Impaired Thrombolytic Status Predicts Adverse Cardiac Events in Patients Undergoing Primary Percutaneous Coronary Intervention

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Summary

Antithrombotic medications reduce thrombosis but increase bleeding. Identification of ST-elevation myocardial infarction (STEMI) patients at risk of recurrent thrombosis could allow targeted treatment with potent antithrombotic medications, with less potent agents in others, to reduce bleeding. Conventional platelet function tests assess platelet reactivity only, yet there is increasing evidence that endogenous thrombolytic potential determines outcome following thrombus initiation. We investigated whether assessing both platelet reactivity and endogenous thrombolysis, could identify STEMI patients at high-risk of recurrent thrombotic events. Thrombotic status was assessed in STEMI patients, before and after primary percutaneous coronary intervention (PPCI), at discharge and at 30 days; with 12 months' follow-up. The time to form an occlusive thrombus under high shear (occlusion time, OT), and time to restore flow by endogenous thrombolysis (lysis time, LT) was measured using the point-of-care Global Thrombosis Test (GTT) in the cardiac catheterisation laboratory. Impaired endogenous thrombolysis (prolonged LT≥3000s), seen in 13% patients pre-PPCI, was related to major adverse cardiac events, MACE (HR:3.31, 95%CI:1.02-10.78, p=0.045), driven by cardiovascular death (HR:4.17, 95%CI:0.99-17.51, p=0.05). Enhanced (rapid) endogenous thrombolysis (LT<1000s) was associated with spontaneous reperfusion, ST-segment resolution and Thrombolysis In Myocardial Infarction 3 flow pre-PPCI. Baseline OT was shorter in those with MACE (especially recurrent myocardial infarction and stroke) than those without (253±150s vs. 354±134s, p=0.017). Endogenous thrombolysis, when impaired, is associated with increased cardiovascular risk, and when enhanced, with spontaneous reperfusion. Endogenous thrombolysis may be a novel target for pharmacological intervention, and allow targeting of potent antithrombotic medications to high-risk patients.

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Key words: percutaneous coronary intervention, thrombosis, endogenous thrombolysis, myocardial infarction, coronary reperfusion

Abbreviations

ACS = acute coronary syndrome

CVD = cardiovascular death

GPI = glycoprotein IIb/IIIa inhibitors

GTT = global thrombosis test

LT = lysis time

MACE = major adverse cardiovascular events

MI = myocardial infarction

NOACs = non-vitamin K antagonist oral anticoagulants

OT = occlusion time

PFTs = platelet function tests

PPCI = primary percutaneous coronary intervention

STEMI = ST-elevation myocardial infarction

Introduction

Thrombotic occlusion of a coronary artery upon a background of atherosclerotic plaque rupture is considered the ultimate and key step in the pathogenesis of myocardial infarction (MI) (1). The emergency treatment of ST-elevation myocardial infarction (STEMI) is therefore two-pronged; namely, the pharmacological treatment and prevention of recurrent thrombosis (through antithrombotic and antiplatelet medications), and the mechanical relief of the atherosclerotic stenosis through primary percutaneous coronary intervention (PPCI). Even after mechanical relief of obstruction, there are many patients in whom there is failure to establish Thrombolysis in Myocardial Infarction (TIMI) 3 flow and a persistent pro-thrombotic state contributes to ongoing microvascular thrombosis and extension of infarction. Introduction of increasingly potent antithrombotic and antiplatelet agents has reduced the risk of recurrent thrombosis. Yet despite such treatment, some 10 to 15% of MI patients go on to have a major adverse cardiac event (MACE) after the index event, which is due predominantly to thrombotic complications (2-5). Contemporary, newer antithrombotic and antiplatelet agents, whilst reducing recurrent thrombosis, have significantly increased the risk of bleeding (5-8). Identification of individuals at high-risk of future thrombotic complications would be highly desirable, since this group could be targeted with more potent antithrombotic medications, allowing perhaps the use of less potent agents (thereby reducing bleeding risk) in lower risk groups.

Although many tests of platelet function tests (PFTs) are available, favourably modifying the results of PFTs through pharmacological modulation has not translated into a reduction in adverse thrombotic events. This may be due to intrinsic limitations of PFTs which differ from the *in vivo* situation (9), using citrated blood and measuring the response of platelets to only

specific agonists (10), which prevents assessment of thrombin generated by activated platelets or the effect of high shear (10), and importantly, spontaneous (endogenous) thrombolytic activity is not assessed. The innate ability of blood to induce lysis of a formed thrombus is an important defence mechanism against lasting arterial occlusion. Understandably, MI has been considered a result of the failure of timely spontaneous thrombolysis (11). The ideal test of thrombotic status should, in addition to assessing platelet reactivity, also measure the endogenous thrombolytic activity. Global assays, simultaneously assessing proaggregatory and fibrinolytic pathways, could play a role in risk stratification and in identifying impaired fibrinolysis as a potential target for pharmacological modulation (12).

The Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK), is a highly physiological, automated point-of-care test that assesses platelet reactivity, coagulation (thrombin generation) and endogenous thrombolysis from a native, non-anticoagulated blood sample. Impaired endogenous thrombolysis, as assessed by the GTT, has been shown to predict MACE in patients with non ST-elevation MI (13), and in patients with end-stage renal disease (14). As this test is performed from native (non-anticoagulated) blood, and assesses global thrombosis and thrombolysis, it is genuinely different from other PFTs which employ citrate-anticoagulated blood and cannot assess thrombolysis (10). We aimed to identify patients with STEMI undergoing PPCI who, despite dual antiplatelet medication, remain at risk of thrombotic complications and MACE, due to their inability to dissolve thrombus, namely impaired endogenous thrombolysis.

Materials and Methods

Study design and population

This prospective study was approved by the local research ethics committee. Consecutive eligible patients presenting with STEMI for emergency angiography with a view to PPCI, between the hours of 8 a.m. and 6 p.m. Monday-Friday, over the course of a year, were enrolled after obtaining informed consent. We included adults (≥18 years) with a presumed diagnosis of STEMI based on clinical presentation and ECG criteria (namely: new ST-elevation at the J point in at least 2 contiguous leads of ≥2 mm (0.2 mV) in men or ≥1.5 mm (0.15 mV) in women in leads V2–V3 and/or of ≥1 mm (0.1 mV) in other contiguous chest leads or the limb leads, or ST-depression in ≥2 precordial leads (V1–V4), or new or presumably new left bundle branch block (LBBB) in patients with clinical suspicion of ongoing myocardial ischaemia (15, 16)). All patients had received a loading dose of aspirin 300mg and clopidogrel 600mg in the ambulance or in the emergency department upon diagnosis of STEMI, within the preceding 20-60 min, in line with current local standard of care. The exclusion criteria listed in Table 1 were applied. Clinical characteristics of patients are shown in Tables 2 and 3. Unfractionated heparin (UFH) was given at a dose of 70 to 100 units per kilogram immediately before PPCI. Use of the glycoprotein IIb/IIIa inhibitor (GPI) abciximab (Reopro, Eli Lilly, Indianapolis, Indiana, USA) or bivalirudin (Angiomax, The Medicines Company, Parsippany, NJ, USA) was allowed at the operator's discretion, in conjunction with UFH. Decisions regarding access site, thrombus aspiration, and stent type were left to physician preference. Procedural characteristics are shown in Table 4. All patients received dual antiplatelet medication for 12 months as part of local protocol.

Blood sampling

Blood samples for thrombotic status were taken from all consented patients at 4 time points: 1) baseline upon admission (after loading with dual antiplatelet therapy, DAPT), through the arterial sheath, prior to heparin or GPI administration and before PPCI, 2) approximately 30-60 min after the administration of abciximab (if used), 3) at clinical stabilisation, just prior to hospital discharge, and 4) at 30 days' follow-up. Fasting was not required.

The first 2 time-point blood samples were taken from a 6-F radial or femoral sheath using a 2-syringe technique. For the initial sample, the sheaths were only ever flushed with normal saline (and not heparinised saline) prior to sampling. The third and fourth samples were taken from an antecubital vein using an 18-G butterfly cannula also using a 2-syringe technique, taking care to avoid prolonged tourniquet time. The two syringe technique involved using the first 5 ml blood for routine blood tests and using the second 5 ml for assessment of thrombotic status.

