coagulation Index; DAPT, Dual antiplatelet therapy; GTT, Global Thrombosis Test; LT, Lysis time; MA, Maximal amplitude; NOAC, Non-vitamin K antagonist oral anticoagulant; NSTEMI, Non-ST-segment elevation myocardial infarction; OAC, Oral anticoagulation; OT, Occlusion time; PPCI, Primary percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction; TEG, Thromboelastography; VLDR, Very low dose rivaroxaban.

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or low shear. Further studies are needed to determine whether alternative antithrombotic regimens can enhance endogenous fibrinolysis.

1. Introduction

The likelihood of coronary occlusion is determined by the balance between factors which propagate thrombosis, through platelet aggregation and activation of coagulation, and the effectiveness of the inherent defence mechanism of endogenous thrombolysis/fibrinolysis (1). Although there is increasing evidence to support the reduction of the potency or duration of dual antiplatelet therapy (DAPT) in patients at high bleeding risk (2), the majority of patients with acute coronary syndromes (ACS) are routinely treated with DAPT for 12 months (3,4), and despite this, some 15 % of patients experience recurrent adverse cardiovascular events within the first year. The mechanism driving this "residual risk" is not fully understood.

There is increasing evidence that impaired endogenous fibrinolysis is a strong independent risk factor for recurrent cardiovascular events (5). In patients with ACS, impaired fibrin clot lysis, measured in plasma using turbidimetry, was an independent predictor of cardiovascular risk (6). Other studies, assessing endogenous fibrinolysis in whole blood, have shown that patients with non-ST-segment elevation ACS (NSTE-ACS) who exhibit impaired endogenous fibrinolysis have a significantly increased risk of cardiovascular death, myocardial infarction or stroke (7), and in patients with ST-segment elevation myocardial infarction (STEMI), impaired fibrinolysis was associated with a 9-fold increased risk of subsequent adverse cardiovascular events (8). Recently, higher platelet-fibrin clot strength and lower fibrinolytic activity in ACS patients were also associated with excess cardiovascular risk (9).

While DAPT treatment addresses platelet activation, it does not affect endogenous fibrinolysis (8–10). Whether oral anticoagulant treatment, in addition to DAPT, can improve fibrinolysis and reduce the "residual risk" in ACS is unknown (11). Since anticoagulation increases the risk of bleeding, use of a personalised approach, targeting only high ischaemic risk patients with impaired fibrinolysis with more potent antithrombotic therapy could reduce cardiovascular risk, whilst avoiding excess bleeding in low risk patients (12).

We aimed to assess whether use of VLDR in addition to DAPT in ACS patients with impaired fibrinolysis, can improve fibrinolytic profile, compared to DAPT alone.

2. Methods

2.1. Study design and conduct

The VaLiDate-R study (ClinicalTrials.gov Identifier: NCT03775746, EudraCT: 2018-003299-11) was an investigator-initiated, randomised, open-label, single centre trial comparing the effects of aspirin combined with (i) ticagrelor, (ii) clopidogrel and (iii) clopidogrel together with VLDR, on fibrinolytic status in patients with ACS. The full methods have been published previously (protocol provided in Appendix) (13). Although guidelines recommend the use of potent $P2Y_{12}$ inhibitors prasugrel or ticagrelor, over clopidogrel in patients with ACS (14), the guidelines also allow the option to use clopidogrel, particularly if the patient is at high bleeding risk or elderly. During the study period, in our institution and in most of the UK, ticagrelor was used and preferred over prasugrel. The benefit of ticagrelor, over clopidogrel, in reducing ischaemic endpoints in the PLATO trial in the first 30 days post-ACS was modest (4.8 % vs. 5.4 %, $p = 0.045$) and most of the benefit of ticagrelor was seen in the period between 31 and 360 days post-infarct, where the effect of ticagrelor in reducing ischaemic events was highly significant ($p < 0.001$) (15). Therefore, we felt that the short treatment time of 4 weeks with clopidogrel in group 2, versus more potent antithrombotic treatment in groups 1 and 3 was not unreasonable in this study.

The study was sponsored by East and North Hertfordshire NHS Trust and funded by Bayer AG (Leverkusen, Germany) as an investigator-initiated study. Bayer AG had no input into the design, conduct, data analysis or write up of the results. The study was approved by the UK Health Research Authority and the local Research and Development Board, and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

The study design is shown in Fig. 1. All patients provided written informed consent.

A data safety monitoring board was established to review patient safety data at 6-monthly intervals and reported to a trial steering committee, which included independent cardiologists, statisticians and two lay members, to oversee the conduct of the study.

Unfortunately, impacted by the COVID-19 pandemic, some of the patients originally recruited were unable to attend for their subsequent study visits and some were advised to isolate/shield at home. Therefore, an extension was sought and granted, to increase the sample size when COVID-19 restrictions were eased, to reach the required sample size.

2.2. Study population

This was an all-comers clinical trial enrolling patients presenting with ACS. Consecutive patients aged 18 years or older, admitted with ACS (including those with STEMI, NSTE-ACS and unstable angina, see Supplement for definitions) and who fulfilled the inclusion and exclusion criteria (Table 1) were approached for consent.

A two-stage consent process was adopted whereby an eligible patient was first consented to a screening blood test to assess endogenous fibrinolysis, before proceeding to a full consent to allow randomisation and trial-related procedures. If fibrinolysis was impaired on the first test, then the patient was approached for full informed consent for randomisation.

In patients presenting with STEMI for primary percutaneous coronary intervention (pPCI), a delayed consenting approach was undertaken, with ethical permission, whereby screening blood tests for assessment of thrombotic status were taken at the same time as standard-of-care blood samples on arrival, and patients were consented for the study after the pPCI procedure when they were stable and able to carefully consider the study. If after the emergency procedure the patient declined to participate or was unable to consent, the patient was not included in the study.

2.3. Randomisation and study groups

In patients whose screening blood test showed that fibrinolytic status was impaired (LT >2000s), full written informed consent was obtained, and patients were randomised to one of 3 treatment arms, using a web-based block randomisation process, in a 1:1:1 ratio, to clopidogrel 75 mg daily (Group 1); clopidogrel 75 mg daily plus rivaroxaban 2.5 mg twice daily (Group 2); or ticagrelor 90 mg twice daily (Group 3). For patients randomised to a new $P2Y_{12}$ inhibitor, for example patients initially treated with ticagrelor who were subsequently randomised to clopidogrel, a loading dose of the new medication was given (clopidogrel 300 mg or ticagrelor 180 mg). The duration of rivaroxaban was 30 days, after which the patient stopped rivaroxaban and continued clopidogrel only or was switched to ticagrelor (including with loading), as decided by the clinical care team. The total duration of $P2Y_{12}$ inhibitor treatment was determined by the clinical care team. All patients were treated with aspirin 75 mg daily, and all other treatments were continued in accordance with standard of care, at the discretion of the clinical team.

2.4. Study follow-up

Follow up visits were conducted at weeks 2, 4 and 8 to assess thrombotic status and record any adverse events. At each visit, participants were asked about compliance and asked to return any unused medication. Additionally, patients in the rivaroxaban arm had Factor Xa levels assessed to confirm compliance during Visit 2 and 4, taken during the peak effect of rivaroxaban (between 2 and 5 h post-dose). A telephonic follow-up was performed at 6 months to assess for further clinical events.

2.5. Study related procedures

A single blood draw was performed to assess thrombosis and thrombolysis. The first 5 mL blood was used for routine clinical measurements and the subsequent sample used for the Global Thrombosis Test (GTT) assay, thromboelastography (TEG), and citrated plasma stored at −80 °C for subsequent analysis.

2.6. Blood sampling

For patients presenting with STEMI undergoing emergency coronary angiography, blood samples were taken upon arrival, before coronary intervention, from a 6 Fr arterial sheath, after DAPT loading but prior to the administration of heparin or other antithrombotic medication. Antiplatelet loading for these patients was usually aspirin 300 mg and clopidogrel 600 mg, administered in the ambulance. Some patients had additional loading with 180 mg of ticagrelor on arrival, at the discretion of the admitting physician.

For patients with NSTE-ACS, blood samples were taken at presentation using an 18G butterfly needle, after DAPT loading, and before anticoagulation. Care was taken to avoid prolonged tourniquet time and a large bore (usually antecubital) vein was used.

For all patients, the first 5 mL of blood was used for routine clinically-indicated tests. Of the subsequent 15 mL drawn for the assessment of thrombotic status, the first 5 mL of native (non-anticoagulated) blood was immediately tested within 15 s of withdrawal using the point-of-care Global Thrombosis Test (GTT), and the subsequent sample used for Thromboelastography (TEG). The remaining 5 mL blood was citrated, centrifuged at 2300 ×*g* for 10 min to yield platelet-poor-plasma, and stored at −80 °C for subsequent assessment.

