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Impaired Endogenous Fibrinolysis in STEMI patients undergoing PPCI is a predictor of recurrent cardiovascular events -the RISK PPCI study

Short title: Impaired Fibrinolysis in PPCI- RISK-PPCI Study

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Abstract

Aims The endogenous fibrinolytic system serves to prevent lasting thrombotic occlusion and infarction following initiation of coronary thrombosis. We aimed to determine whether impaired endogenous fibrinolysis can identify patients with ST-elevation myocardial infarction (STEMI) who remain at high cardiovascular risk despite dual antiplatelet therapy (DAPT).

Methods and Results A prospective, observational study was conducted in 496 patients presenting with STEMI for primary percutaneous coronary intervention (PPCI). Blood was tested on arrival pre-PPCI, at discharge and at 30 days to assess thrombotic status using the automated point-of-care Global Thrombosis Test and patients followed for 1 year for major adverse cardiovascular events (MACE). Endogenous fibrinolysis was significantly impaired (baseline Lysis Time [LT] ≥2500s) in 14% of patients, and was highly predictive of recurrent MACE (HR:9.1, 95%CI:5.29-15.75, p<0.001), driven by cardiovascular death (HR:18.5, 95%CI:7.69-44.31, p<0.001) and MI (HR:6.2, 95%CI:2.64-14.73, p<0.001), particularly within 30 days. Fibrinolysis remained strongly predictive of MACE after adjustment for conventional risk factors (HR:8.03, 95%CI:4.28-15.03, p<0.001). Net reclassification showed that adding impaired fibrinolysis improved the prediction of recurrent MACE by >50% (p<0.001). Patients with spontaneous ST-segment resolution pre-PPCI had more rapid, effective fibrinolysis (LT 1050[1004-1125]s vs. 1501[1239-1997]s, p<0.001) than those without. LT was not altered by standard of care STEMI treatment including DAPT and was unchanged at 30 days.

Conclusion Endogenous fibrinolysis assessment can identify patients with STEMI who remain at very high cardiovascular risk despite PPCI and DAPT. Further studies are needed to assess whether these patients may benefit from additional, personalised antithrombotic/anticoagulant medication to reduce future cardiovascular risk.

Key words: STEMI, fibrinolysis, thrombosis, PPCI, risk.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02562690

Abbreviations

CV: coefficient of variation

CVD: cardiovascular death

DAPT: dual antiplatelet therapy

GPI: glycoprotein IIb/IIIa inhibitor

GTT: Global Thrombosis Test

LT: lysis time

MACE: major adverse cardiovascular event

MI: myocardial infarction

NOACs: non-vitamin K antagonist oral anticoagulants

OT: occlusion time

PPCI: primary percutaneous coronary intervention

STEMI: ST-segment elevation myocardial infarction

TEG: thromboelastography

TIMI: Thrombolysis In Myocardial Infarction

Introduction

Despite primary percutaneous coronary intervention (PPCI) and optimal medications including dual antiplatelet therapy (DAPT), some 15% of patients presenting with an ST-segment elevation myocardial infarction (STEMI) experience a major adverse cardiovascular event (MACE) after the index episode, due predominantly to thrombotic complications.[1] More potent antithrombotic medications, which may reduce thrombosis, significantly increase bleeding.[2,3] Identification of individuals at risk of future arterial thrombosis would be highly desirable, since this group could be targeted for pharmacological intervention.

The likelihood of a thrombotic event is determined by the balance between prothrombotic drivers on the one hand, and the effectiveness of the fibrinolytic system, which protects against persisting occlusive thrombosis.[4,5] There is increasing evidence that impaired fibrinolytic capacity contributes to ongoing thrombosis risk in patients with cardiovascular disease.[6,7] Current DAPT strategy fails to modify the residual risk of thrombosis resulting from impaired endogenous fibrinolysis. This is supported by the ATLAS-ACS-2[3] and COMPASS[8] studies showing additional risk reduction with addition of an antithrombotic/anticoagulant strategy in addition to antiplatelet therapy. Laboratory studies have shown abnormally dense fibrin structures,[9,10] with clots that are more dense, less permeable and more resistant to lysis in patients with stent thrombosis,[11] family history of premature coronary disease,[12] diabetes,[11,13] renal failure[11] and ischaemic stroke.[11,14] However, fibrin clot structure assessment is cumbersome and lacks standardisation.[15] Individual components of the fibrinolytic pathway (such as t-PA, PAI-1, thrombin activatable fibrinolysis inhibitor, d-dimer) are, at best, weakly predictive of cardiovascular risk, and technical limitations and difficulty in interpretation of the relative contribution of any individual marker to overall fibrinolytic status greatly limit usefulness.[16] Low plasma fibrinolytic potential, found in 10% of the population, was found to increase the risk of