Assessment of global thrombotic status

The Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK), an automated point-of-care test, was used to assess both platelet reactivity and endogenous thrombolysis from a native, non-anticoagulated blood sample. The instrument has 4 independent measuring channels, and was positioned in the cardiac catheterisation laboratory. After blood sample was obtained, it was introduced into the disposable GTT cartridge, within 15 seconds 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 thrombolysis (lysis time, LT). The principle of the GTT has previously been described in detail (13, 17). In brief, blood flows under high shear

conditions through narrow gaps inside the conical tube where thrombus formation occurs and the instrument measures the time (d) between 2 consecutive blood drops downstream of this. This time interval increases gradually as flow slows down and at an arbitrary point (d ≥15s, before reaching complete occlusion), the end point of the measurement is displayed (occlusion time [OT], in seconds). Restart of blood flow after occlusion is due to spontaneous thrombolysis (lysis time [LT], in seconds). If lysis does not occur until >6,000s (LT cut-off time), "no lysis" is displayed and recorded. The coefficient of variation (cv) was assessed by testing a cohort of 10 stable patients on 2 occasions, 48 hours apart.

ECG and Angiographic Analyses

ECG and angiographic analyses were performed by two investigators blinded to clinical characteristics and outcome. Standard 12-lead ECGs were obtained on arrival in the cath lab and compared to that at baseline presentation (in ambulance or emergency department). All ECGs were analyzed using a hand-held caliper. The ST-segment was measured 20 ms after the J point, and the sum of ST deviation was measured as previously described (18). The percent resolution of ST deviation from baseline to arrival was calculated, and categorized using Schröder's 3-component definition: complete (≥70%), partial (30% to 70%) and no (≤30%) ST resolution (18). Flow in the infarct-related artery was reported using the TIMI flow grading system and patency defined as TIMI grade 2 or 3 flow.

Data collection and follow-up

Patients were recruited during their index admission and their 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 months and 1 year by telephonic follow up, for the occurrence of MACE, defined as the composite of cardiovascular death (CVD), nonfatal MI or stroke. The secondary endpoint was major bleeding. For all endpoints, source documents were obtained and diagnosis verified by 2 independent clinicians blinded to the GTT results. The end points of the study were as follows: CVD, recurrent non-fatal MI, stroke, and major bleeding, classified as type 3-5 according to the Bleeding Academic Research Consortium (BARC) definition (19). For definitions of endpoint events, please see Supplement 1.

Statistical analysis

A clinical trial assessing whether LT is a predictor of MACE in STEMI, based on an effect size (HR) of 2.5 (13), assuming a MACE rate of 10% and an attrition rate of 10% over the follow-up period, would require about 500 patients to achieve 80% power. Based on previous published guidance (20), a pilot study sample should include at least 10% of the sample projected for the larger study. We opted for 17% (85 patients) to allow for suitable numbers for the analysis. Paired and unpaired t tests were used for comparison of normally distributed and Mann-Whitney U test used for non-normally distributed variables. Where necessary, log transformations were employed. Dichotomous variables were compared using chi-square test with continuity correction or Fisher's exact test, as appropriate. Correlations were analysed using Spearman's rank test. The OT and LT at baseline were related to the OT and LT at other time points. The OT and LT at the 4 time points were separately related to study end points of MACE or major bleeding. Separate analysis was used to compare the OT and LT of patients who had spontaneous coronary reperfusion to those who did not. Simple regression was used to examine the relationship between baseline OT and LT and pain to balloon time.

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 log-rank tests were used. In addition, to investigate the relationship between increasing LT and MACE, univariate Cox proportional hazard regression was performed on LT. Univariate and multivariate hazard regression models of Cox were used, respectively, to identify risk factors for clinical end points and to adjust for potential confounders that were associated with clinical end points on univariate analysis. Net reclassification improvement (NRI) was used to assess the added predictive ability of LT ≥3,000 for MACE. Tests were two-sided, and significance was fixed at 0.05 level. Analyses were performed with Stata V.11.2 (StataCorp, College Station, Texas, USA).

Results

Out of 85 eligible patients, 2 patients met one or more exclusion criteria, and 1 patient declined consent. The clinical characteristics are shown in Tables 2 and 3. Angiographic, procedural and echocardiographic characteristics are shown in Table 4. The coefficient of variation (cv) was 8% for OT and 10% for LT. There was no relationship between baseline OT (r=0.03, p=0.592) or baseline LT (r=0.71, p= 0.283) and the time of blood sampling.

Distribution and change in occlusion time

Baseline OT was normally distributed and OT at the 4 sampling time points is shown in Figure 1. OT was markedly prolonged after GPI/UFH treatment (mean±SD; 743±157s vs. 330±136s, p=0.00001) and after bivalirudin/UFH (707±121s vs. 381±97s, p=0.0004) compared to baseline, and remained prolonged at hospital discharge (536±195s vs. 330±136s, p=0.00001)

and at 30 days (515±147s vs. 330±136s, p=0.00001) compared to baseline. OT was similar at hospital discharge and at 30 days' follow-up (536±195s vs. 515±147s, p=0.695).

Distribution and change in lysis time

The distribution of LT at the 4 time points is shown in Figure 2. Baseline LT was positively skewed and many patients exhibited impaired endogenous thrombolysis with prolonged lysis times. LT was significantly reduced after GPI/UFH compared to baseline (median[IQR] 747 [579-950] s vs. 1075 [882-1682] s, p=0.00001). LT was similar at hospital discharge (1172 [931-2202] s vs. 1075 [882-1682] s, p=0.321), and at 30 days' follow-up (1059 [806-1569] s vs. 1075 [882-1682] s, p=0.801) compared to baseline [Figure 3-B]. At 30 days, LT was similar to LT at hospital discharge (1059 [806-1569] s vs. 1172 [931-2202] s, p=0.112).

Recurrent adverse clinical events and OT and LT

There were a total of 13 MACE events experienced by 13 patients, namely 8 CVD, 3 nonfatal MI (of which 1 attributable to acute stent thrombosis within 24 hours and 1 attributable to late stent thrombosis at 76 days), and 2 ischaemic strokes (not associated with atrial fibrillation). There were a total of 2 major bleeding events (BARC type 3a). There were no reported non-compliance issues with antiplatelet medication in those patients who experienced an adverse event.

Patients who went on to experience a MACE event, demonstrated a baseline OT that was significantly shorter (more thrombotic) than the OT of patients who did not experience an adverse event (253±150s vs. 354±134s; p=0.017), particularly with respect to nonfatal MI (OT 135±45s vs. 346±137s; p=0.009), and ischaemic stroke (OT 133±31s vs. 343±138s; p=0.036) [Figure 4]. Baseline OT did not differ between patients who experienced a major bleed and those who did not (297±121s vs. 339±141s; p=0.682), but the number of events was small.

ROC curve analysis showed that no particular threshold of baseline OT was a good binary discriminator of MACE risk [Figure 5-A]. Survival analysis demonstrated no relationship between baseline OT and MACE (hazard ratio [HR]: 0.99, 95%CI:0.989-0.999) or major bleeding. At other pre-specified time points, OT was not related to MACE or major bleeding.

Baseline LT level significantly discriminated between patients with and without MACE, with an area under the ROC curve of 0.59 (95% confidence interval [CI]:0.471-0.693; $p<0.05$) [Figure 5-B]. LT≥2901s was the optimal cut-point to predict MACE (rounded to 3000s for clinical ease), with sensitivity 31% and specificity 89% [Figure 5-B]. There were no LT readings between 2900s and 3000s that could have been wrongly classified based on rounding the cut-point to 3000s. Survival analysis demonstrated that LT≥3000s at baseline was strongly related to MACE (HR:3.31, 95%CI:1.02-10.78, p=0.045) [Figure 6], driven by CVD (HR:4.17, 95%CI:0.99-17.51, p=0.05) [Figure 7-B and Table 5]. LT≥3000s at baseline was not related to an increase in major bleeding, but the number of events was small. LT at the other pre-specified time points was not related to MACE or major bleeding.

Spontaneous coronary reperfusion and ST-resolution

Five STEMI patients had spontaneous coronary reperfusion, defined as complete or partial resolution of ST-elevation and chest pain before PPCI. All five patients had short baseline LT (<1000s), TIMI-3 flow at presentation, and uneventful 12-month outcomes. Lysis time was inversely correlated with spontaneous coronary reperfusion before PPCI (r= -0.3, p=0.022). The baseline OT in those with spontaneous coronary perfusion was no different to those patients without spontaneous coronary reperfusion (306±156s vs. 340±140s, p=0.609).