2.7. Global thrombosis test

The GTT (Thromboquest Limited, London, UK) is point-of-care test of thrombotic status that utilises native (non-anticoagulated) blood to assess thrombosis (occlusion time) and thrombolysis (lysis time). The technique has previously been described (16). A 4 mL blood sample was introduced into the cartridge and the measurement is fully automated. Blood flows under the influence of gravity at 37 °C, and is exposed to high shear stress, resulting in platelet activation. Further downstream, low shear and turbulent flow favour large platelet aggregate formation leading to the generation of thrombin and onset of thrombosis. Flow then carries these fibrin-coated platelet aggregates downstream, leading to thrombotic occlusion. Downstream, a light-interruption sensor detects the blood drops; the instrument measures the time between two consecutive drops (d). At a predefined point ($d \geq 15$ s), the occlusion time (OT, s) is detected. After a 15 s stabilisation period, during which the light sensor is inactive, the instrument detects the restart of flow due to endogenous fibrinolysis and this is recorded as the lysis time (LT, s).

2.8. Thromboelastography

Whole blood was also assessed using the point-of-care TEG (Haemonetics Corporation, USA). This measures the viscoelastic properties of blood as it clots under low shear stress, based on pin-and-cup technology. Whole blood is placed in the cup which oscillates 4°45′ every 5 s while a pin on a torsion wire is suspended in the blood. As the viscoelastic strength of the clot increases, more rotation is transmitted to the torsion wire and is detected by an electromagnetic transducer. The kinetic changes during clot formation and lysis are shown graphically. (17) The measured parameters include the kinetics (K index, a measure of platelet function), reaction time (R) - the time taken until the first detectable clot formation, coagulation index (CI), angle (A) which indicates the rate of fibrin-formation, maximal amplitude (MA) reflecting the platelet contribution to clot formation, and clot lysis time (CLT),

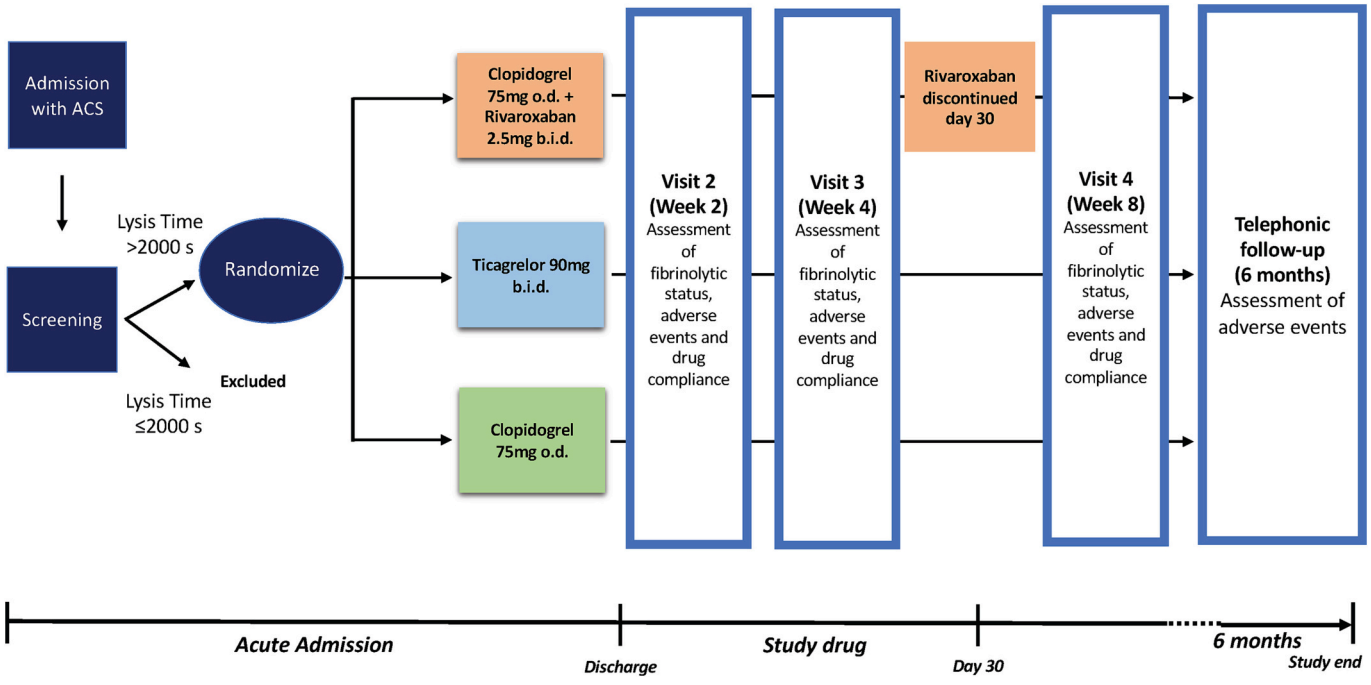

Fig. 1. Outline of study and follow-up procedures.

Table 1
VaLiDate-R inclusion/exclusion criteria.

Inclusion	Exclusion
1. Male and female patients aged 18 years or over	1. Male and female participants aged <18 years of age.
2. Have a diagnosis of acute coronary syndrome requiring treatment with dual antiplatelet therapy	2. Patient unwilling or unable to give informed consent
3. Be willing and able to understand the Participant Information Sheet and provide informed consent	3. Patients who might be pregnant or are breast-feeding
4. Agree to comply with the drawing of blood samples for the assessments	4. Active clinically significant bleeding
5. Not meet any of the exclusion criteria	5. Patient who, in the opinion of the investigator, has condition considered to be a significant risk for major bleeding (such as current or recent gastrointestinal ulceration, presence of malignant neoplasm at high risk of bleeding, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial haemorrhage, known or suspected oesophageal varices, arteriovenous malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities) 6. Hepatic disease associated with coagulopathy and clinically relevant bleeding risk including cirrhotic patients with Child Pugh B and C 7. Patient with any contraindications to use of antiplatelet agents or anticoagulants 8. Hypersensitivity to the active substance or to any of the excipients listed in Section 6.1 of Summary of Product Characteristics (SmPC) of Rivaroxaban 9. Concomitant treatment with any other anticoagulants e.g. unfractionated heparin (UFH), low molecular weight heparins (enoxaparin, dalteparin, etc.), heparin derivatives (fondaparinux, etc.), oral anticoagulants (warfarin, dabigatran, apixaban etc.) except under specific circumstances of switching anticoagulant therapy or when UFH is given at doses necessary to maintain an open central venous or arterial catheter 10. Concomitant treatment of ACS with antiplatelet therapy in patients with a prior stroke or a transient ischaemic attack (TIA) 11. Patient with ongoing active alcohol or substance abuse or demonstrates signs or clinical features of active substance abuse. 12. Patient with any major bleeding diathesis or blood dyscrasia at baseline (platelets<70 × 10^9/l, Hb < 80 g/l, INR > 1.4, APTT> x 2UNL, leucocyte count<3.5 × 10^9/l, neutrophil count<1 × 10^9/l) 13. Patient currently enrolled in an investigational drug trial

which is also assessed at 30 and 60 min, and recorded as Lysis 30 (LY30) and Lysis 60 (LY60) (Supplementary Table 1).

2.9. FXa inhibition

The impact of rivaroxaban on factor X (FX) levels in patients in Group 2 (clopidogrel plus rivaroxaban) was measured at the week 2 visit, at 4 h after the last rivaroxaban dose, using a specific chromogenic assay for anti-FXa activity with rivaroxaban calibrators, using an amidolytic anti-FXa assay with drug-specific calibrators (STA®-Liquid Anti-Xa, STAGO, Sulhamstead, UK) on a STAR Max 2 analyser (STAGO, Sulhamstead, UK). The rivaroxaban anti-FXa levels were measured by investigators blinded to the GTT and TEG results.

2.10. Primary and secondary endpoints

The primary outcome was the change in fibrinolytic status as measured by lysis lime (LT) from admission to follow-up at 4 weeks post-discharge. Secondary outcomes were measures of fibrinolysis using the TEG and the effect on thrombotic occlusion time at high shear (OT).

In addition, the occurrence of major adverse cardiovascular events (composite of recurrent myocardial infarction, stroke or cardiovascular death) and bleeding events (evaluated by BARC criteria) were evaluated up to 6 months (18).