arterial thrombosis 2-fold.[17] Early data indicate that assessment of global fibrinolytic status may identify patients at increased risk of arterial thrombosis. Impaired endogenous fibrinolysis has been shown to predict MACE in non-ST elevation myocardial infarction[18] and end-stage renal disease.[19]

The ability to predict future thrombosis would be most important in those at highest risk, such as those with STEMI, where there is a genuine need for "personalised" treatment to reduce thrombotic complications, whilst avoiding unnecessary bleeding in low-risk groups. Blocking the thrombin pathway in addition to platelet inhibition may translate into a survival advantage, by inhibiting coagulation and improving fibrinolytic potential where this is impaired.[20] Our preceding pilot study in 80 STEMI patients indicated that endogenous fibrinolysis, when impaired, was associated with increased cardiovascular risk, and when enhanced, with spontaneous reperfusion.[21] Impaired endogenous fibrinolytic status may thus identify at-risk patients, who may benefit from additional antithrombotic medication.[6,22] We aimed to assess the usefulness of impaired endogenous fibrinolysis as a predictor of increased thrombosis risk, and as a potential target for pharmacological modulation to improve outcomes.

Methods

Study design and population

We conducted a prospective, observational, single-centre study in 496 patients in accordance with the Declaration of Helsinki and Good Clinical Practice. The study was approved by National Research Ethics Service and the UK Health Research Authority (ClinicalTrials.gov identifier: NCT02562690).

Consecutive eligible patients presenting with STEMI to our Heart Attack Centre (HAC) with a view to PPCI, were recruited. We enrolled adults (≥18 years) with a presumed diagnosis of STEMI based on clinical presentation and ECG criteria.[23] Patients receiving oral anticoagulation, those with known coagulation disorders, sepsis, platelet count $<100x10^9$/L, haemoglobin <80g/L, active malignancy, inability to take DAPT, or those previously enrolled in the study were excluded.

A delayed consent strategy was used, with ethical approval. 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 PPCI as clinically indicated. Surviving patients were subsequently approached for consent. Patients who died before consent could be obtained were excluded (n=11).

Antiplatelet therapy consisted of aspirin 300mg and either clopidogrel 600mg or ticagrelor 180mg orally in the ambulance or emergency department upon diagnosis. Patients receiving clopidogrel pre-arrival received additional ticagrelor 180mg loading peri-PPCI and continued on this post-procedure. Unfractionated heparin 70-100IU/kg was given immediately before PPCI. Use of the glycoprotein IIb/IIIa inhibitor (GPI) tirofiban (Aggrastat, Correvio UK Ltd, London, UK) or bivalirudin (Angiomax, The Medicines Company, Parsippany, NJ, USA),

decisions regarding access site, thrombus aspiration, and stent type were left to physician preference.

Blood sampling

Non-fasting blood samples were taken at 3 time-points: 1) baseline upon arrival, after DAPT loading, prior to heparin or GPI/bivalirudin administration and before PPCI, 2) at clinical stabilisation, just prior to hospital discharge, and 3) at 30-days' follow-up. The rationale for measuring thrombotic status at discharge and 30 days was to assess for any change in over time that may be explained by an acute phase response or modification of thrombotic status by DAPT. Initial samples were taken from a 6-F radial or femoral sheath, which was flushed with non-heparinised saline before insertion. Subsequent samples were taken from an antecubital vein using an 18-G butterfly cannula. A 2-syringe technique was employed, using the first 5ml for routine tests and the second 5ml for thrombotic status assessment, avoiding prolonged tourniquet time. Prior data showed no difference in thrombotic status between simultaneously collected arterial and venous samples.