Influence of patient characteristics on OT and LT and outcomes

There was a weak positive correlation between baseline OT and the time from symptom onset (r=0.2, p=0.04) but no relationship between baseline LT and time from symptom onset. The following variables were interrogated (using parametric or non-parametric tests, as appropriate) for effects on baseline OT and LT: all patient characteristics in Table 2; haematological and biochemical profiles on admission in Table 3; the echocardiographic, angiographic, and interventional characteristics in Table 4. Smokers exhibited a shorter (more thrombotic) OT than non-smokers (294±105s vs. 365±153s, p=0.026). There was a weak negative correlation between baseline OT and creatinine level on admission (r= -0.4, p=0.057), and a weak positive correlation between baseline OT and pre-PCI TIMI-3 flow (r=0.2, p=0.063). None of the other variables including haematocrit, platelet count, troponin I, or left ventricular function correlated with baseline OT or LT. Female sex and patients with raised creatinine level on admission had significantly more baseline LT≥3000s values measured [Table 2 and Table 3]. Of the characteristics in Tables 2 and 3, only the following were related to MACE: increasing age (p=0.008), statin therapy (p=0.012), prior aspirin use (p=0.001), beta-blocker treatment (p=0.024), angiotensin-converting enzyme (ACE) inhibitor treatment (p=0.0008), calcium antagonists (p=0.001), prior renal insufficiency (p=0.002), high serum creatinine (p=0.001), and right coronary artery culprit vessel (p=0.020). None of the variables were related to major bleeding outcome, but the number of events was small.

The following variables were then entered into the final baseline multivariate Cox proportional hazard model: age (HR:1.07, 95%CI:1.02-1.13, p=0.005), statin therapy (HR:6.39, 95%CI:1.95-20.99, p=0.002), and two traditional risk factors, namely diabetes (HR:0.51, 95%CI:0.15-1.71, p=0.276) and hypertension (HR:0.51, 95%CI:0.16-1.60, p=0.246). None of these basic covariates were correlated either with LT or its dichotomized version, such as sex, which would have increased the standard error of the HR in the Cox proportional hazard

model. We did not include prior aspirin use in the model, as although in our small sample it was not related to OT or LT, we felt biologically it was likely to have influenced baseline platelet reactivity and thus not a truly independent variable. Multivariate analysis including the baseline covariates showed that baseline LT≥3000s remained strongly related to MACE after adjustment for risk factors (HR:4.26, 95%CI:1.13-16.01, p=0.032). Net reclassification improvement (NRI) showed that the inclusion of baseline LT≥3000s in a model containing three baseline predictors (age, statin therapy and diabetes) significantly added to the model effectiveness (NRI estimate 0.347, p=0.018).

Discussion

Impaired (prolonged) endogenous thrombolysis, seen in 13% STEMI patients at presentation, prior to PPCI, was predictive of an increased risk of adverse cardiovascular events, in particular cardiovascular death. Enhanced (rapid) endogenous thrombolysis was seen in patients with STEMI who demonstrated spontaneous coronary reperfusion, with spontaneous ST-segment resolution and TIMI-3 flow prior to intervention. Enhanced platelet reactivity, as demonstrated by short OT, was seen in patients with recurrent adverse events of nonfatal MI and stroke. Whilst the treatment with DAPT and PPCI, and the patient journey over time, resulted in OT prolongation; LT was similar pre-PPCI, at 30 days' follow-up and at hospital discharge. This would suggest that LT could be tested at any point up to 30 days, provided GPI is not used, and would not need to necessarily be tested prior to PPCI. However, although overall LT was similar at these time-points, only LT pre-PPCI was predictive of adverse cardiovascular events. This may at least in part be explained by the fact that many events occurred in the first 30 days [Figure 6] and that the "tail" of LT distribution, where some patients exhibited LT>6000s, was no longer apparent at 30 days. The AUC although significant, is overall small and while LT as a predictor of MACE is highly specific, its sensitivity is low, such that LT on its own is not a very good independent predictor of events. This may be better assessed in a larger study with a broad spectrum of patients which would avoid bias and allow for control of confounders, as well as allow calculation of the additive value of LT to other predictive markers in the assessment of LT performance as an additional predictive test.

It would seem that antiplatelet medications favourably alter (prolong) OT to reduce platelet reactivity over time, but LT is unaffected by standard chronic pharmacological treatment and

coronary intervention. However, in the acute phase, administration of GPI results in marked prolongation of OT and reduction in LT, an effect that wears off by the time the patient leaves the hospital, when LT has returned to baseline.

Prior work has shown that aspirin at low doses does not alter LT. More potent antiplatelet agents such as clopidogrel have negligible effects on LT (21). In patients with atrial fibrillation, the administration of non-vitamin K antagonist oral anticoagulants (NOACs), but not warfarin, favourably modulates (reduces) LT (22, 23). Previously in a study of patients with coronary disease, treatment with the PAR1 inhibitor vorapaxar resulted in reduction of LT (24). Our current work lends further support to the notion that agents that inhibit thrombin, but not antiplatelet agents, can exert a favourable effect on endogenous thrombolysis. This may underlie the observed findings of adding NOACs on top of standard dual antiplatelet in patients with ACS, where in APPRAISE-2, RE-DEEM, and ATLAS ACS 2–TIMI 51 trials, a reduction in recurrent ischaemic events was observed when a NOAC was used (25-27).

Our data are supported by prior studies which have demonstrated that impaired endogenous thrombolysis is a risk factor for adverse cardiac events in a number of patient cohorts, including non-ST-elevation MI (13), end-stage renal failure (14), stroke (28) and diabetes (29). The cut-point of LT that predicts MACE appears to be consistent in our study and prior studies (13, 14). Furthermore, D-dimer levels on admission were associated with a higher incidence of no reflow (30) and higher risk of MACE in STEMI patients undergoing PPCI (31) and elevated levels post-PPCI were related to impairment of myocardial function (32). Furthermore, when intracoronary thrombi aspirated during PPCI were examined, increased von Willebrand factor, P-selectin and fibrin content were related to unfavourable thrombus characteristics rendering the thrombus resistant to lytic therapy (33) and denser plasma fibrin clots were independently associated with high fibrin content (34). Recently, a crucial role of fibrinolysis

in determining infarct outcome was shown by combined inhibition of thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1, prior to transient cerebral artery occlusion in mice, which reduced cerebral infarct size by 50% (35).

Baseline LT≥3000s was more commonly seen in females in our study, and data show that women with STEMI tend to have a higher mortality than men, despite PPCI, and this is likely to be at least in part attributable to a worse risk profile (36-38). An earlier report assessing global thrombotic status showed no difference in LT between male and female healthy volunteers (39), thus whether the excess risk in women with STEMI may in part be related to worse thrombolytic status in the setting of STEMI, requires further study.

Others have shown that high residual platelet reactivity (HRPR) on DAPT as assessed with the VerifyNow technique was predictive of recurrent events, especially in ACS patients undergoing PCI (40, 41). However, those tests were uniformly performed on therapeutic DAPT, whereas our initial sample was performed before therapeutic levels of DAPT had been achieved, and our third sample was done at follow up, when almost all events had occurred. It would have been the pre-discharge sample that we would have expected to correlate with MACE. The discrepancy between earlier findings of HRPR and our results can be explained by the different techniques used. ADP-induced platelet aggregation (VerifyNow) is more sensitive than the GTT in detecting ADP antagonist effect. ADP-induced platelet aggregation measurement is based on the concept that ADP plays a crucial role in platelet aggregation and thrombus formation. The GTT measures thrombus formation at high shear stress. However, there is strong evidence that platelet aggregation induced by high shear (relevant to arterial shear rates *in vivo*) occurs independently of ADP (42, 43), which explains the lower sensitivity of GTT technique to ADP antagonism. The fact that ADP

mediated thrombus formation does not underlie all arterial thrombotic events also helps explain the low specificity (53%) reported with the Verify Now technique (44) and may support the validity of our results. The lower specificity of HRPR as measured by VerifyNow compared with LT measurement is further supported by emerging evidence showing the important role of $P2Y_{12}$ receptor signalling not in the growth rate but in the stability of the evolving thrombus. This effect can be measured by detecting disaggregation of ADP-induced aggregates and low disaggregation identified patients on clopidogrel at risk of MI with equal or better specificity than did the VerifyNow (45). The concept that alteration of thrombus stability plays a major role in the mechanism of the antithrombotic effect of antiplatelet medication is gaining momentum (46).

The main limitation of our study is the small sample size. As such, this data is hypothesis generating and requires validation in suitably powered, large, prospective clinical study. In addition, because of the need to consent patients pre-procedure, we excluded those who were *in extremis*, such as those with cardiogenic shock or out-of-hospital cardiac arrest, who were at very high risk of MACE and who may have had even worse thrombotic profile than the patients studied. Another limitation is that optimal antithrombotic medication in the context of STEMI comprises of aspirin combined with newer $P2Y_{12}$ inhibitors, namely ticagrelor or prasugrel. Whilst these medications are increasingly used, in much of the UK and elsewhere, clopidogrel and aspirin administration in the ambulance remains the "standard of care" due to financial constraints. It is unlikely that prasugrel or ticagrelor administration would have impacted on endogenous thrombolysis, but assessment of the effect of these newer and more potent antiplatelet medications would have been desirable. The study could not assess the relationship between thrombotic status and bleeding events,

since the number of bleeds was very small, probably helped by the majority (>90%) of interventions being performed radially. Furthermore, the timing of the first (baseline) sample was such that absorption of antiplatelet agents was unreliable and therapeutic doses were likely not have been reached. Although this may be a criticism of our study, it also reflects real-time thrombotic status at the time of PPCI.