2.11. Sample size calculation

Sample size was calculated using Stata version 15.1 (StatCorp, College Station, TX, USA). Assuming $\alpha = 0.05$ for two sided tests, to detect a difference of LT = 90s with a power $1\text{-}\beta = 0.80$, a sample size of 45 in each group was required. Earlier published work had shown that in patients with ACS, LT has a standard deviation with an upper limit of 150 s (7). Given the range of typical LT scores, and the indication of a transition in health benefits below 300 s, a 90 s difference in LT at the primary assessment point, as the basis for the study design, was felt to provide a reasonable starting point in the absence of any other data. Accounting for a 10 % drop-out, withdrawal and loss to follow up, we calculated that 50 patients per group would be needed, resulting in a sample size of 150. Due to the impact of the COVID-19 pandemic, some of the patients originally recruited were unable to attend hospital for their study visits and therefore, following the easing of COVID-19 restrictions, ethics permission was sought and granted to increase the total sample size to 180.

2.12. Statistical analysis

We aimed to compare the effects of 3 different antithrombotic treatment strategies on endogenous fibrinolysis. The study evaluated the extent to which the combination of very low dose rivaroxaban and clopidogrel (Group 2) impacted on LT, compared to clopidogrel (Group 1) or ticagrelor (Group 3). An intention-to-treat analysis was used and the data of patients who decided to not attend for some of the study, due to the need to shield during the COVID 19 pandemic, was still included in the analysis. ANOVA was used to compare the means of all three treatment groups, with separate two-sample comparisons used to evaluate univariable effects between the study groups at 2, 4 and 8 weeks. Similarly, the influence of very low dose rivaroxaban was evaluated by considering change over time from baseline using two sample comparisons between groups. The size of the effect between groups was measured using Cohen's d, both for absolute and change from baseline values. These analyses were repeated for the secondary outcome, OT. Mean scores and change in scores from baseline for TEG outcomes (R, K, A, MA, CI, LY30, LY60, time to MA [TMA], CLT) were also compared at each time point. Continuous variables were expressed as mean ± standard deviation (SD) or as median [interquartile range (IQR)], while categorical variables were presented as absolute numbers and frequencies (%). Analyses were performed using Stata version 15.1 (StatCorp, College Station, TX, USA) and $p < 0.05$ taken as significant.

3. Results

A total of 549 patients were screened and of these, 180 patients were randomised (Fig. 2). Patients in the three groups were well matched for baseline clinical and laboratory characteristics, and medications on discharge (excepting study drug) (Table 2). In total 98 % of patients had angiography, and of those 92 % had significant coronary disease (one or more epicardial stenoses at or >50 %). The decision to withdraw patients from the study following randomisation was due to either the development of an indication for oral anticoagulation, predominantly atrial fibrillation or left ventricular thrombus, or due to the patient's

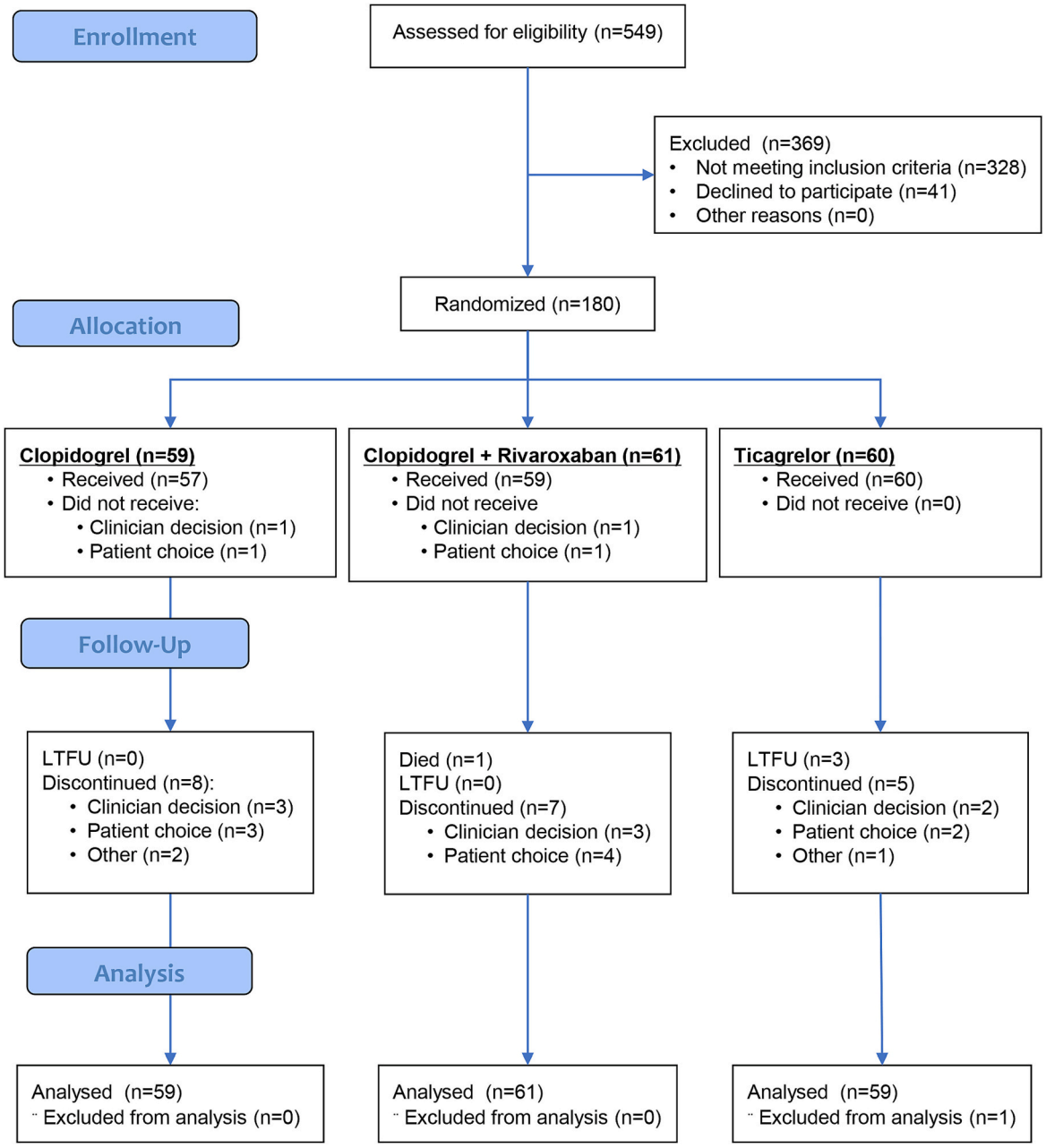

Fig. 2. Consort diagram showing recruitment and withdrawal from study.

wishes (almost exclusively due to the wish to shield and avoid the hospital environment and associated risk of infection during the COVID-19 pandemic). The new need for full anticoagulation in some patients identified after randomisation, accounts for the fact that not all patients were treated with aspirin and/or a $P2Y_{12}$ inhibitor at discharge.

3.1. Endogenous fibrinolysis

There was no difference in lysis time between the patients in the three groups at baseline, but in all three groups there was a reduction in LT at week 2 compared to baseline values (Tables 3 and 4). There was a significant difference in LT at week 2 between the groups, with those patients on ticagrelor having the shortest LT and the greatest reduction in LT compared to baseline (Table 4). By week 4, there was no difference in LT between the three groups and no overall change in LT compared to baseline (Table 4 and Fig. 3 [Visual Abstract]). At week 8, there was no difference in LT between the groups, no difference compared to baseline, and no difference compared to week 4.

3.2. Occlusion time

There was no difference in occlusion time between the patients in the three groups at baseline (Supplementary Table 2). However, at 2 weeks and 4 weeks, there were significant differences in OT between the three groups, with the longest OT observed in patients on clopidogrel plus rivaroxaban (Supplementary Tables 2 and 3). There was also a difference in the change in OT compared to baseline, between the three groups, with the greatest prolongation in OT compared to baseline, at weeks 2 and 4, being observed in patients on clopidogrel plus rivaroxaban.

3.3. Thromboelastography

The results of TEG are shown in Supplementary Table 4. There were

Table 2
Baseline clinical and laboratory characteristics of study population.