Assessment of thrombotic status

Global Thrombosis Test (GTT)

The GTT (Thromboquest Ltd., London, UK) assesses both platelet reactivity and endogenous fibrinolysis from native, non-anticoagulated blood, and the principle of the technique has previously been described.[18] The instrument was positioned in the catheterisation laboratory, ready to use. The blood sample was introduced into the GTT cartridge within 15 seconds (s) of withdrawal and the automated measurement begun. The instrument assesses the time taken to form an occlusive thrombus under high-shear stress (occlusion time, OT), and the time required to restore flow through endogenous fibrinolysis (lysis time, LT). The inter- and

intra-assay coefficients of variation (CV) were assessed by testing 10 stable patients on 2 occasions, 48 hours apart and with samples run on 2 simultaneous channels of the instrument.

Thromboelastography (TEG)

In a subgroup of 60 patients, to assess the relationship between clot strength in the TEG and lysis in the GTT, blood was also tested with thromboelastography (TEG 5000 Hemostasis Analyser System, Haemonetics, Watford, UK). Two TEG tests were performed in parallel for each patient; whole blood and whole blood plus kaolin.

ECG and Angiographic Analyses

ECG and angiographic analyses were performed by an investigator blinded to thrombotic status results and not involved in the PPCI procedure. Standard 12-lead ECGs obtained in the ambulance or emergency department (baseline), and on arrival to the HAC were analyzed using a hand-held caliper. The ST-segment was measured 20ms after the J-point, and the sum of ST deviation measured as previously described.[24] The percent resolution of ST deviation from baseline to arrival was categorized using Schröder's 3-component definition: complete (≥70%), partial (30-70%) and no (≤30%) ST-segment resolution. Flow in the infarct-related artery was graded using the Thrombolysis in Myocardial Infarction (TIMI) classification and patency defined as TIMI 2 or 3 flow.

Scanning electron microscopy

In a cohort of representative patients, two blood samples per patient (n=10) at the time of acute STEMI presentation were run in parallel channels in the GTT. Whilst in the first channel the measurement was allowed to proceed as normal, in the second channel, the test was terminated after OT. The thrombus formed between the 2 ball-bearings was carefully extracted, immediately washed in Na-cacodylate buffer and fixed with 2.5% glutaraldehyde,

before point-critical dehydration with ethanol (5-100%) and hexamethyldisilazane. Samples were coated with gold palladium and imaged using a Phenom ProX (EDS) scanning electron microscope (SEM) (Lambda Photometrics Ltd., Hertfordshire, UK).

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 casenotes.

Study endpoints

The primary endpoint was the occurrence of MACE, defined as the composite of cardiovascular death (CVD), nonfatal myocardial infarction (MI) including stent thrombosis (defined according to the Academic Research Consortium criteria), or stroke. These are defined in the Supplement. The secondary endpoint was major bleeding classified as type 3-5 according to the Bleeding Academic Research Consortium (BARC) definition. For all endpoints, source documents were obtained and diagnosis verified by 2 independent clinicians blinded to thrombotic status results.

Statistical analysis

This study aimed to assess whether LT is a predictor of MACE in patients with STEMI. Based on an effect size (hazard ratio, HR) of 3.3 from our pilot,[21] assuming a two-sided alpha of 0.01, 10% MACE and 10% attrition rate, using the Cox proportional hazard model we calculated that 464 patients would be required to achieve 90% power. A lower α level and higher power were employed, to adequately address the study question.

Data are presented as mean (standard deviation) or median and inter-quartile range (IQR). Dichotomous variables were compared using chi-square test or Fisher's exact test. Correlations were analysed using Spearman's method. Ability of the test to discriminate between patients with and without MACE was evaluated by receiver-operating characteristic (ROC) curve analysis. Kaplan-Meier survival methods with logrank tests were used. Only events occurring beyond the point of LT testing were related to the test's ability to predict MACE. To investigate the relationship between LT and MACE, univariate and multivariable hazard regression models of Cox were used. The bootstrap technique using one thousand samples was used as a way to account for final multivariable model uncertainty. All study variables were first analysed with univariate analysis and those that showed a significant interaction ($p<0.05$) were entered into the multivariable analysis. Multiple fractional polynomial models were used to illustrate the nonlinear association between LT and MACE. Net reclassification improvement (NRI) was used to assess the change in discrimination after adding LT ≥2500 to the combination of the 3 baseline predictors (age, diabetes, TIMI score). The NRI uses reclassification tables to examine whether there is an additive benefit gained from reclassifying patients into different categories based on the addition of new markers. Analyses were performed with Stata V.15 (StataCorp, College Station, TX, USA).