For the first time, we show that impaired endogenous thrombolysis in STEMI patients is predictive of a high risk of adverse events despite good angiographic outcome, whilst patients with spontaneous coronary reperfusion and ST-segment resolution demonstrate enhanced (short) endogenous thrombolysis. Large clinical studies are needed to see if the addition of potent antithrombotic medications such as NOACs to standard care with DAPT in those with impaired endogenous thrombolysis, can reduce adverse events. The GTT may serve as a risk stratification tool to identify patients with impaired endogenous thrombolysis, and to target those who are most pro-thrombotic with selective escalation of antithrombotic medication, whether to further target platelets or thrombin; and to avoid excessive unnecessary antithrombotic agents in those who are at low risk.

What is known on this topic

- The outcome of a thrombotic event depends on the balance between prothrombotic drivers and the effectiveness of the intrinsic endogenous thrombolytic potential.
- Contemporary antithrombotic and antiplatelet agents, whilst reducing recurrent thrombosis, have significantly increased the risk of bleeding.
- Identification of patients at high-risk of future thrombotic complications would be highly desirable, since this group could be targeted with more potent antithrombotic medications, allowing use of less potent agents (thereby reducing bleeding risk) in lower risk groups.

What this paper adds

- in patients with STEMI presenting for PPCI, impaired endogenous thrombolysis, as measured in the catheterisation laboratory with the point-of-care Global Thrombosis Test, is predictive of recurrent adverse.
- Patients with STEMI who demonstrate spontaneous reperfusion and spontaneous ST-segment resolution have enhanced (rapid) endogenous thrombolysis.
- Endogenous thrombolysis may be a novel target for pharmacological intervention in STEMI, and allow targeting of potent antithrombotic medications to high-risk patients.

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Conflict of interest

DAG is related through family to a company director of Thromboquest Ltd., which manufactures the Global Thrombosis Test, but she, her spouse, and her children have no financial involvement or equity interest in, and have received no financial assistance, support, or grants from Thromboquest Ltd. Thromboquest Ltd. has no involvement in the design, conduct, or the finance of this review. The other authors have reported no relationships relevant to the contents of this paper to disclose.

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Figure legends

Figure 1: Distribution of Occlusion Time at 4 time-points

(A) Baseline, upon admission and before PPCI, (B) after administration of glyocoprotein IIb/IIIa inhibitor (GPI), (C) at clinical stabilisation and just prior to hospital discharge, and (D) at 30-days post hospitalisation. OT=occlusion time. Y axis shows % patients.

Figure 2: Distribution of Lysis Time at 4 time-points

(A) Baseline, upon admission and before PPCI, (B) after administration of glyocoprotein IIb/IIIa inhibitor (GPI), (C) at clinical stabilisation and just prior to hospital discharge, and (D) at 30-days post hospitalisation. LT=occlusion time. Y axis shows % patients.

Figure 3: Occlusion Time and Lysis Time (mean ± SD)

Occlusion time (OT) was prolonged after treatment with glyocoprotein IIb/IIIa inhibitor (GPI)/unfractionated heparin (UFH) or after bivalirudin (Bival)/UFH, at discharge from hospital, and at 30 days post hospitalisation compared to baseline OT. Lysis time (LT) was shorter after GPI/UFH than at baseline. *$p<0.05$, compared to baseline.

Figure 4: MACE and major bleeding according to baseline Occlusion Time

Baseline OT in patients who did not have an adverse outcome is indicated by open bars; baseline OT in patients who had an adverse outcome is indicated by solid bars. *$p<0.05$.

MI = myocardial infarction; CVA = cerebrovascular accident; CVD = cardiovascular death

Figure 5: ROC curves for baseline Occlusion Time and Lysis Time

Receiver-operating characteristic (ROC) curves for (A) occlusion time (OT), and (B) lysis time (LT) at baseline. An LT≥3000s was identified as the optimal cut point to predict MACE outcome, with sensitivity of 31% and specificity of 89%.

Figure 6: Probability of event-free survival according to baseline LT

Kaplan-Meier curves showing probability of event-free survival (survival free of MACE) in STEMI patients based on the baseline LT. An LT≥3000s at baseline was associated with a hazard ratio of 3.31. Solid line indicates LT<3000s and dotted line indicates LT≥3000s.

Figure 7: Major adverse cardiovascular events and major bleeding according to baseline LT

(A) Baseline LT in patients who did not have an outcome is indicated by open bars; baseline in patients who had an outcome is indicated by solid bars, (B) Baseline LT<3000s is indicated by open bars; baseline LT≥3000s is indicated by solid bars. *p<0.05. †p=0.05.

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

Figure 8: HR for major adverse cardiovascular events according to baseline LT

Hazard ratios (HR) for cardiovascular death (CVD), nonfatal myocardial infarction (MI), and stroke according to baseline lysis time (LT). The 95% confidence interval is shown in brackets. n: number of patients. *p<0.05.

Table 1: Exclusion Criteria

Inability to consent
Current participation in another study
Less than 18 years of age
Known diagnosis of malignancy
Sepsis
Bleeding diathesis
Thrombolysis, warfarin, heparin, or GPI inhibitor treatment before sampling
Blood dyscrasia (platelets <100x10^9/L, haemoglobin <80g/L, international normalized ratio >1.4, activated partial thromboplastin time more than twice upper limit of normal, leukocyte count <3.5x10^9/L, neutrophil count <1x10^9/L)
Intolerance or contraindication to aspirin or clopidogrel
Complete follow-up over 1-year period not likely
Life expectancy <12 months due to known non-cardiovascular condition

Table 2: Baseline Patient Characteristics

	Overall Group (n=82)	Baseline LT <3000s (n=71)	Baseline LT ≥3000s (n=11)	p Value
Age, yrs	63±12	63±12	64±10	0.444
Male	67 (81.7)	61 (85.9)	6 (54.5)	0.025
Diabetes mellitus	13 (15.9)	12 (16.9)	1 (9.1)	1.000
Active smoker	31 (37.8)	29 (40.8)	2 (18.2)	0.193
Hypertension	39 (47.6)	33 (46.5)	6 (54.5)	0.749
Prior CAD	12 (14.6)	10 (14.1)	2 (18.2)	0.660
Renal insufficiency	4 (4.9)	2 (2.8)	2 (18.2)	0.085
PVD	2 (2.4)	2 (2.8)	0	1.000
Prior CVA	1 (1.2)	1 (1.4)	0	1.000
Prior aspirin use	15 (18.3)	12 (16.9)	3 (27.3)	0.414
Medications on discharge				
Aspirin	80 (97.6)	70 (98.6)	10 (90.9)	0.252
Clopidogrel	79 (96.3)	69 (97.2)	10 (90.9)	0.355
VKA	4 (4.9)	4 (5.6)	0	1.000
Beta-blocker	64 (78.1)	56 (78.9)	8 (72.7)	0.699
ACE inhibitor	68 (82.9)	61 (85.9)	7 (63.6)	0.087
Calcium antagonists	12 (14.6)	9 (12.7)	3 (27.3)	0.199
Statin	69 (84.2)	61 (85.9)	8 (72.7)	0.369
Nitrate	9 (11.0)	8 (11.3)	1 (9.1)	1.000
Insulin	5 (6.1)	4 (5.6)	1 (9.1)	0.523
Metformin	5 (6.1)	5 (7.0)	0	1.000

Values are mean ± standard deviation or n (%). Renal insufficiency was defined by creatinine levels >177 µmol/L (13). Prior aspirin use was defined as aspirin use before hospitalisation.

ACE: angiotensin-converting enzyme, CAD: coronary artery disease, CVA: cerebrovascular accident, LT: lysis time, PVD: peripheral vascular disease, VKA: vitamin K antagonist.

Table 3: Blood Tests on Admission

	Overall Group (n=82)	Baseline LT <3000s (n=71)	Baseline LT ≥3000s (n=11)	p value
Haemoglobin (g/L)	139±20	140±19	130±27	0.115
Haematocrit (%)	42±6	42±6	39±6	0.077
Platelet count (x 10^9/L)	257±71	253±70	286±72	0.154
Creatinine (µmol/L)	94±39	91±29	117±76	0.039
C-reactive protein (mg/L)	13±20	13±18	17±30	0.571
Troponin T (ng/L), peak at 12 hrs	20,200±2770	21,300±2850	9,800±1670	0.336
Total Cholesterol (mmol/L)	4.8±1.2	4.8±1.2	4.9±1.2	0.711

Values are mean ± standard deviation.

