Point-of-care biomarkers of thrombotic status predict spontaneous reperfusion in ST-segment elevation MI and clinical outcomes

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ABSTRACT

Background

Spontaneous reperfusion, seen in ~20% of patients with ST-segment elevation myocardial infarction (STEMI), manifests as normal epicardial flow in the infarct-related artery (IRA), with or without ST-segment resolution, before percutaneous coronary intervention (PCI). The drivers mediating this are unknown.

Objectives

To relate spontaneous reperfusion to thrombotic profile.

Methods

In a prospective study, blood from STEMI patients (n=801) was tested pre-PCI to assess *in vitro,* point-of-care, occlusion (OT) and endogenous fibrinolysis (LT) times. Spontaneous reperfusion was defined as IRA TIMI III flow pre-PCI. Patients were followed for major cardiovascular events (MACE; death, myocardial infarction, stroke).

Results

Spontaneous reperfusion was associated with longer OT (435s vs. 366s, $p<0.001$) and shorter LT (1257s vs. 1616s, $p<0.001$), lower troponin and better LV function. LT was superior to OT for predicting spontaneous reperfusion (AUC for LT: 0.707, 95% CI 0.661-0.753; AUC for OT: 0.629, 95% CI 0.581-0.677). Amongst patients with spontaneous reperfusion, those with complete, versus partial ST-segment resolution, had longer OT ($p=0.002$) and shorter LT ($p<0.001$). Spontaneous reperfusion was unrelated to clinical characteristics or pain-to-angiography times.

Over 4-years, patients with spontaneous reperfusion experienced fewer MACE than those without (4.1% vs. 10.6%, p=0.013), especially in those with both spontaneous reperfusion *and* complete ST-segment resolution (1.5% vs. 10.1%, p=0.029).

Conclusions

We demonstrate a novel haematological signature in STEMI patients with spontaneous reperfusion, namely reduced platelet reactivity and faster endogenous fibrinolysis, relating to smaller infarcts and improved survival. This indicates a role for modulating thrombotic status early following STEMI-onset, to facilitate spontaneous reperfusion and improve outcomes.

Word count 250

Condensed abstract

Spontaneous reperfusion, seen in ~20% of STEMI patients, manifests as normal flow in the infarct-related artery, with or without ST-segment resolution, pre-PCI. The drivers mediating this are unknown. In blood samples from STEMI patients pre-PCI, we measured global thrombotic status, as occlusion time and endogenous fibrinolysis time, using native blood with a point-of-care technique. Spontaneous reperfusion correlated with reduced platelet reactivity and faster endogenous fibrinolysis, smaller infarct size and improved clinical outcomes. The relation between thrombotic status and spontaneous reperfusion indicates a role for modulating thrombotic status early following STEMI-onset, to facilitate reperfusion and improve outcomes.

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Keywords:

Spontaneous reperfusion, myocardial infarction, thrombotic status, platelet reactivity, endogenous fibrinolysis

Abbreviations:

IRA = infarct related artery

LT = lysis time

MACE = major adverse cardiovascular events

OT = occlusion time

PCI = percutaneous coronary intervention

STEMI = ST-segment elevation myocardial infarction

TIMI = thrombolysis in myocardial infarction

INTRODUCTION

Amongst patients presenting with ST-segment elevation myocardial infarction (STEMI), 15-25% have spontaneous reperfusion of the infarct related artery (IRA), before mechanical or pharmacological intervention, depending on the definition used.[1, 2] Spontaneous reperfusion manifests as resolution of the ST-segment elevation on the ECG (transient STEMI) and/or normal coronary flow (thrombolysis in myocardial infarction [TIMI] III) in the IRA at angiography before percutaneous coronary intervention (PCI).

Analysis of data from the CADILLAC and HORIZONS-AMI trials showed lower 1 year mortality in patients with spontaneous reperfusion,[3] with studies also reporting smaller infarct size and lower rates of in-hospital death, heart failure and length of stay.[4, 5] Further, patients with spontaneous reperfusion may not benefit from immediate revascularisation, since myocardial salvage has already occurred, as reported by registries[4] and the TRANSIENT[6] small randomised controlled trial. Analysis of the 4 Primary Angioplasty in Myocardial Infarction (PAMI) studies showed that TIMI III flow pre-intervention predicted not only procedural success, but low mortality, which was independent of post-procedural TIMI flow.[5] The mechanism underlying the spontaneous reperfusion in some patients, but not others, is not understood.