Results

Between April 2015 and March 2017, 543 patients were eligible for recruitment. Six patients were already enrolled in the trial and 41 met ≥1 exclusion criteria. All survivors were followed for a median of 12 months. Clinical characteristics are shown in Table 1 and

baseline bloodwork, angiographic, procedural and echocardiographic characteristics in Supplementary Tables 2 and 3.

Thrombotic status over 30-days

The inter- and intra-assay CVs were 8% and 9% for OT and 10% and 10% for LT, respectively. There was a negative correlation between baseline OT and baseline LT (r=-0.4, p<0.001). Compared to baseline, OT increased significantly at hospital discharge and 30-days [Supplementary Figure 1-A]. Compared to baseline, LT did not change at discharge (p=0.761) or 30-days (p=0.652). Baseline LT correlated with LT at discharge (r=0.2, p<0.001) and LT at 30-days (r=0.2, p<0.001) [Supplementary Figure 1-B].

Relationship between patient characteristics and thrombotic status

Patient characteristics in Table 1 and supplementary Tables 1 and 2 were interrogated for relation to baseline OT and LT. Baseline OT was related to pre-PPCI TIMI-3 flow (r=0.2, p<0.001), peak troponin (r=-0.2, p=0.002) and neutrophil count (r=-0.2, p<0.001). Baseline LT was related to pre-PPCI TIMI-3 flow (r=-0.2, p<0.001). Impaired endogenous fibrinolysis (baseline LT≥2500s) was significantly more frequent in patients with cardiogenic shock, prior MI or PCI, low albumin, raised fibrinogen, raised high-sensitivity C-reactive protein, and high TIMI score [Table 1, Supplementary Tables 1 and 2]. There was a very weak but significant correlation between baseline LT and peak troponin (r=0.14, P=0.016). There was no correlation between baseline OT or baseline LT and time of day of blood sampling or pain-to-balloon time.

Thrombotic status and cardiovascular outcomes

There were 53 MACE events among 36 patients [Table 2]. Patients who experienced MACE demonstrated baseline OT that was significantly shorter (more thrombotic) than patients without MACE (268[180-333]s vs. 367[278-484]s; p<0.001), particularly with respect to MI (231[106-300]s vs. 363[275-483]s; p<0.001), and ischaemic stroke (186[125-288]s vs. 360[270-482]s; p=0.003) [Supplementary Figure 2]. The OT at hospital discharge did not differ between patients who experienced MACE or major bleed and those who did not, and OT was not predictive of MACE [Figure 1-A].

Baseline LT significantly discriminated between patients with and without MACE, with a c-index of 0.78 (95%CI:0.691-0.859; p<0.05) [Figure 1-B]. LT≥2483s was the optimal cut-point to predict MACE (rounded to 2500s for ease), with sensitivity 60% and specificity 90.5% [Figure 1-B]. There were no LT readings between 2483s and 2500s that could have been wrongly classified based on rounding the cut-point. Baseline LT≥2500s was strongly related to MACE (HR:9.1, 95%CI:5.29-15.75, p<0.001) [Figure 2], driven by CVD (HR:18.5, 95%CI:7.69-44.31, p<0.001) and MI (HR:6.2, 95%CI:2.64-14.73, p<0.001) [Figures 3-B and 5][Table 2] and probability of MACE increased with increasing baseline LT [Figures 2-B and 4]. LT at subsequent timepoints was not related to MACE.

Prognostic value of impaired endogenous lysis time

Of the patient characteristics [Table 1, Supplementary Tables 1 and 2], the following were related to MACE: age (p<0.001), prior MI (p=0.003), prior PCI (p=0.013), prior stroke (p=0.016), prior statin (0.005), prior aspirin (p=0.002), cardiogenic shock (p=0.003), TIMI score (p<0.001), low haemoglobin (p<0.001), low haematocrit (p=0.001), low albumin (p<0.001), high creatinine (p=0.009), high C-reactive protein (p=0.003), short baseline OT (p<0.001), number of diseased vessels on angiography (p=0.007), left ventricular dysfunction

(p<0.001), and absence of spontaneous ST-resolution pre-PPCI (p=0.032) (Supplementary Table 4).