Normal values: haemoglobin 130-180 g/L in adult males and 115-165 g/L in adult females; haematocrit 40-52 % in adult males and 36-47 % in adult females; platelet count 150-400 x10^9/L; creatinine 60-110 µmol/L in adult males and 45-90 µmol/L in adult females; C-reactive protein 0-5 mg/L; troponin T <14 ng/L; total cholesterol ≤4.0 mmol/L.

Table 4: Angiographic, Interventional, and Echocardiographic Patient Characteristics

	Overall Group (n=82)	Baseline LT <3000s (n=71)	Baseline LT ≥3000s (n=11)	p value
1-vessel disease	43 (52.4)	39 (54.9)	4 (36.4)	0.260
2-vessel disease	22 (26.8)	17 (23.9)	5 (45.5)	0.141
3-vessel disease	16 (19.5)	14 (19.7)	2 (18.2)	0.902
Culprit vessel				
LAD	25 (30.9)	24 (33.8)	1 (9.1)	0.131
Diagonal	2 (2.5)	2 (2.8)	0	0.901
LCA	9 (11.1)	6 (8.5)	3 (27.3)	0.080
OM	3 (3.7)	3 (4.2)	0	0.923
RCA	42 (51.9)	35 (49.3)	7 (63.6)	0.381
GPI use	58 (70.7)	52 (73.2)	6 (54.5)	0.285
Bivalirudin use	4 (4.9)	3 (4.2)	1 (9.1)	0.444
Balloon predilatation	57 (69.5)	49 (69.0)	8 (72.7)	1.000
Stent implantation				
BMS	19 (24.4)	15 (21.1)	4 (36.4)	0.273
DES	59 (75.6)	52 (73.2)	7 (63.6)	0.514
TIMI-3 flow				
Before PCI	14 (17.5)	13 (18.3)	1 (9.1)	0.461
After PCI	69 (86.3)	59 (83.1)	10 (90.9)	0.520
LV function after index event				
Normal (EF ≥ 55%)	45 (59.2)	37 (52.1)	8 (72.7)	0.212
Mildly impaired (EF 45-54%)	17 (22.4)	16 (22.5)	1 (9.1)	0.333

Moderately impaired (EF 36-44%)	13 (17.1)	13 (18.3)	0	0.264
Severely impaired (EF ≤ 35%)	1 (1.3)	1 (1.4)	0	0.673
Door to balloon time, min	24±16	23±16	26±14	0.519
Call to balloon time, min	100±18	100±19	98±14	0.940

Values are n (%).

BMS: bare metal stent, DES: drug eluting stent, EF: ejection fraction, GPI: glycoprotein IIb/IIIa inhibitor, LAD: left anterior descending coronary artery, LCA: left circumflex coronary artery, LT: lysis time, LV: left ventricular, OM: obtuse marginal coronary artery, PCI: percutaneous coronary intervention, RCA: right coronary artery, TIMI: Thrombolysis in Myocardial Infarction.

Table 5: Clinical outcome at 12 months' follow-up

	Overall Group (n=82)	Baseline LT <3000 s (n=71)	Baseline LT ≥3000 s (n=11)	HR (95% CI)	p value
Cardiovascular death, nonfatal MI, and stroke	13 (15.9)	9 (12.7)	4 (36.4)	3.31 (1.02-10.78)	0.047
Cardiovascular death*	8 (9.8)	5 (7.0)	3 (27.3)	4.18 (0.99-17.51)	0.05
Nonfatal MI	3 (3.7)	2 (2.8)	1 (9.1)	3.96 (0.36-43.94)	0.263
Stroke**	2 (2.4)	2 (2.8)	0	NA	NA
Major bleeding	2 (2.4)	1 (1.4)	1 (9.1)	8.79 (0.55-140.46)	0.124

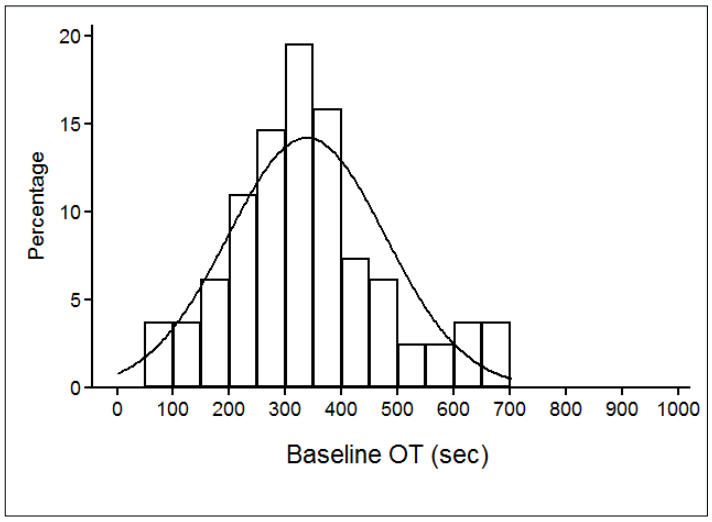
Values are n (%).

CI: confidence interval, HR: hazard ratio, LT: lysis time, MI: myocardial infarction, NA: not applicable.

* Cardiovascular deaths defined as death in the presence of acute coronary syndrome, significant cardiac arrhythmia, or refractory congestive heart failure, or death attributed to cardiovascular cause at post-mortem.

** Causes of stroke were ischaemic stroke.

(A)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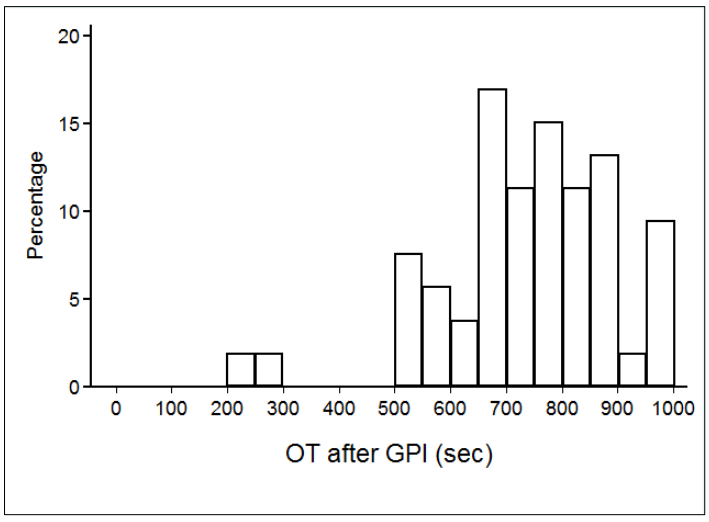
Baseline OT (sec)