Coronary thrombosis has been described as a failure of timely spontaneous, endogenous thrombolysis,[7] and a few studies have linked plasma markers of thrombosis and fibrinolysis with spontaneous reperfusion. A small study of STEMI patients undergoing angiography showed lower pre-treatment levels of the main potentiator of thrombolysis, plasma tissue-type plasminogen activator (t-PA) antigen, and higher prothrombin fragments 1+2 and soluble fibrin levels in patients with spontaneous reperfusion, compared to patients without,

with no difference in the levels of the primary inhibitor of thrombolysis, namely plasminogen activator inhibitor 1.[8] In a retrospective analysis of 998 patients with STEMI, the 23% of patients with spontaneous reperfusion had a significantly lower plasma homocysteine level compared to patients without spontaneous reperfusion.[9] Hyperhomocysteinaemia has been associated with reduced t-PA activity,[10] and in a dose-dependent manner linked with adverse clot architecture with more densely packed fibrin strands.[11] Clots with dense fibrin composition have been linked to impaired endogenous fibrinolysis *in vitro*.[12] Higher levels of the serum peptide apelin, which can inhibit thrombin and collagen-induced platelet activation, have been detected in patients with spontaneous reperfusion compared to those without.[13] More recently, assessment of global thrombotic status in whole blood has shown that impaired (prolonged) endogenous fibrinolysis time *in vitro* is a strong, independent predictor of future cardiovascular events in patients with acute myocardial infarction.[12, 14]

The aim of our study was to assess the relationship between global thrombotic status, in particular endogenous fibrinolysis, and spontaneous reperfusion in patients with STEMI, in relation to 1) pre-PCI IRA patency; 2) resolution of ST-segment elevation on the ECG; 3) infarct size; and 4) adverse cardiovascular outcomes.

METHODS

Study design and population

We conducted a prospective, observational, single-centre study of consecutive patients aged 18 years or older, presenting with STEMI and undergoing emergency coronary angiography.

Patients were excluded if they were on concomitant oral anticoagulation, involved in an investigational trial of a medicinal compound, suffered from known coagulopathy, thrombocytopaenia, end-stage renal failure, sepsis, alcohol dependence or other condition known to effect coagulation. Bloods samples were taken on presentation and a delayed consenting strategy was used, with ethical permission, following primary PCI. All enrolled patients gave written informed consent. The study, together with the delayed consenting strategy used, was approved by the local ethics committee of the UK Health Research Authority and the local Research and Development Board and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The cohort comprises of patients enrolled into two observational studies in acute coronary syndromes (ClinicalTrials.gov identifier: NCT02562690 and UK Independent Research Application System ID no. 260786).

Patients with spontaneous coronary artery reperfusion were identified during emergency diagnostic angiography based on the presence of TIMI III flow in the IRA. This may or may not have been accompanied by resolution or reduction of symptoms and of ST-segment elevation (when compared with the initial ECG performed by the paramedic crew/emergency department). Complete, partial and no ST-segment resolution were defined by ≥70%, 30%-70%, and ≤30% resolution respectively.[15]

Blood sampling

Blood samples were obtained immediately before emergency angiography, and taken from a 6 Fr arterial sheath, immediately after its insertion, and before diagnostic imaging or the administration of heparin or other anticoagulant, but after dual antiplatelet therapy loading. In most cases, this comprised aspirin 300mg and clopidogrel 600mg, administered in the

ambulance by paramedics, but some patients received additional loading with 180mg of ticagrelor in the heart attack centre or emergency department prior to angiography, at the treating physician's discretion. The first 10 ml of blood withdrawn was used for routine clinical measurements, whilst the subsequent 5 ml was used to assess thrombotic status.

Assessment of thrombotic status

Thrombotic status was assessed with the Global Thrombosis Test (GTT, Thromboquest Ltd, London, UK). Whole, non-anticoagulated blood was injected into a cartridge according to the manufacturer's instructions and the automated measurement commenced. The technique has been described previously.[16] Briefly, within the GTT cartridge, are two ball bearings, and adjacent to each there are narrow gaps between the ball bearing and walls of the cartridge. As blood passes through the very narrow gaps adjacent to the first ball bearing, it is subjected to high shear, leading to platelet activation. In the area between the two ball bearings, platelet aggregates are formed, which gradually occlude the narrow gaps adjacent to the second ball bearing, leading to arrest of flow. Blood flow is detected by a downstream photosensor, and the time taken from introduction of blood, to arrest of flow, is termed the occlusion time (OT). The time taken for the spontaneous restart of blood flow is termed lysis time (LT) and is a measure of endogenous fibrinolysis. Samples were injected into the cartridges within 15 seconds (s) of withdrawal.

Patient demographics and baseline characteristics

Patient demographics, medical history, drug therapy (prior to admission, and up to discharge), results of ECGs, blood work on admission, angiography and revascularisation procedural details were collected and recorded into electronic case report forms.

Follow-up and clinical end-points

Patients were followed-up either face-to-face or by telephone, for up to 4 years, for the occurrence of cardiovascular events, including death, myocardial infarction and stroke (ischaemic and haemorrhagic). These events were classified according to standard definitions (supplementary material). All events were investigated by formal review of patients' notes by two independent clinicians and reported individually and as a combined outcome of major adverse cardiovascular events (MACE).

Statistical analysis

Sample size was calculated using the methodology described by O'Keeffe et al. for positively skewed data,[17] and the median LT (1405 s) and standard deviation (1118 s) reported by the RISKPPCI study,[18] assessing endogenous fibrinolysis in patients admitted to hospital with STEMI. Assuming a 25% incidence of spontaneous reperfusion as reported by recent studies, and a two-sided alpha of 0.05, we required a sample size of 800 patients to detect a 12% difference in LT, with 80% power and a 5% drop-out/loss to follow-up rate.