The following were then entered into the final multivariable Cox proportional hazard model: age, prior stroke, prior statin, prior aspirin, creatinine, number of diseased vessels and left ventricular dysfunction. None of these covariates were significantly correlated either with LT or its dichotomized version. Although low haemoglobin and haematocrit levels were not related to LT, these were not included in the model, as we felt they may be related at the extremes. Baseline LT≥2500s remained significantly predictive of MACE after adjustment for other risk factors (HR:8.03, 95%CI:4.28-15.03, p<0.001) and after bootstrap resampling. It remained significant after the addition of diabetes into the final model (HR:8.44, 95%CI:4.47-15.93, p<0.001) and also after excluding patients with cardiogenic shock (HR:8.07, 95%CI 4.55-14.34, p<0.001) or severe LV dysfunction (HR:7.14, 95%CI 3.77-13.49, p<0.001) from the analysis.

NRI showed that inclusion of baseline LT≥2500s in a model containing three baseline predictors (age, diabetes and TIMI score) significantly added to the model effectiveness (NRI estimate 0.514, p<0.001).

Spontaneous coronary reperfusion and ST-resolution

Patients with spontaneous reperfusion [Supplementary Table 2], manifesting as complete or partial resolution of ST-elevation pre-PPCI, all had TIMI 2-3 flow at presentation, shorter baseline LT (1050[1004-1125]s vs. 1501[1239-1997]s, p<0.001) and longer baseline OT (448[371-586]s vs. 338[245-454]s, p<0.001) than patients without spontaneous reperfusion. Spontaneous reperfusion correlated with OT (r=0.3, p<0.001) and inversely with LT (r=-0.4, p<0.001).

Scanning electron microscopy

Increasing lysis time *in vitro* was associated with increasing density of the fibrin network on SEM [Figure 6] and the association was discernible in each tested patient. Images are illustrative of the possible pathophysiological mechanism underlying the observed resistance to lysis and should be interpreted in the context of the limitations pertaining to the small number of samples analysed as well as possible inter-observer variability.

Thromboelastography

Baseline OT correlated with the following whole blood TEG indices [explained in Supplementary Table 3]: R (r=0.4, p=0.002), K (r=0.2, p=0.049), TMA (r=0.3, p=0.027), and MA (r=-0.2, p=0.050). Similar relationships were observed with kaolin: R (r=0.4, p=0.001), K (r=0.4, p=0.009), TMA (r=0.3, p=0.038) and MA (r=-0.4, p=0.005). Baseline LT did not correlate with any TEG indices, including LY30 and LY60 using whole blood with or without kaolin.

Discussion

In patients with STEMI presenting for PPCI, impaired endogenous fibrinolysis detected using an automated point-of-care test in the catheterisation laboratory, is a novel risk factor for subsequent MACE with significant predictive value.

Whilst platelet reactivity fell from admission to hospital discharge and further by day 30 (shown by increasing OT), likely representing the effects of antiplatelet medications and plaque stabilisation, endogenous fibrinolysis by comparison, did not change from admission to 30-days. Endogenous fibrinolysis was significantly impaired in 14% of patients and was highly predictive of recurrent MACE, driven by CVD and MI. As in other contemporary

PPCI trials, most events occurred in hospital, and the majority of the rest within the first 30-days, and thus because of the small number of events, the study was underpowered to assess the predictive power of LT measured at discharge and 30-days. Prolonged lysis time was associated with increasing fibrin network density with reduced pore size on SEM, which likely explains the resistance to lysis, in line with earlier studies.[7,9,11,12]

Our data showing impaired endogenous fibrinolysis to be highly predictive of outcome, confirms findings from our STEMI pilot,[21] and is supported by data in a lower-risk cohort with non-ST elevation MI, where impaired fibrinolysis was predictive of increased cardiovascular risk (HR 2.5).[18] Our findings are strongly supported by a very recent publication analysing stored plasma samples from >4000 ACS patients in the PLATO trial, showing that clot lysis time, measured using a turbidimetric assay, was an independent predictor adverse outcome, with risk increasing as lysis time increased and this was independent of antiplatelet medications.[25] However, unlike the specialised turbidimetric assay performed in a centralised laboratory, we report results with a point-of-care test that could influence clinician decision making in a timely manner in patients with impaired fibrinolysis, allowing personalisation of treatment before hospital discharge to reduce 30-day MACE.

The lack of a linear relationship between LT and outcome likely reflects the small number of patients in the 3rd and 4th LT quartiles (n=21 and n=30 respectively), such that the error of estimation is large and compromises the ability to evaluate the relationship between the magnitude of LT and the risk of MACE. However, we postulate that there would have been a linear relationship had we had a larger sample size.