	Entire population ($n = 180$)	Clopidogrel ($n = 59$)	Clopidogrel plus rivaroxaban ($n = 61$)	Ticagrelor ($n = 60$)	*P* value
Clinical characteristics					
Age, years (SD)	62.4 (12.0)	62.0 (11.5)	62.3 (12.3)	62.9 (12.4)	0.915
Male, n (%)	146 (81.1)	51 (86.4)	46 (75.4)	49 (81.7)	0.305
Race					0.226
Caucasian, n (%)	161 (89.4)	55 (93.2)	52 (85.3)	54 (90.0)	
Black, n (%)	3 (1.7)	1 (1.7)	0 (0.0)	2 (3.3)	
Asian, n (%)	16 (8.9)	3 (5.1)	9 (14.8)	4 (6.7)	
BMI, kg/m^2 (SD)	28.3 (5.1)	28.1 (5.0)	28.3 (4.9)	28.4 (5.4)	0.934
Current smoker, n (%)	51 (28.7)	14 (24.1)	21 (35.0)	16 (26.7)	0.396
Alcohol, units/week (SD)	4.1 (13.7)	4.9 (11.1)	2.7 (6.0)	4.8 (20.2)	0.608
Hypertension, n (%)	60 (33.7)	16 (27.6)	20 (33.3)	24 (40.0)	0.365
Diabetes, n (%)	33 (18.5)	12 (20.7)	11 (18.3)	10 (16.7)	0.855
Hypercholesterolaemia, n (%)	40 (22.5)	13 (22.4)	11 (18.3)	16 (26.7)	0.554
Prior Angina, n (%)	12 (6.7)	4 (6.9)	4 (6.7)	4 (6.7)	0.998
Prior MI, n (%)	16 (9.0)	5 (8.6)	6 (10.0)	5 (8.3)	0.945
Prior PCI, n (%)	16 (9.0)	5 (8.6)	5 (8.3)	6 (10.0)	0.945
Prior CABG, n (%)	5 (2.8)	1 (1.7)	1 (1.7)	3 (5.0)	0.456
HF, n (%)	2 (1.1)	1 (1.7)	1 (1.7)	0 (0.0)	0.602
CKD, n (%)	4 (2.3)	1 (1.7)	1 (1.7)	2 (3.3)	0.787
CVA/TIA, n (%)	4 (2.3)	2 (3.5)	1 (1.7)	1 (1.7)	0.757
PAD, n (%)	3 (1.7)	0 (0.0)	2 (3.3)	1 (1.7)	0.376
Prior bleeding, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–
SBP, mmHg (SD)	137 (24.1)	136 (22.5)	136 (27.3)	138 (22.5)	0.885
DBP, mmHg (SD)	80 (14.8)	81 (15.0)	80 (14.2)	80 (15.3)	0.960
ACS presentation					0.588
STEMI, n (%)	77 (44.5)	22 (39.3)	30 (51.7)	25 (42.4)	
NSTEMI, n (%)	90 (52.0)	32 (57.1)	25 (43.1)	33 (55.9)	
UA, n (%)	6 (3.5)	2 (3.6)	3 (5.2)	1 (1.7)	
Laboratory characteristics					
Hb, g/dL (IQR)	144 (130–153)	145 (135–153)	143 (125–155)	144 (134–152)	0.454
WCC, x10^9/L (IQR)	9.9 (7.6–12)	9.9 (7.6–12.2)	9.9 (8.2–11.8)	9.8 (7.3–11.5)	0.891
Neutrophil, % (IQR)	6.69 (4.99–8.85)	6.82 (4.82–9.10)	6.69 (5.16–8.72)	6.06 (5.00–8.56)	0.975
Platelets, x10^9/L (IQR)	237 (194–286)	238 (190–286)	236 (201–278)	239 (191–292)	0.856
INR, (IQR)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	0.953
PT, s (IQR)	10.7 (10.3–11.1)	10.8 (10.3–11.2)	10.6 (10.3–11.0)	10.8 (10.3–11.1)	0.989
APTT, s (IQR)	23.1 (21.3–24.6)	23.6 (21.3–24.8)	23.0 (21.3–24.3)	23.0 (21.6–24.6)	0.322
Fibrinogen, g/l (IQR)	3.5 (3.0–4.1)	3.7 (2.7–4.2)	3.5 (3.1–4.0)	3.5 (3.0–4.2)	0.618
Creatinine, μmol/L (IQR)	82 (71–97)	83 (72–97)	86 (75–97)	76 (68–93)	0.230
Albumin, g/L (IQR)	43 (41–45)	44 (41–45)	43 (41–45)	43 (42–46)	0.541
CRP, mg/L (IQR)	4 (2–9)	4.0 (1.7–10.7)	4.2 (2.0–8.3)	4.0 (1.0–9.0)	0.417
Total cholesterol, mmol/L (IQR)	4.7 (4.1–5.6)	5.2 (4.1–5.7)	4.6 (4.2–5.6)	4.7 (3.8–5.2)	0.352
HDL cholesterol, mmol/L (IQR)	1.07 (0.95–1.33)	0.96 (0.84–1.32)	1.09 (1.02–1.19)	1.15 (1.01–1.46)	0.361
LDL cholesterol, mmol/L (IQR)	2.79 (1.97–3.44)	3.08 (1.87–3.93)	2.79 (2.24–3.44)	2.46 (1.96–3.23)	0.675
Triglycerides, mmol/L (IQR)	1.65 (1.24–2.42)	1.83 (1.24–2.75)	1.66 (1.36–2.33)	1.54 (1.12–2.28)	0.406
Discharge medications					
Aspirin, n (%)	168 (93.3)	53 (89.8)	58 (95.1)	57 (95.0)	0.735
Clopidogrel, n (%)	112 (62.2)	54 (91.5)	58 (95.1)	0 (0.0)	NA
Ticagrelor, n (%)	57 (31.7)	0 (0.0)	0 (0.0)	57 (95.0)	NA
Beta-blocker, n (%)	140 (77.9)	43 (72.9)	48 (78.7)	49 (81.7)	0.789
ACEi, n (%)	129 (71.7)	43 (72.9)	42 (68.9)	44 (73.3)	0.557
ARB, n (%)	13 (7.2)	2 (3.4)	6 (9.8)	5 (8.3)	0.410
Statin, n (%)	149 (82.8)	45 (76.3)	52 (85.2)	52 (86.7)	0.560
Insulin, n (%)	13 (7.2)	6 (10.2)	5 (8.2)	2 (3.3)	0.299
Nitrate, n (%)	14 (7.8)	4 (6.8)	4 (6.6)	6 (10.0)	0.760

Values are presented as mean (SD), median (IQR) or n (%). CKD defined as creatinine >177 μmol/L. Prior aspirin defined as regular use pre-hospitalization. Family history of premature CAD defined as a diagnosis of CAD in a first-degree relative <60 years. Values in bold are significant (i.e. $p < 0.05$).
Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACS, acute coronary syndrome; AF, atrial fibrillation; APTT, activated partial thromboplastin time; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CKD, chronic kidney disease; CRP, high sensitivity c-reactive protein; CVA, cerebrovascular accident; DBP, diastolic blood pressure; Hb, haemoglobin; HF, heart failure; INR, international normalized ratio; IQR, interquartile range; LDL, low density lipoprotein; MI, myocardial infarction; NSTEMI, non-ST segment elevation myocardial infarction; OT, occlusion time; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; PT, prothrombin time; SBP, systolic blood pressure; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction; UA, unstable angina; WCC, white cell count.

some observed baseline differences between the 3 groups, for example in native blood with respect to the rate and strength of clot formation.

At two weeks, the rate of clot formation (A) was lowest with clopidogrel plus rivaroxaban, but the change from baseline was not different between the groups. With kaolin, the speed of clot formation (K) was longest with clopidogrel plus rivaroxaban, with the change from baseline different between the groups.

At four weeks, with kaolin, the rate of clot formation (A) was lowest with clopidogrel plus rivaroxaban, but the change from baseline was not different between the groups. With kaolin, the speed of clot formation (K) was longest with clopidogrel plus rivaroxaban, but the change from baseline was not different between the groups.

Table 3
Lysis time at baseline and during follow-up.

	Study Arm			P value (ANOVA)
	Clopidogrel	Clopidogrel + rivaroxaban	Ticagrelor	
	Mean (SD)	Mean (SD)	Mean (SD)	
Visit 1 (baseline)				
Lysis time *(seconds)*	3066.4 (1107.3)	2978.8 (1211.9)	3149.0 (1233.4)	0.733
Visit 2 (2 weeks)				
Lysis time	2788.7 (1211.1)	2920.2 (1220.1)	2307.8 (742.8)	**0.019**
Difference from baseline	−302.9 (1357.7)	−168.5 (1471.4)	−912.5 (1365.7)#	**0.029**
Visit 3 (4 weeks)				
Lysis time *(seconds)*	2781.4 (1278.1)	2877.8 (1394.8)	2420.2 (976.6)	0.172
Difference from baseline	−338.5 (1463.5)	−27.4 (1483.3)	−692.4 (1319.4)#	0.084
Visit 4 (8 weeks)				
Lysis time *(seconds)*	2186.8 (854.1)	2249.6 (795.8)	2331.3 (1032.7)	0.774
Difference from baseline	−852.1 (983.6)#	−876.2 (1486.2)#	−779.4 (1427.3)#	0.943
Difference from visit 3 (week 4)	−463.7 (1273.2)#	−515.9 (1003.0)#	45.9 (1048.9)	0.058

p-values < 0.05 (paired t-tests; within group differences).