(B)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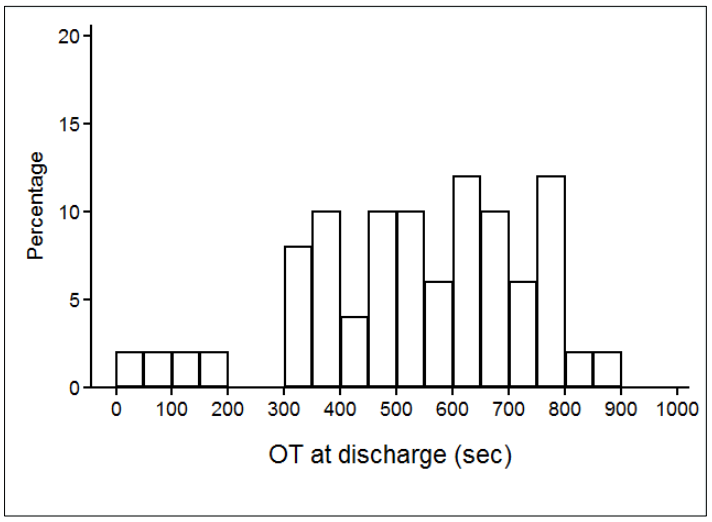
(C)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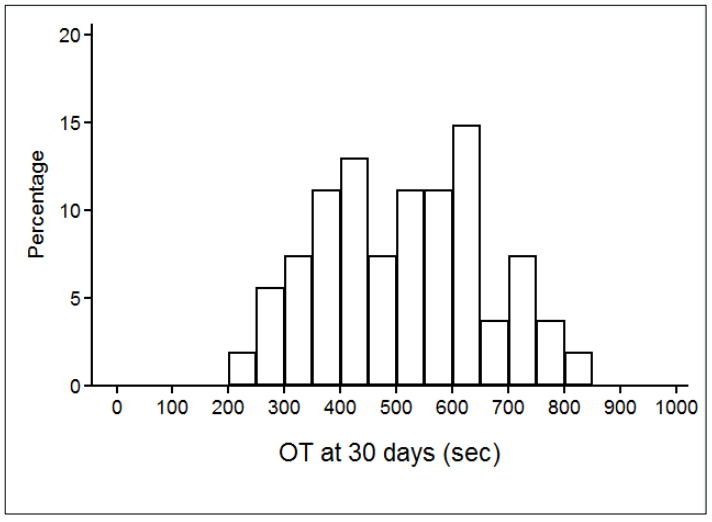
(D)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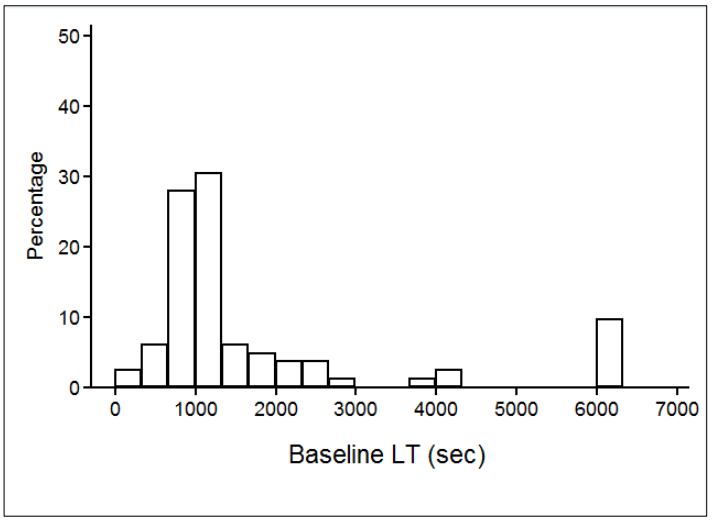
OT at 30 days (sec)

(A)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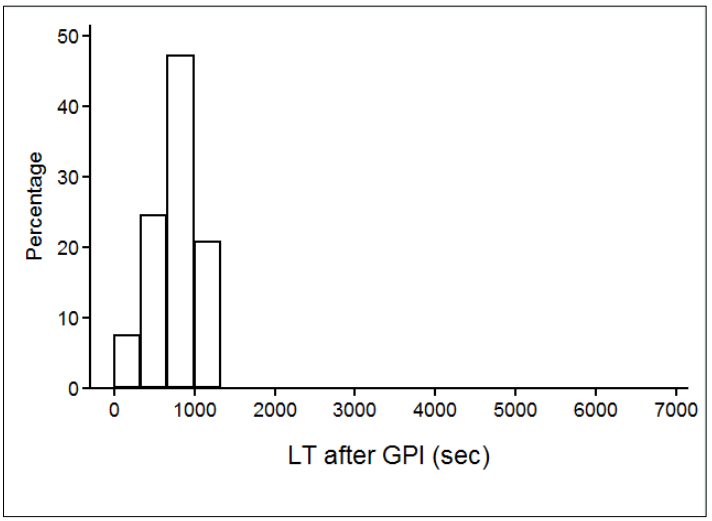
Baseline LT (sec)

(B)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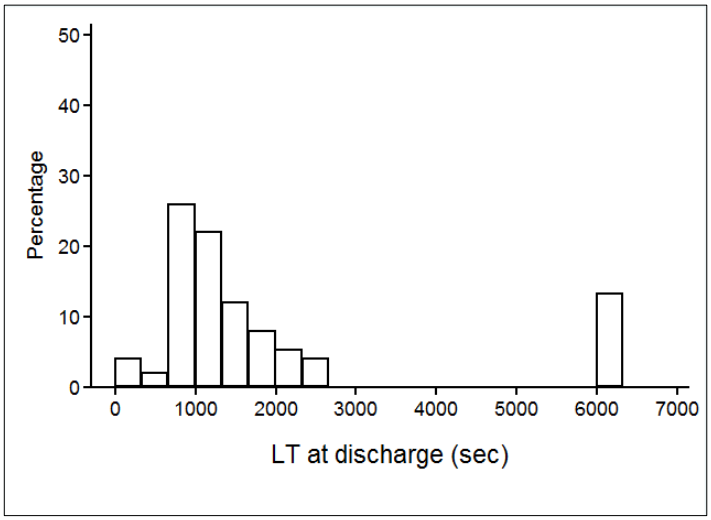
LT after GPI (sec)

(C)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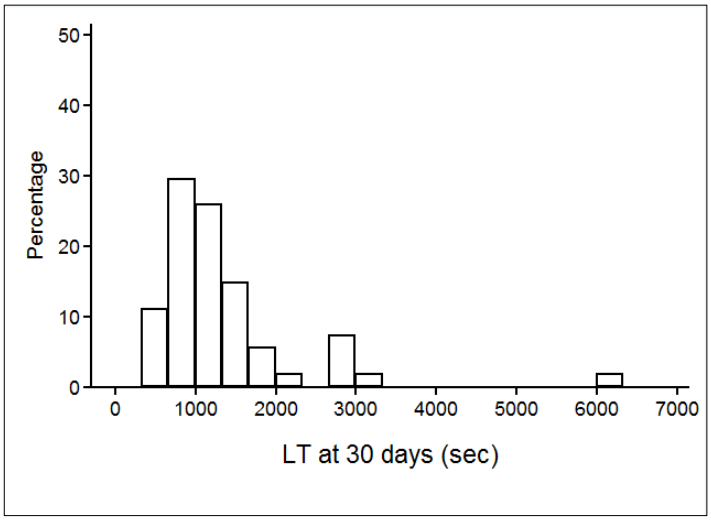
(D)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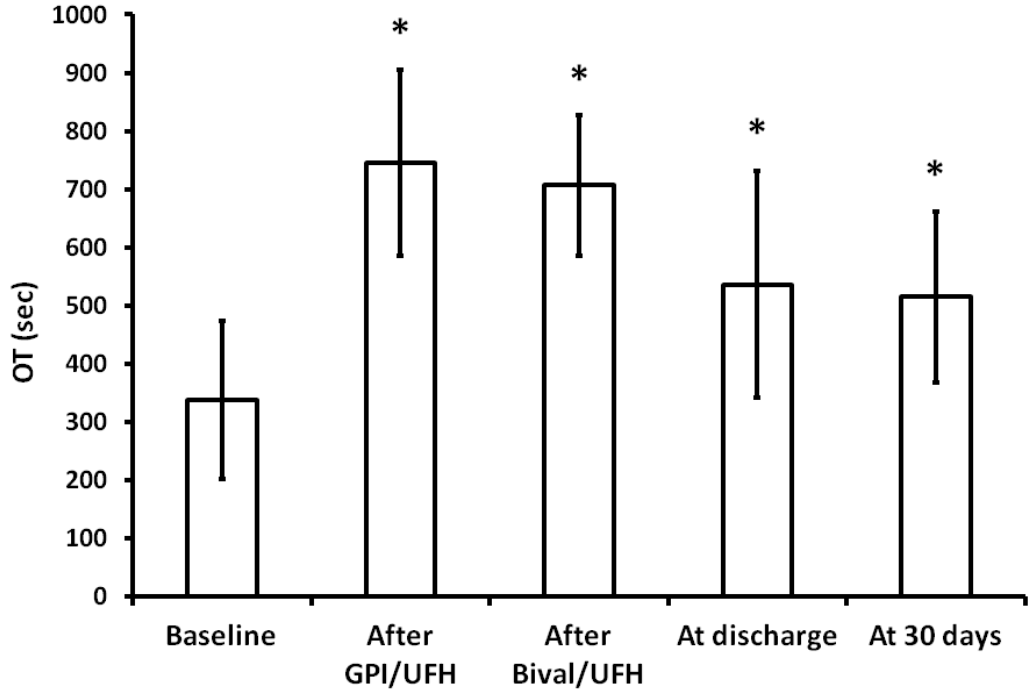
LT at 30 days (sec)

(A)