There were no cardiovascular risk factors that clearly related to LT, but numbers were too small for subgroup analysis. However, amongst patients with very high-risk, namely those with cardiogenic shock, high TIMI scores, low albumin and high C-reactive protein, significantly more patients exhibited impaired fibrinolysis than lower risk patients. The

relationship between cardiogenic shock and prolonged lysis time is supported by data from the recently published PLATO substudy also showing an association between NT-proBNP levels and lysis time.[25] Whilst we did not measure NT-proBNP, the proportion of patients with cardiogenic shock increased with increasing lysis time. Such patients are known to be at very high risk. However, lysis time remained a significant predictor of recurrent MACE even after excluding patients with cardiogenic shock. Unlike the PLATO substudy, we saw only a weak relationship between troponin and LT. However, any conclusions drawn from this have to be tempered by the unusual observation that peak troponin was not predictive of outcome in our population. Impaired fibrinolysis appears not to simply mirror conventional cardiovascular risk profile, but to be a novel risk factor, which when added to traditional risk factors very significantly improved the prediction of MACE.

An important issue is whether impaired endogenous fibrinolysis is a modifiable risk factor. Whilst antiplatelet medications reduce platelet reactivity over time, LT appears unaffected by oral antiplatelet medications or plaque stabilisation. Previous work suggests that non-vitamin K antagonist oral anticoagulants (NOACs)[26] and the PAR-1 inhibitor vorapaxar[27] can favourably modulate (reduce) LT. The COMPASS trial showed that among patients with stable atherosclerosis, those assigned to ultra-low dose rivaroxaban plus aspirin had lower risk of the composite of CVD, stroke, or MI and more major bleeding events than those assigned to aspirin alone.[8] In ACS patients, addition of a NOAC, such as rivaroxaban in ATLAS-ACS-2[3] or vorapaxar[2] to DAPT can reduce the risk of recurrent ischaemic events, although offset by a significant increase in bleeding. Since this strategy clearly cannot be recommended for all ACS patients, assessing endogenous fibrinolysis could identify those at highest risk, who may benefit most from additional anticoagulation.

Spontaneous reperfusion was observed in 15% of patients before PPCI, who had more rapid (efficient) endogenous fibrinolysis and reduced platelet reactivity, supporting the signal seen

in our pilot. We postulate that this may indicate a cohort of patients where complete thrombotic vessel occlusion can occur due to a very potent thrombotic stimulus, but in whom the endogenous fibrinolytic system is much more effective than in others, and sufficient to spontaneously restore coronary flow.

In our sub-study measures of clot lysis with TEG did not relate to endogenous fibrinolysis with the GTT. TEG is a global test of coagulation, simultaneously assessing clot development, stabilisation and dissolution under low-shear stress and is useful for assessing clot strength, but its value in assessing spontaneous fibrinolytic status is questionable, as it fails to assess the procoagulant (thrombin-generating) and fibrinolysis-inhibiting (PAI-1, TAFI) properties of platelets.[6] It more closely resembles the low-shear environment of venous stasis than the high-shear situation in the GTT , which likely explains the lack of correlation between these tests.

Although baseline fibrinogen levels were higher in patients with impaired fibrinolysis, fibrinogen is an acute phase protein and levels normalise following an acute event. C-reactive protein and albumin may similarly reflect acute illness. On the other hand, endogenous fibrinolysis did not change over time. Previous studies have measured individual proteins involved in fibrinolysis, including t-PA, PAI-1, alpha-2 antiplasmin, alpha2-antiplasmin-plasmin complex, D-dimer, TAFI and Lp(a). Technical limitations, combined with challenges in knowing the relative importance and contribution of individual variables to overall fibrinolytic status, limit the usefulness of this approach.[16] In this study, the predictive power of endogenous fibrinolytic activity measured with a global test was considerably higher than that of plasma PAI-1 level,[28,29] perhaps because PAI-1 release from the platelet core of a thrombus is localised, and not necessarily reflected by systemic PAI-1 antigen levels.