At eight weeks, the rate of clot lysis (percentage clot lysis by 60 min) was numerically greatest in patients treated with clopidogrel plus rivaroxaban, but the change from baseline in clot lysis was not significantly different between the groups.

Importantly, during the 4 weeks of randomised treatment allocation, we saw no difference between the three treatments groups with regard to any change in the indices of fibrinolysis compared to baseline, namely clot lysis time (CLT) or percentage of clot that had lysed after 30 or 60 min (LY30 and LY60, respectively).

3.4. Rivaroxaban anti-FXa levels

Rivaroxaban anti-FXa levels varied widely amongst patients in Group 2 and were on average 58.38+/− 62 (range 0 to 385) ng/mL with undetectable levels in 15 % of the group, and levels at or below 50 ng/mL in 62 % of patients. Within this group, there was no correlation between the anti-FXa level and LT at 2 weeks or 4 weeks (Supplementary Table 5). Furthermore, in Group 2, there was no correlation between anti-FXa levels and the change in LT between baseline and week 2, or the change in LT between baseline and week 4. There was no correlation between anti-FXa level and OT. At 2 weeks, there was no relationship between TEG indices and anti-FXa levels. At 4 weeks, anti-FXa levels correlated modestly with LY30 ($r = -0.591$, $p = 0.021$) and LY60 ($r = -0.536$, $p = 0.039$) in the presence of kaolin but not native blood.

3.5. Cardiovascular events

There were no significant differences between the groups with respect to cardiovascular events or bleeding events during the follow-up period (Table 5).

4. Discussion

In this prospective randomised trial assessing the effect of different combinations of antithrombotic therapies in patients with ACS with prolonged endogenous fibrinolysis at baseline, we saw no difference in endogenous lysis time between patients assigned to the three different groups, at either 2 weeks or 4 weeks of treatment. We also did not observe a difference in clot lysis time with TEG. Although only a secondary analysis, we observed longer OT in patients assigned to clopidogrel plus rivaroxaban, compared to the other groups, and the greatest prolongation of OT, compared to baseline, was seen in response to clopidogrel plus rivaroxaban. Similarly, this group had the lowest maximal amplitude of clot formation when assessed with thromboelastography, representing the lowest fibrin clot strength, likely due to the impact of clopidogrel and rivaroxaban on platelets aggregation and fibrin formation. However, other studies have shown that prolongation of OT by antithrombotic medication may not be reflected in changes in LT, as seen in both patients with STEMI (8) and in patients with stable coronary disease (19).

The lack of effect of adding rivaroxaban to clopidogrel, on either endogenous fibrinolysis (assessing thrombus formation under conditions of high shear) or clot lysis (assessing thrombus formation at low shear), is unexpected and is at odds with our predictions. However, despite good compliance reported by patients on direct questioning, rivaroxaban levels were unexpectedly low in the majority of patients assigned to clopidogrel plus rivaroxaban group, which likely represents non-compliance. Although patients were closely followed-up, the open label design of the study, together with the explicit statement in the patient consent that rivaroxaban may increase bruising and bleeding, may have contributed to non-compliance in this cohort. The variation in anti-FXa levels is large and overwhelms any potential treatment effect. There remains the possibility that there are other more complex confounding effects that cannot be evaluated with the current dataset. The

Table 4
Effect size of the observed lysis time (unadjusted).

	Comparison					
	Clopidogrel + Rivaroxaban vs Clopidogrel		Ticagrelor vs Clopidogrel		Clopidogrel + Rivaroxaban vs Ticagrelor	
	Mean (95 % CI)	Cohen's d' (95 % CI)	Mean (95 % CI)	Cohen's d' (95 % CI)	Mean (95 % CI)	Cohen's d' (95 % CI)
Visit 2 (2 weeks)						
Lysis time *(seconds)*	131.5 (−387.7, 650.7)	0.1 (−0.3, 0.5)	−480.9 (−917.5, −44.4)*	−0.5 (−0.9, −0.1)	612.5 (192.8, 1032.1)*	0.6 (0.2, 1.0)
Difference from baseline	134.5 (−471.5, 740.5)	0.1 (−0.3, 0.5)	−550.8 (−943.7, 221.4)	−0.4 (−0.8, 0.0)	685.2 (102.4, 1268.1)*	0.5 (0.1, 0.9)
Visit 3 (4 weeks)						
Lysis time *(seconds)*	96.4 (−479.2, 671.9)	0.1 (−0.4, 0.5)	−361.3 (−840.0, 117.5)	−0.3 (−0.7, 0.1)	457.7 (−44.1, 959.4)	0.4 (0.0, 0.8)
Difference from baseline	311.1 (−321.7, 943.9)	0.2 (−0.2, 0.6)	−298.7 (−883.0, 285.6)	−0.2 (−0.6, 0.2)	609.8 (33.5, 1186.0)*	0.4 (0.0, 0.9)
Visit 4 (8 weeks)						
Lysis time *(seconds)*	62.8 (−302.0, 427.7)	0.1 (−0.4, 0.5)	144.5 (−280.7, 569.6)	0.2 (−0.3, 0.6)	−81.6 (−485.6, 322.4)	−0.1 (−0.5, 0.3)
Difference from baseline	−24.1 (−578.7, 530.6)	0.0 (−0.5, 0.4)	62.6 (−404.6, 732.1)	0.1 (−0.4, 0.5)	−86.7 (−735.7, 562.3)	−0.1 (−0.5, 0.4)

* $p < 0.05$ (two-sample t-tests).