OT (sec)
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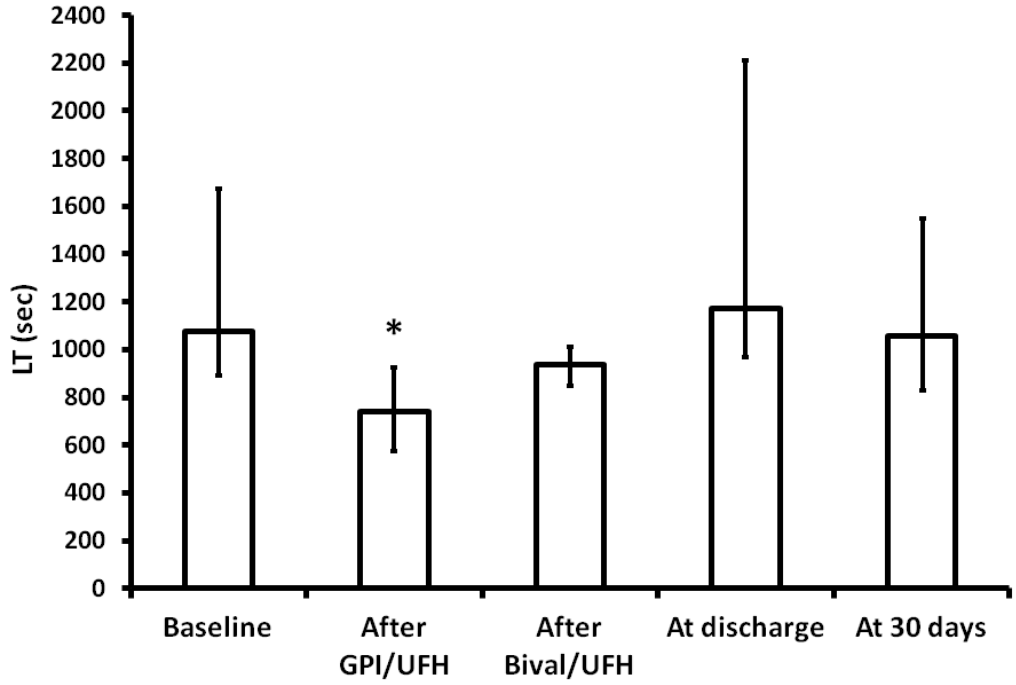
Baseline
After GPI/UFH
After Bival/UFH
At discharge
At 30 days

(B)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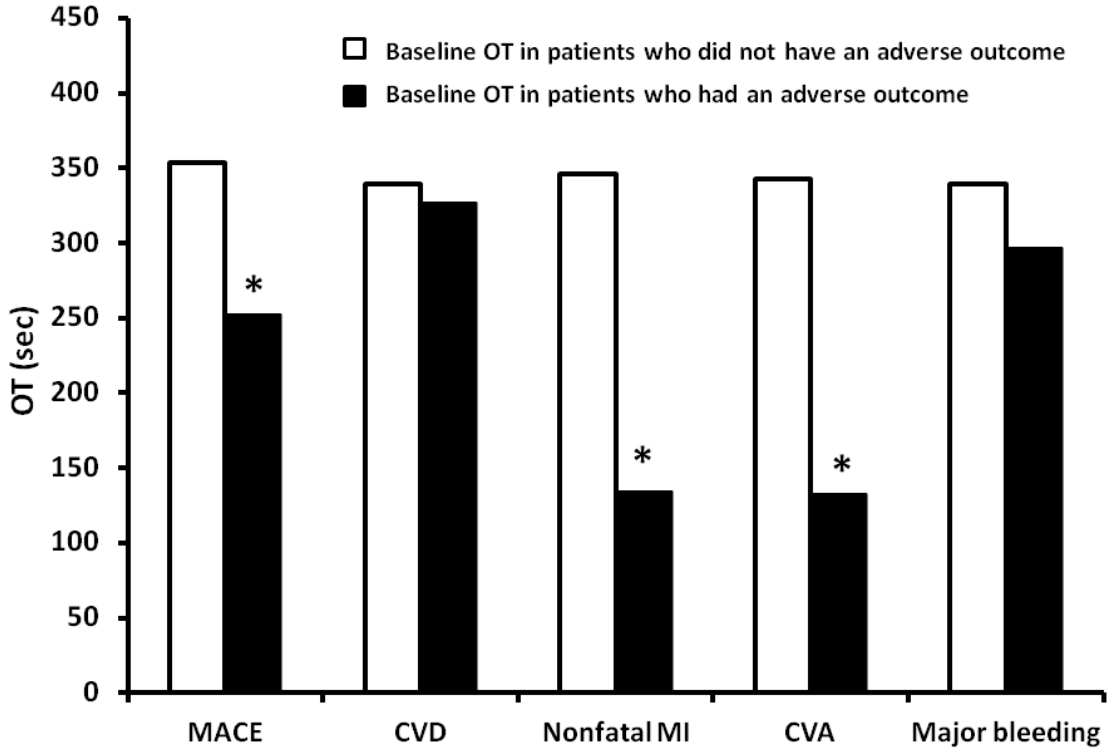
*
Baseline
After GPI/UFH
After Bival/UFH
At discharge
At 30 days


Baseline OT in patients who did not have an adverse outcome
Baseline OT in patients who had an adverse outcome
OT (sec)
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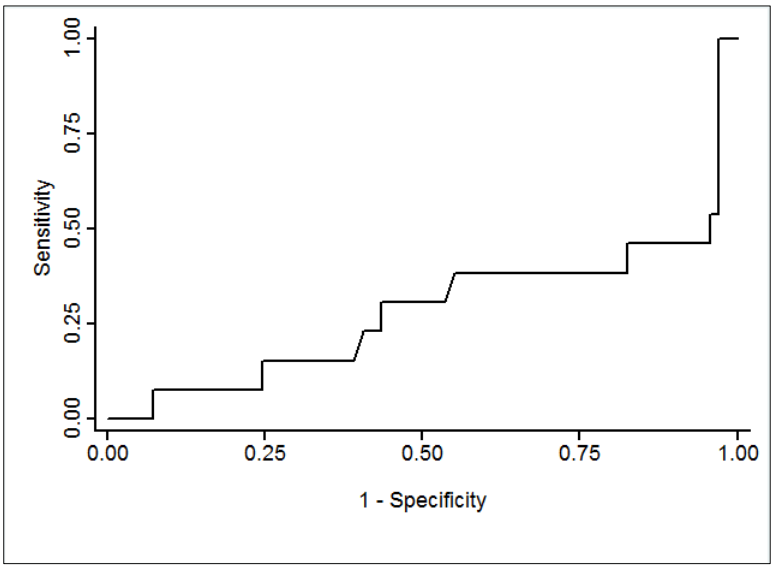
*
MACE
CVD
Nonfatal MI
CVA
Major bleeding