The main limitations of our study are the sample size and recruitment from a single centre. The numbers are too small to assess relationships between endogenous fibrinolysis and

established cardiovascular risk factors. Because of the need to obtain informed consent, some of the sickest patients who died before giving consent, were excluded. Optimal antithrombotic medication for STEMI comprises of aspirin with ticagrelor or prasugrel, but due to financial constraints in much of the UK, the ambulance crew often administer clopidogrel and aspirin. However, such patients were additionally given ticagrelor on arrival and it is unlikely that prasugrel or ticagrelor administration in the ambulance instead of clopidogrel would have impacted on endogenous fibrinolysis. Baseline samples were obtained before the acutely administered antiplatelet agents would have had maximal effect and so reflect real-time thrombotic status at the time of PPCI. The study was underpowered to assess the relationship between thrombotic status and bleeding events, since very few bleeds were observed at least in part due to the majority of interventions being performed radially and the exclusion of anticoagulated patients.

Conclusion

Impaired endogenous fibrinolysis in patients with STEMI presenting for PPCI, detected using an automated point-of-care technique in the catheterisation laboratory, identifies those at high risk of recurrent MACE. This novel risk factor improved the prediction of recurrent MACE by >50% when added to conventional predictors. Further studies are needed to assess whether impaired endogenous fibrinolysis is a modifiable risk factor, targetable by pharmacotherapy, to reduce cardiovascular risk.

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Figures

Figure 1. Receiver-operating characteristic curves for Occlusion Time and Lysis Time for recurrent adverse events

Baseline LT≥2500s predicted MACE, with 60% sensitivity and 91% specificity.

Figure 2. Probability of event-free survival according to baseline Lysis Time

Kaplan-Meier curves showing probability of (A) event-free survival according to baseline LT. Baseline LT≥2500s was associated with a hazard ratio of 9.1, (B) MACE based on baseline LT in quartiles.

Figure 3. Major adverse cardiovascular events and major bleeding according to baseline Lysis Time

(A) Baseline LT in patients with (solid bars) versus those without MACE (open bars), (B) Baseline LT<2500s (open bars); baseline LT≥2500s (solid bars). *p<0.001. †p<0.05.

MI: myocardial infarction; CVA: cerebrovascular accident; CVD: cardiovascular death.

Figure 4. Major adverse cardiovascular events according to baseline Lysis Time

Hazard ratios for cardiovascular death (CVD), myocardial infarction (MI), and cerebrovascular accident (CVA) according to baseline lysis time (LT). The 95% confidence interval shown in brackets at top of bars. n: number of patients, E: number of events. *p<0.001.

Figure 5. Multiple fractional polynomial curves showing major adverse cardiovascular event rates according to baseline Lysis Time

Solid lines are shown as estimate and 95% CI. Dotted line indicates LT≥2500s.

Figure 6. Composition of *in vitro* formed thrombus at the time of acute myocardial infarction

Scanning electron microscope x1400 (upper panel) and x9000 (lower panel) magnification, from patients with LT 990s (A1, A2), LT 3160s (B1, B2) and LT>6000s (C1, C2). Increasing lysis time *in vitro* was associated with increasing density of the fibrin network.