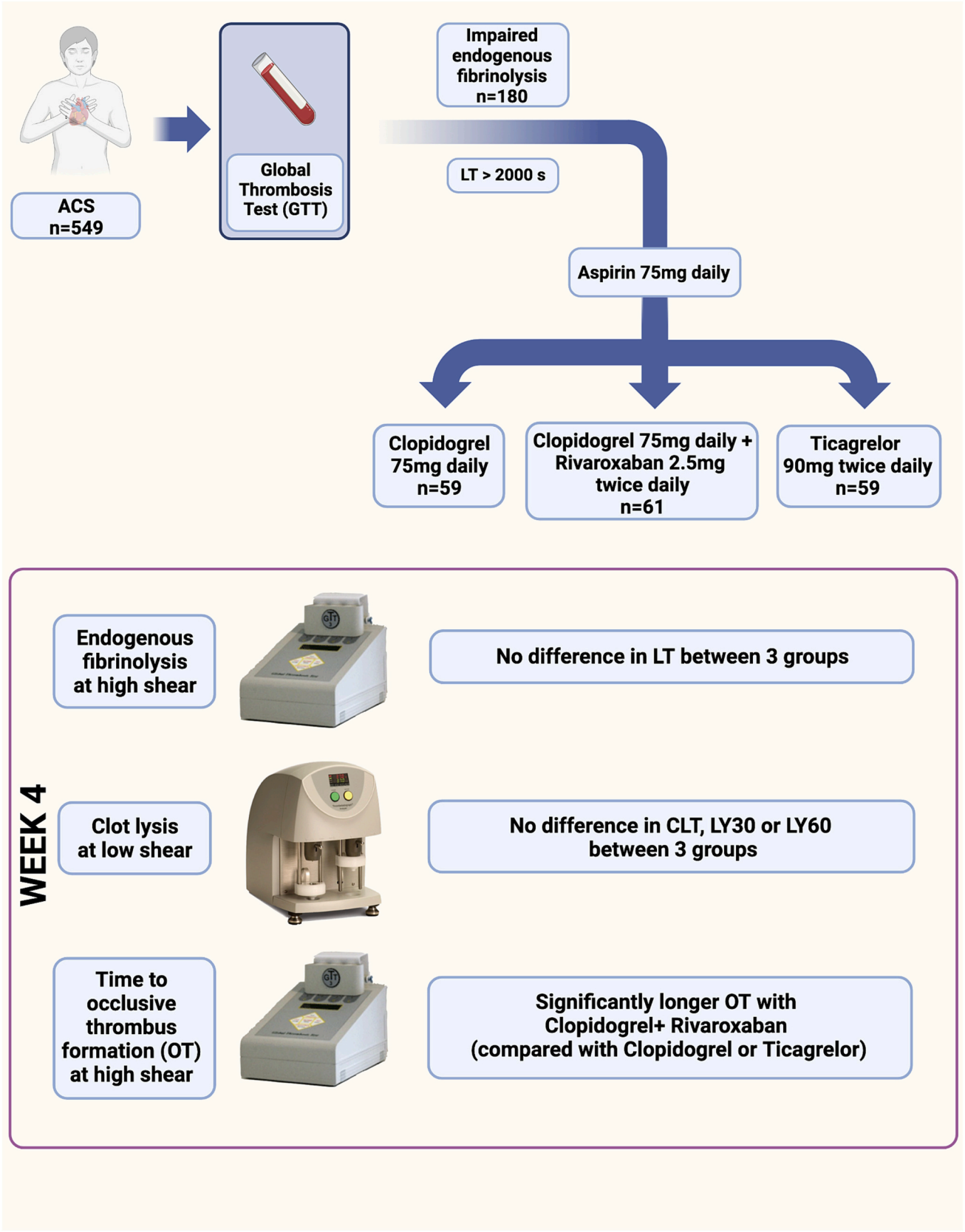

Fig. 3. (Visual abstract). We randomly allocated 180 patients with acute coronary syndromes (ACS) with lysis time above 2000 s, to one of three antithrombotic regimens (clopidogrel, ticagrelor, or clopidogrel plus rivaroxaban 2.5 mg twice daily), in addition to aspirin, for 4 weeks. At the end of 4 weeks of treatment, there was no difference in endogenous fibrinolysis of thrombus formed at either high or low shear. Thrombus formation at high shear was much more attenuated in patients on clopidogrel plus rivaroxaban, compared to those with clopidogrel or ticagrelor. Abbreviations: GTT, Global Thrombosis Test; OT, occlusion time; LT, lysis time; CLT, clot lysis time; LY30, percentage of clot which has lysed after 30 min; LY60, percentage of clot which has lysed after 60 min.

Table 5
Clinical outcomes at 6 months' follow-up.

	Entire population (n = 180)	Clopidogrel (n = 59)	Clopidogrel + rivaroxaban (n = 61)	Ticagrelor (n = 60)	p-value
MACE (cardiovascular death, re-infarction, CVA)	3 (1.67)	1 (1.69)	1 (1.64)	1 (1.67)	1.000
Cardiovascular death	1 (0.56)	0 (0.0)	1 (1.64)	0 (0.0)	0.375
New myocardial infarction or re-infarction	1 (0.56)	1 (1.69)	0 (0.0)	0 (0.0)	0.357
Cerebrovascular accident	1 (0.56)	0 (0.0)	0 (0.0)	1 (1.67)	0.366
Major bleeding (BARC type 3–5)	1 (0.56)	0 (0.0)	0 (0.0)	1 (1.67)	0.366
Minor bleeding (BARC type 1–2)	2 (1.11)	0 (0.0)	1 (1.64)	1 (1.67)	0.611
Non-cardiovascular death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n/a

Values are n (%). MACE: major adverse cardiovascular events, CVA: cerebrovascular accident, BARC: Bleeding Academic Research Consortium.

fact that the majority of patients report taking rivaroxaban as prescribed may also indicate that some patients had a sub-therapeutic dose.

Impaired (prolonged) endogenous fibrinolysis is a recently recognised risk factor for ongoing cardiovascular risk in patients with ACS, and indeed, rapid (effective) endogenous fibrinolysis has been shown to be associated with improved outcome and reduced infarct size in patients with STEMI (20). The measurement of endogenous fibrinolysis in clinical practice is feasible, since it is a point-of-care test that requires no specialist laboratory expertise and the instrument is commercially available.

In a previous study of STEMI patients, treatment with DAPT did not appear to reduce LT, either at hospital discharge or at 30 days, compared to admission (8). Overall, this was confirmed in this study, even using clopidogrel plus rivaroxaban, with no significant change in LT compared to baseline across the 3 groups.

Rivaroxaban is a direct inhibitor of free and clot-bound FXa, which thereby prevents thrombin generation. Thrombin is a key protein in fibrin clot formation, and an important determinant of the strength and stability of the fibrin clot and its resistance to fibrinolysis (21). Since thrombin is also the most potent activator of platelet aggregation in vivo (22), rivaroxaban would be expected to also reduce platelet aggregation in part by reducing thrombin generation, and in part by blocking the direct effects of FXa via protease activator receptor signalling (22). Therefore, rivaroxaban would be expected to reduce endogenous fibrinolysis time and reduce platelet aggregation. The impact on platelet aggregation is likely reflected here in the prolongation of occlusion time and reduction in the maximum amplitude of clot formation, but an impact on fibrinolysis time was not seen. It is also possible that the dose of rivaroxaban used here was too low to impact endogenous fibrinolysis.

In a small study in patients with atrial fibrillation, treatment with non-vitamin K antagonist oral anticoagulation (full treatment dose) appeared to favourably enhance endogenous fibrinolysis (23). All non-vitamin K antagonist oral anticoagulants (NOACs) studied (dabigatran, rivaroxaban, apixaban) exhibited a trend toward enhancing endogenous thrombolytic status, although this was significant only for apixaban, and notably all agents were given at the full treatment dose licensed for thromboprophylaxis of atrial fibrillation, unlike the "vascular" dose of rivaroxaban used in the present study, which was a quarter of the dose used for conventional thromboprophylaxis in atrial fibrillation. Treatment with apixaban 5 mg twice daily improved endogenous fibrinolysis time (LT pre-treatment 2204 [1779–2738] vs. on-treatment 1882 [1607–2374] s, $p = 0.0003$), whereas apixaban did not alter TEG indices, except for a small reduction in the rate of clot formation with kaolin (68.4 vs. 67, $p = 0.026$) TEG (24). Change in LT (ΔLT) with apixaban correlated with baseline LT ($r = 0.77$, $p < 0.0001$). The magnitude of effect of apixaban on reducing LT was greatest in those with the longest LT at baseline. It is possible that while full dose anticoagulation with a NOAC may significantly lower LT, the low "vascular" dose rivaroxaban of 2.5 mg twice daily had little impact on LT, and we may have seen a significant effect had 15 mg or 20 mg daily doses been used.

In addition to the lack of effect on endogenous fibrinolysis, we also did not observe any consistent or significant effect of rivaroxaban plus clopidogrel, compared to ticagrelor or clopidogrel alone, on any of the TEG parameters. This finding is supported by a systematic review (25) on the impact of direct oral anticoagulants on viscoelastic testing, including 31 studies which assessed rivaroxaban, 13 of which employed TEG. Four studies used the new cartridge based anti-FXa (AFXa) channel of TEG 6 s which is not yet commercially available. Plasma concentration of rivaroxaban was strongly related to R using the new AFXa channel, whilst a moderate to strong correlation was seen with citrated kaolin channel. There was no other impact on any of the other TEG parameters. Three studies found no significant effect of rivaroxaban on any of the TEG 5000 parameters. Thus, whilst a NOAC specific assay shows good correlation between R and rivaroxaban level, non-specific TEG assays are not sensitive enough to detect an effect of rivaroxaban. Furthermore, those results were obtained using full treatment dose rivaroxaban, not the vascular dose used in our study. Given the lack of difference in TEG measurements of lysis between the 3 groups, at either 2 weeks or 4 weeks of treatment, and the lack of change in LY30 or LY60 in Group 2 between baseline and weeks 2 or 4, it is likely that the isolated observation of a relationship between LY30/LY60 and anti-FXa level at week 4 (with kaolin but not in native blood, and not at week 2) is a spurious result due to the play of chance.

The impact of rivaroxaban plus clopidogrel on OT is significant. In a previous study of STEMI patients who were tested on admission (and were therefore effectively aspirin and $P2Y_{12}$ inhibitor naïve), there was a significant increase in OT at discharge compared to that on admission, presumably due to the effect of DAPT (26). This was again borne out in our study here, with the greatest prolongation of OT seen with clopidogrel plus rivaroxaban. Recent studies in patients with stable atherosclerotic disease have shown that compared to treatment with DAPT, switching to dual pathway inhibition with antiplatelet therapy combined with VLDR resulted in enhanced $P2Y_{12}$ inhibition and reduced thrombin generation, with similar platelet-mediated global thrombogenicity (27–29). Earlier we showed that the principal determinants of endogenous fibrinolysis in whole blood under high shear are fibrinogen and shear-induced platelet reactivity, the latter directly related to the speed of thrombin generation (30). Yet here, the prolonged OT seen in Group 2, did not translate into a reduction in LT. There are few studies which have assessed the effect of FX inhibition on platelet reactivity. Ex vivo addition of rivaroxaban inhibited platelet aggregation induced by tissue factor and to a lesser extent induced by thrombin (31). Even low dose rivaroxaban 2.5 mg b.i.d. reduced platelet-dependent thrombin generation and coagulation-dependent thrombus-formation in patients treated with aspirin plus $P2Y_{12}$ inhibitor, whereas pure platelet-dependent thrombus formation was not affected (31). Indeed, FXa inhibition appears to have no significant effect on platelets, including in response to adenosine diphosphate, collagen, thrombin receptor-activating peptide, or arachidonic acid (32).