(A)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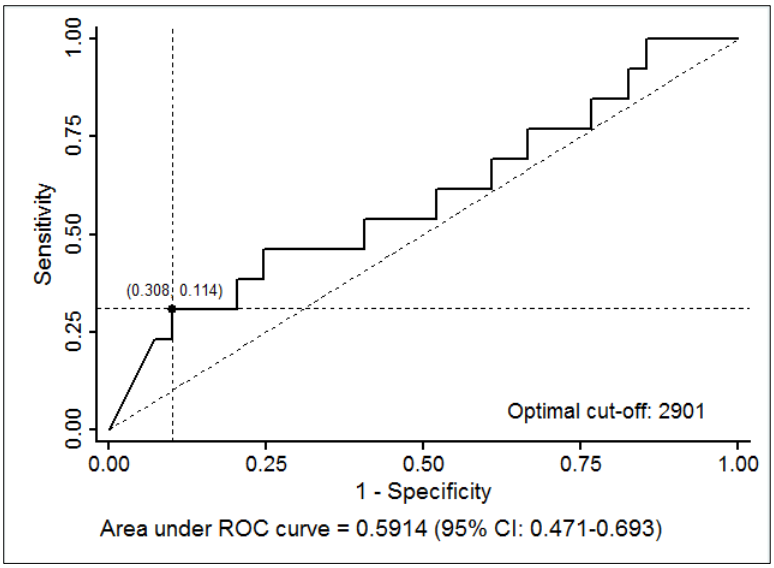
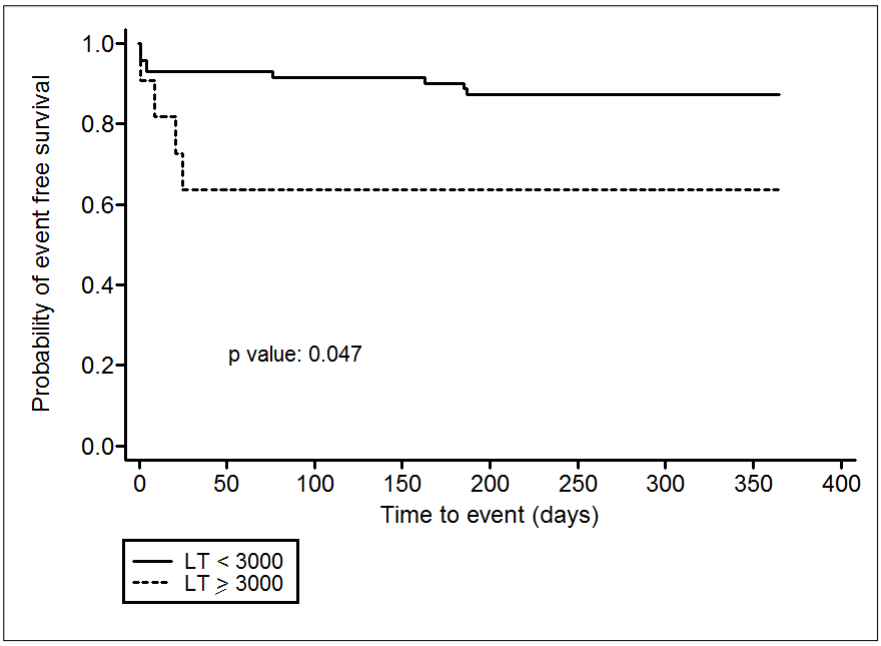
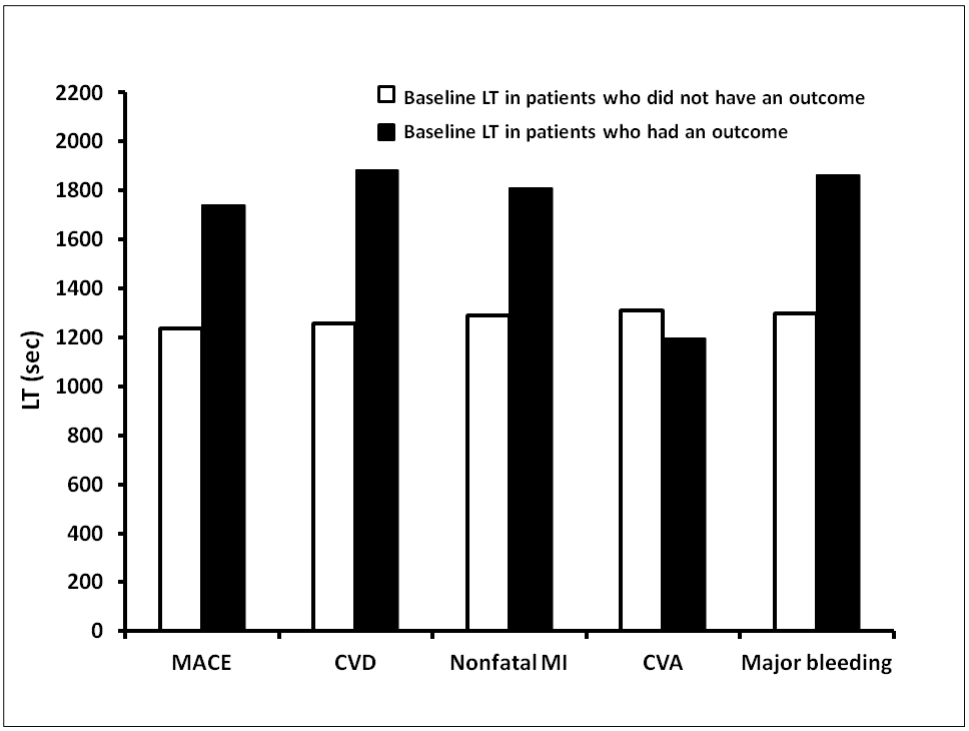
1.00
1 - Specificity

(B)

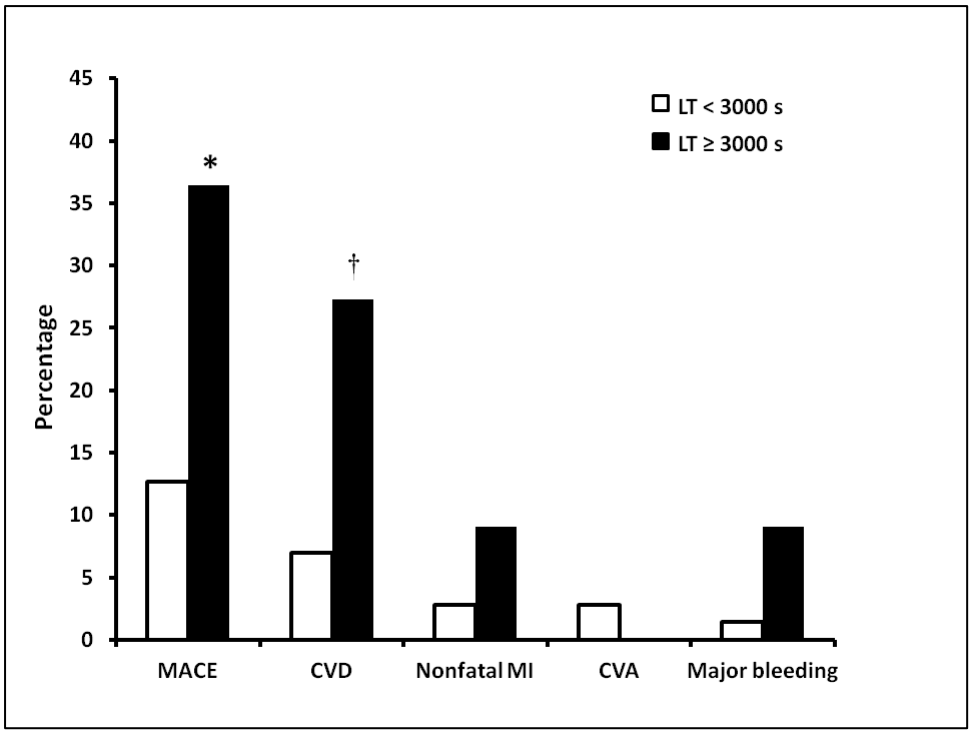

Sensitivity
(0.308, 0.114)
Optimal cut-off: 2901
1 - Specificity
Area under ROC curve = 0.5914 (95% CI: 0.471-0.693)

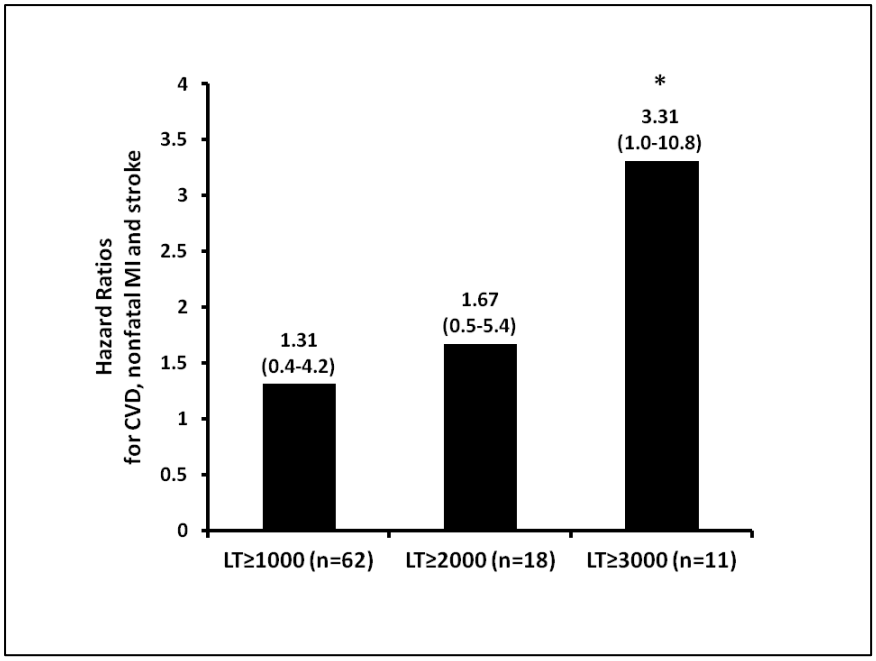

Probability of event free survival
1.0
0.8
0.6
0.4
0.2
0.0
p value: 0.047
0
50
100
150
200
250
300
350
400
Time to event (days)
LT < 3000
LT ⩾ 3000

(A)


Baseline LT in patients who did not have an outcome
Baseline LT in patients who had an outcome
LT (sec)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
MACE
CVD
Nonfatal MI
CVA
Major bleeding

(B)


LT < 3000 s
LT ≥ 3000 s
Percentage
45
40
35
30
25
20
15
10
5
0
*
†
MACE
CVD
Nonfatal MI
CVA
Major bleeding


Hazard Ratios
for CVD, nonfatal MI and stroke
4
3.5
3
2.5
2
1.5
1
0.5
0
1.31
(0.4-4.2)
1.67
(0.5-5.4)
*
3.31
(1.0-10.8)
LT≥1000 (n=62)
LT≥2000 (n=18)
LT≥3000 (n=11)