Table 1. Baseline patient characteristics

	Whole Group (n=496)	Baseline LT <2500 (n=426)	Baseline LT ≥2500 (n=70)	P Value
Age, yrs.	63[55-74]	63[54-72]	67[56-79]	0.082
Male	386(77.8)	332(77.9)	54(77.1)	0.877
Caucasian	447(90.1)	382(89.7)	65(92.9)	0.710
BMI	26.7[23.9-30.1]	26.8[23.9-30.2]	26.1[23.7-29.9]	0.487
Cardiogenic shock *	18(3.6)	12(2.8)	6(8.6)	**0.029**
TIMI score	3[1-5]	3[1-4]	4[1-6]	**<0.001**
GRACE score	115[92-140]	115[92-135]	125[101-174]	**0.011**
Diabetes mellitus	96(19.4)	77(18.1)	19(27.1)	0.101
Active smoker	164(33.1)	137(32.2)	27(38.6)	0.337
Hypertension	247(49.8)	213(50.0)	34(43.6)	0.898
Family history of premature IHD	206(41.5)	178(41.8)	28(40.0)	0.795
Prior MI	61(12.3)	47(11.0)	14(20.0)	**0.048**
Prior PCI	56(11.3)	41(9.6)	15(21.4)	**0.007**
Prior CABG	7(1.4)	5(1.2)	2(2.9)	0.258
Renal insufficiency	25(5.0)	21(4.9)	4(5.7)	0.768
PVD	20(4.0)	17(3.9)	3(4.3)	0.753
Prior CVA	23(4.6)	17(3.9)	6(8.6)	0.117
Prior statin use	134(27.0)	114(26.8)	20(28.6)	0.772
Prior aspirin use	91(18.3)	73(17.1)	18(25.7)	0.096
Prior $P2Y_{12}$ inhibitor use	22(4.4)	16(3.8)	6(8.6)	0.069
Initial $P2Y_{12}$ inhibitor loading agent				
Clopidogrel	407(82.1)	347(81.5)	59(84.3)	0.620
Ticagrelor	78(15.7)	69(16.2)	9(12.9)	0.491
Cangrelor	11(2.2)	9(2.1)	2(2.9)	1.000
Morphine prior to blood sample	327(65.9)	276(64.8)	51(72.9)	0.221
Time from $P2Y_{12}$ inhibitor loading to first blood sample (min)	46[37-60]	45[36-60]	50[40-60]	0.145
Medications prior to hospital discharge				
Aspirin	472(95.2)	408(95.8)	64(91.4)	0.130
Clopidogrel	81(16.3)	68(15.9)	13(18.6)	0.601
Ticagrelor	388(78.2)	337(79.1)	51(72.9)	0. 274
Prasugrel	2(0.4)	2(0.5)	0	1.000
Beta-blocker	438(88.3)	380(89.2)	58(82.9)	0.157
ACE inhibitor	433(87.3)	376(88.3)	57(81.4)	0.123
Calcium antagonist	29(5.8)	26(6.1)	3(4.3)	0.606
Statin	466(93.9)	403(94.6)	63(90.0)	0.169
Nitrate	18(3.6)	15(3.5)	3(4.3)	0.999
Insulin	42(8.5)	35(8.2)	7(10.0)	0.642

Values are median[IQR] or n(%). Renal insufficiency defined as creatinine >177 μmol/L. Prior statin, aspirin or $P2Y_{12}$ inhibitor defined as regular use pre-hospitalisation. Family history of premature IHD defined as a diagnosis of IHD in a first-degree relative <60 years.

ACE: angiotensin-converting enzyme, BMI: body mass index, CABG: coronary artery bypass grafting, CVA: cerebrovascular accident, IHD: ischaemic heart disease, MI: myocardial infarction, PCI: percutaneous coronary intervention, PVD: peripheral vascular disease, TIMI: Thrombolysis in Myocardial Infarction.

*Patients artificially ventilated or with reduced conscious level received rectal aspirin 300mg and either intravenous cangrelor 30mcg/kg bolus followed by 4mcg/kg/min infusion (n=11), or ticagrelor 180mg via nasogastric tube (n=7) on arrival.

Table 2. Clinical outcomes at 12 months' follow-up

	Whole Group (n=496)	Baseline LT <2500 (n=426)	Baseline LT ≥2500 (n=70)	HR (95% CI)	P Value
MACE (cardiovascular death, re-infarction, CVA)	53(10.7)	23(5.4)	30(42.9)	9.1(5.29-15.75)	**<0.001**
Cardiovascular death	25(5.0)	7(1.6)	18(25.7)	18.5(7.69-44.31)	**<0.001**
New myocardial infarction or re-infarction	21(4.2)	11(2.8)	10(14.3)	6.23(2.64-14.73)	**<0.001**
Definite Stent thrombosis					
Acute (<24 hours)	6(1.2)	3(0.7)	3(4.3)	5.58(1.13-27.63)	**0.046**
Subacute (24 hours to 30 days)	4(0.8)	2(0.5)	2(2.9)	7.32(1.02-52.14)	0.064
Late (>30 days)	3(0.6)	2(0.5)	1(1.4)	4.36(0.39-48.34)	0.281
Cerebrovascular accident	7(1.4)	5(1.2)	2(2.9)	2.71(0.52-14.01)	0.273
Major bleeding (BARC type 3-5)	7(1.4)	5(1.2)	2(2.9)	3.23(0.62-16.70)	0.204
Minor bleeding (BARC type ≤2)	19(3.8)	17(3.9)	2(2.9)	1.03(0.24-4.47)	0.967
Non-cardiovascular death	9(1.8)	7(1.6)	2(2.9)	2.42(0.50-11.68)	0.312

Values are n(%). MACE: major adverse cardiovascular events, CVA: cerebrovascular accident, BARC: Bleeding Academic Research Consortium. CI: confidence interval, HR: hazard ratio, LT: lysis time.