The addition of OAC to APT in patients with ACS has also been assessed in earlier studies.

In the APPRAISE-2 trial in patients with ACS, addition of apixaban 5 mg twice daily versus placebo, on top of standard antiplatelet therapy (81 % of patients were on DAPT, with clopidogrel as the main $P2Y_{12}$ inhibitor) increased major bleeding, leading to premature termination of

the trial (33). Whilst there was no signal for reduction in ischaemic events with apixaban, the premature trial termination limits conclusions that can be drawn about efficacy.

Full dose OAC, in addition to antiplatelet therapy, was assessed in patients with atrial fibrillation (AF) and concomitant coronary disease. In the AUGUSTUS trial, patients with AF, with concomitant ACS (62 %) or PCI were randomised in a 2 × 2 factorial design to apixaban or vitamin K antagonist (VKA) and to aspirin or placebo (34). Ticagrelor was the main $P2Y_{12}$ inhibitor used. The secondary endpoint of ischaemic events occurred similarly with VKA and apixaban, and with aspirin and placebo. In the PIONEER AF-PCI trial (35), patients with AF undergoing PCI were randomised to rivaroxaban 15 mg daily plus a $P2Y_{12}$ inhibitor, VLDR (2.5 mg twice daily) plus DAPT, or VKA plus DAPT. Whilst both doses of rivaroxaban reduced clinically significant bleeding compared to VKA, the secondary endpoint of cardiovascular death, myocardial infarction, or stroke was similar in the three groups, indicating that low-dose rivaroxaban or VLDR may offer similar ischaemic protection.

There have been 2 studies evaluating VLDR (2.5 mg twice daily) in patients with cardiovascular disease. These provided insight that whilst addition of OAC to antiplatelet therapy can increase bleeding, such a regimen could reduce ischaemic risk. In the ATLAS ACS-2 TIMI 51 study, addition of VLDR to DAPT in ACS patients significantly reduced the composite of cardiovascular death, myocardial infarction, or stroke compared to placebo but increased the risk of major and intracranial haemorrhage (36). However, although the mean follow up period was 13 months, the primary efficacy endpoint of cardiovascular death, myocardial infarction or stroke was most noticeably different between the VLDR and placebo groups beyond 1 year, and most marked in those on aspirin monotherapy, rather than those on DAPT. In patients with stable cardiac or vascular disease in the COMPASS trial, the addition of VLDR to aspirin reduced the composite of cardiovascular death, stroke or myocardial infarction (4.1 vs 5.4 %, $p < 0.001$) albeit at a cost of increased bleeding. (37) However, in that study, VLDR was added to aspirin only, whilst in the current VALIDATE-R study, VLDR was added to DAPT. It is possible that the reason why we did not see a reduction in ischaemic events with VLDR in our study, is that VLDR has negligible additional ischaemic benefit when the patient is already on DAPT.

4.1. Limitations

There are a number of limitations to our study. The main limitation is the sample size, which may have been insufficient to detect a small effect of rivaroxaban on LT. A significant limitation is that, despite verbal assurance of compliance by patients, the surprisingly low rivaroxaban levels in the patients allocated to clopidogrel plus rivaroxaban suggest non-compliance with rivaroxaban, and this is likely to have impacted the results. There were significant baseline differences between the groups with respect to TEG baseline measurements which may have confounded the results analysing a change in response to treatment. Furthermore, the sample size is too small to exclude the impact of confounding variables such as age, sex, ethnicity or ACS type. The study was impacted by the COVID 19 pandemic and we had some patients withdraw from follow up as they did not wish to attend for blood sampling in hospital, to minimise their risk of infection. This resulted in a number of patients missing follow-up appointments. There was a higher-than-expected withdrawal rate from the study, due to a relatively high incidence of left ventricular thrombus formation, and new atrial fibrillation. Since we did not assess plasma levels of clopidogrel metabolite or ticagrelor, nor urinary levels of aspirin metabolite, we cannot be certain of compliance and rely on patient reports. It would also have been useful to assess fibrin levels to assess the relationship between this and lysis time. Lastly, it is possible that endogenous fibrinolysis is not implicated in the pathogenesis of ACS, since data supporting that hypothesis have come from prior observational studies.

5. Conclusions and outlook

The results suggest that the addition of rivaroxaban 2.5 mg b.i.d. to the combination of aspirin plus clopidogrel in patients with ACS, does not affect endogenous fibrinolysis of thrombus formed at high shear, or clot lysis of thrombus formed at low shear. Low compliance with rivaroxaban, may have contributed to the results. Further studies are needed to determine whether the addition of higher doses of rivaroxaban to DAPT, or full dose rivaroxaban in addition to antiplatelet monotherapy, can favourably modulate fibrinolysis.

6. Perspectives

6.1. Competency in medical knowledge

In response to a thrombotic stimulus, the likelihood of lasting coronary occlusion is determined by the balance between pro-aggregatory factors and the effectiveness of the native endogenous fibrinolytic system. Following an ACS, 10–15 % of patients experience further ischaemic events within a year. Impaired endogenous fibrinolysis is a strong independent risk factor that may drive this “residual risk” and is unaffected by dual antiplatelet therapy (DAPT). We demonstrate that addition of very low dose rivaroxaban, on top of DAPT, fails to enhance endogenous fibrinolysis in ACS patients.

6.2. Translational outlook

Treatments that enhance endogenous fibrinolysis in ACS patients could be a potential approach to prevent thrombotic events. Whilst adding oral anticoagulation to DAPT can reduce ischaemic events, it markedly increases bleeding. Personalised pharmacotherapy to enhance fibrinolysis in the highest risk patients, would be desirable. However, addition of low dose rivaroxaban, on top of DAPT, fails to enhance endogenous fibrinolysis. Further studies are needed to assess the impact of alternative antithrombotic strategies on fibrinolysis.

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CRediT authorship contribution statement

Ying X. Gue: Writing – original draft, Investigation, Data curation. **Vassilios Memtsas:** Validation, Project administration, Formal analysis, Conceptualization. **Rahim Kanji:** Investigation, Data curation. **David M. Wellsted:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Formal analysis, Data curation. **Amanda Busby:** Software, Methodology. **Megan Smith:** Project administration. **Enric Vilar:** Project administration. **Alisdair Ryding:** Project administration. **Deepa J. Arachchillage:** Project administration, Methodology. **Diana A. Gorog:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

DAG has received institutional research grants from Bayer, Medtronic, and Boehringer-Ingelheim. She is related through family to a company director in Thromboquest Ltd., but neither she, nor her spouse, nor children have financial involvement or equity interest in and have received no financial assistance, support, or grants. The other authors have no actual or potential conflicts to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2024.02.030.

References

[1] O.N. Okafor, D.A. Gorog, Endogenous fibrinolysis: an important mediator of thrombus formation and cardiovascular risk, J. Am. Coll. Cardiol. 65 (16) (2015 Apr 28) 1683–1699.

[2] D.A. Gorog, J.L. Ferreiro, I. Ahrens, J. Ako, T. Geisler, S. Halvorsen, et al., De-escalation or abbreviation of dual antiplatelet therapy in acute coronary syndromes and percutaneous coronary intervention: a Consensus Statement from an international expert panel on coronary thrombosis, Nat. Rev. Cardiol. 20 (12) (2023 Dec) 830–844.

[3] M. Roffi, C. Patrono, J.P. Collet, C. Mueller, M. Valgimigli, F. Andreotti, et al., 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC), Eur. Heart J. 37 (3) (2016 Jan 14) 267–315.

[4] B. Ibanez, S. James, S. Agewall, M.J. Antunes, C. Bucciarelli-Ducci, H. Bueno, et al., 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. 39 (2) (2018 Jan 7) 119–177.

[5] D.A. Gorog, G.Y.H. Lip, Impaired spontaneous/endogenous fibrinolytic status as new cardiovascular risk factor?: JACC review topic of the week, J. Am. Coll. Cardiol. 74 (10) (2019 Sep 10) 1366–1375.

[6] W. Sumaya, L. Wallentin, S.K. James, A. Siegbahn, K. Gabrysch, M. Bertilsson, et al., Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: a PLATO substudy, Eur. Heart J. 39 (13) (2018 Apr 1) 1078–1085.

[7] S. Saraf, C. Christopoulos, I.B. Salha, D.J. Stott, D.A. Gorog, Impaired endogenous thrombolysis in acute coronary syndrome patients predicts cardiovascular death and nonfatal myocardial infarction, J. Am. Coll. Cardiol. 55 (19) (2010 May 11) 2107–2115.

[8] M. Farag, N. Spinthakis, Y.X. Gue, M. Srinivasan, K. Sullivan, D. Wellsted, et al., Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study, Eur. Heart J. 40 (3) (2019 Jan 14) 295–305.

[9] S.H. Lee, H.K. Kim, J.H. Ahn, M.G. Kang, K.H. Kim, J.S. Bae, et al., Prognostic impact of hypercoagulability and impaired fibrinolysis in acute myocardial infarction, Eur. Heart J. 44 (19) (2023 May 14) 1718–1728.

[10] W. Sumaya, L. Wallentin, S.K. James, A. Siegbahn, K. Gabrysch, M. Bertilsson, et al., Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: a PLATO substudy, Eur. Heart J. 39 (13) (2018 Apr 1) 1078–1085.

[11] N. Spinthakis, M. Farag, B. Rocca, D.A. Gorog, More, more, more: reducing thrombosis in acute coronary syndromes beyond dual antiplatelet therapy-current data and future directions, J. Am. Heart Assoc. 7 (3) (2018 Jan 26).

[12] Y.X. Gue, Y.H. Jeong, M. Farag, N. Spinthakis, D.A. Gorog, Precision treatment in ACS-role of assessing fibrinolysis, J. Clin. Med. 10 (5) (2021 Mar 1) 929.

[13] Y.X. Gue, R. Kanji, D.M. Wellsted, M. Srinivasan, S. Wyatt, D.A. Gorog, Rationale and design of "can very low dose rivaroxaban (VLDR) in addition to dual antiplatelet therapy improve thrombotic status in acute coronary syndrome (VaLiDate-R)" study: a randomised trial modulating endogenous fibrinolysis in patients with acute coronary syndrome, J. Thromb. Thrombolysis 49 (2) (2020 Feb) 192–198.

[14] R.A. Byrne, X. Rossello, J.J. Coughlan, E. Barbato, C. Berry, A. Chieffo, et al., 2023 ESC Guidelines for the management of acute coronary syndromes, Eur. Heart J. 44 (38) (2023 Oct 12) 3720–3826.

[15] L. Wallentin, R.C. Becker, A. Budaj, C.P. Cannon, H. Emanuelsson, C. Held, et al., Ticagrelor versus Clopidogrel in patients with acute coronary syndromes, N. Engl. J. Med. 361 (11) (2009 Sep 10) 1045–1057.

[16] S. Sharma, K. Farrington, R. Kozarski, C. Christopoulos, M. Niespialowska-Steuden, D. Moffat, et al., Impaired thrombolysis: a novel cardiovascular risk factor in end-stage renal disease, Eur. Heart J. 34 (5) (2013 Feb) 354–363.

[17] R.J. Luddington, Thrombelastography/thromboelastometry, Clin. Lab. Haematol. 27 (2) (2005 Apr) 81–90.

[18] R. Mehran, S.V. Rao, D.L. Bhatt, C.M. Gibson, A. Caixeta, J. Eikelboom, et al., Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium, Circulation 123 (23) (2011 Jun 14) 2736–2747.

[19] N. Spinthakis, M. Farag, Y.X. Gue, M. Srinivasan, D.M. Wellsted, D.A. Gorog, Effect of P2Y12 inhibitors on thrombus stability and endogenous fibrinolysis, Thromb. Res. 173 (2019 Jan) 102–108.

[20] R. Kanji, Y.X. Gue, V. Memtsas, N.H. Spencer, D.A. Gorog, Biomarkers of thrombotic status predict spontaneous reperfusion in patients with ST-segment elevation myocardial infarction, J. Am. Coll. Cardiol. 81 (19) (2023 May 16) 1918–1932.

[21] A.S. Wolberg, Thrombin generation and fibrin clot structure, Blood Rev. 21 (3) (2007 May) 131–142.

[22] J.J.N. Posma, J.J. Posthuma, H.M.H. Spronk, Coagulation and non-coagulation effects of thrombin, J. Thromb. Haemost. 14 (10) (2016 Oct) 1908–1916.

[23] M. Farag, M. Niespialowska-Steuden, O. Okafor, B. Artman, M. Srinivasan, A. Khan, et al., Relative effects of different non-vitamin K antagonist oral anticoagulants on global thrombotic status in atrial fibrillation, Platelets 27 (7) (2016 Nov) 687–693.

[24] N. Spinthakis, Y. Gue, M. Farag, M. Srinivasan, D. Wellsted, D.R.J. Arachchillage, et al., Apixaban enhances endogenous fibrinolysis in patients with atrial fibrillation, Europace 21 (9) (2019 Sep 1) 1297–1306.

[25] S.D. Sahli, C. Castellucci, T.R. Roche, J. Rössler, D.R. Spahn, A. Kaserer, The impact of direct oral anticoagulants on viscoelastic testing - a systematic review, Front. Cardiovasc. Med. 9 (2022) 991675.

[26] M. Farag, N. Spinthakis, Y.X. Gue, M. Srinivasan, K. Sullivan, D. Wellsted, et al., Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study, Eur. Heart J. 40 (3) (2019 Jan 14) 295–305.

[27] M. Galli, F. Franchi, F. Rollini, L. Been, P.A. Jaoude, A. Rivas, et al., Pharmacodynamic profiles of dual-pathway inhibition with or without Clopidogrel versus dual antiplatelet therapy in patients with atherosclerotic disease, Thromb. Haemost. 122 (8) (2022 Aug) 1341–1351.

[28] L. Ortega-Paz, F. Franchi, F. Rollini, M. Galli, L. Been, G. Ghanem, et al., Switching from dual antiplatelet therapy with aspirin plus a P2Y12 inhibitor to dual pathway inhibition with aspirin plus vascular-dose rivaroxaban: the Switching Anti-Platelet and Anti-Coagulant Therapy (SWAP-AC) study, Thromb. Haemost. 12 (2023 Jul).

[29] M. Galli, F. Franchi, F. Rollini, L. Been, P.A. Jaoude, A. Rivas, et al., Platelet P2Y12 inhibiting therapy in adjunct to vascular dose of rivaroxaban or aspirin: a pharmacodynamic study of dual pathway inhibition vs. dual antiplatelet therapy, Eur. Heart J. Cardiovasc. Pharmacother. 8 (7) (2022 Sep 29) 728–737.

[30] R. Kanji, Y.X. Gue, M.F. Farag, N.H. Spencer, N.J. Mutch, D.A. Gorog, Determinants of endogenous fibrinolysis in whole blood under high shear in patients with myocardial infarction, JACC Basic Transl. Sci. 7 (11) (2022 Nov) 1069–1082.

[31] O. Borst, P. Münzer, N. Alnaggar, S. Geue, R. Tegtmeyer, D. Rath, et al., Inhibitory mechanisms of very low-dose rivaroxaban in non-ST-elevation myocardial infarction, Blood Adv. 2 (6) (2018 Mar 27) 715–730.

[32] B. Steppich, F. Dobler, L.C. Brendel, G. Hessling, S.L. Braun, A.L. Steinsiek, et al., Effect of the FXa inhibitors Rivaroxaban and Apixaban on platelet activation in patients with atrial fibrillation, J. Thromb. Thrombolysis 43 (4) (2017 May) 490–497.

[33] J.H. Alexander, R.D. Lopes, S. James, R. Kilaru, Y. He, P. Mohan, et al., Apixaban with antiplatelet therapy after acute coronary syndrome, N. Engl. J. Med. 365 (8) (2011 Aug 25) 699–708.

[34] R.D. Lopes, G. Heizer, R. Aronson, A.N. Vora, T. Massaro, R. Mehran, et al., Antithrombotic therapy after acute coronary syndrome or PCI in atrial fibrillation, N. Engl. J. Med. 380 (16) (2019 Apr 18) 1509–1524.

[35] Prevention of Bleeding in Patients with Atrial Fibrillation Undergoing PCI - PubMed [Internet], Cited 2024 Jan 9, Available from: https://pubmed.ncbi.nlm.nih.gov/27959713/.

[36] J.L. Mega, E. Braunwald, S.D. Wiviott, J.P. Bassand, D.L. Bhatt, C. Bode, et al., Rivaroxaban in patients with a recent acute coronary syndrome, N. Engl. J. Med. 366 (1) (2012 Jan 5) 9–19.

[37] J.W. Eikelboom, S.J. Connolly, J. Bosch, G.R. Dagenais, R.G. Hart, O. Shestakovska, et al., Rivaroxaban with or without aspirin in stable cardiovascular disease, N. Engl. J. Med. 377 (14) (2017 Oct 5) 1319–1330.