Basic descriptive statistics were used to report clinical and biochemical characteristics and procedural details in patients with and without spontaneous reperfusion. Differences between groups were assessed using the $\chi 2$ and Fisher's exact test for dichotomous variables, and the

t-test and Mann-Whitney U test for continuous variables that were normally and non-normally distributed respectively. The Spearman's rank correlation coefficient was used to assess correlations in continuous and ordinal variables.

Binary logistic regression was used to model and assess the utility of both OT and LT in predicting spontaneous reperfusion, whilst receiver operating characteristic (ROC) and Kaplan Meier curves, in addition to Cox regression were used to determine the prognostic significance of spontaneous reperfusion, platelet reactivity and endogenous fibrinolysis function. Checks undertaken to assess the assumption of proportional hazards showed that it was reasonable to use this assumption.

Analyses were performed using SPSS Statistics (IBM Corp. Released 2019. IBM SPSS Statistics for Macintosh, Version 26.0. Armonk, NY: IBM Corp) or R version 4.2.2 (R Core Team, 2022). All statistical tests were two-sided with $p < 0.05$ taken to indicate statistical significance.

RESULTS

Baseline clinical and biochemical characteristics

A total of 801 patients were recruited, of whom 148 (18%) had spontaneous reperfusion identified during emergency angiography. There were no differences in clinical characteristics or in antiplatelet therapy pre-angiography between patients with and without spontaneous reperfusion (Table 1). Although patients with spontaneous reperfusion had lower leucocyte counts and a higher platelet-to-lymphocyte ratio, there was no difference in

inflammatory markers including hs-CRP, neutrophil-to-lymphocyte ratio (NLR) and the systemic immune-inflammation index (SII).

Infarct size, as assessed by peak troponin T, was significantly smaller in patients with spontaneous reperfusion (Table 1), with an inverse correlation between initial TIMI flow during angiography and peak troponin T ($r = -0.369$, $p<0.001$).

Relationship between thrombotic status and spontaneous reperfusion

Patients with spontaneous reperfusion had significantly longer occlusion time (OT), reflecting lower platelet reactivity, and significantly shorter lysis time (LT), reflecting more rapid endogenous fibrinolysis (Table 1). A stepwise increase in the incidence of spontaneous reperfusion was seen with increasing OT (Figure 1), and the reverse seen with increasing LT (Figure 2) (Central Illustration). Using ROC curve analysis and Youden's index, we show the optimal cut-points of OT and LT to predict spontaneous reperfusion (Table 3, Supplementary Figures 1 and 2). Amongst patients with a combination of OT ≥398 s and LT ≤1402 s, 43% had spontaneous reperfusion, compared with only 14% among patients with OT <398 s and LT >1402 s. Combining OT and LT was able to predict spontaneous reperfusion with an AUC of 0.703 (95% CI 0.657 to 0.748) (Supplementary Figure 3). Using binary logistic regression and Youden's index, a model using only OT and LT as continuous variables (which were confirmed as being linearly associated with the logit of the outcome) correctly identified the spontaneous reperfusion state for 66.6% of patients (bootstrap 95% CI 51.3% to 77.3%) with sensitivity 62.3% (bootstrap 95% CI 46.6% to 88.1%) and specificity 67.6% (bootstrap 95% CI 44.0% to 83.6%). We observed no difference in prothrombin time (PT), activated partial thromboplastin time (aPTT) international normalised ratio (INR) and platelet count between patients with and without spontaneous reperfusion.

Relationship between thrombotic status and ST-segment resolution on ECG in patients with spontaneous reperfusion

Not all patients with TIMI III flow during emergency angiography (spontaneous reperfusion) had complete ST-segment resolution on ECG. This occurred in only 66 patients (8% of total cohort). These patients had a significantly shorter LT (1062 [1002-1170] s vs. 1463 [1259-1832] s $p<0.001$) and longer OT (493 [392-641] s vs. 413 [304-522] s, $p=0.002$) than those patients with TIMI III flow and partial/no ST-segment resolution. Infarct size was also smaller, as assessed by peak troponin (732 [158-1551] ng/l vs. 1115 [597-2184] ng/l, $p=0.04$, respectively).

Relationship between spontaneous reperfusion and angiographic findings, revascularisation details, and post-infarct LV function

Compared to patients without spontaneous reperfusion, patients with spontaneous reperfusion had a lower coronary disease burden, evidenced by a significantly greater proportion of patients with unobstructed arteries, and a numerically lower proportion with triple-vessel disease (Table 2). Furthermore, no obvious culprit high grade stenosis (epicardial coronary stenosis >50%)[19] was identified in a significant proportion of patients with spontaneous reperfusion. Stent thrombosis was also less frequent in patients with spontaneous reperfusion. Revascularisation with PCI occurred less frequently in patients with spontaneous reperfusion, and fewer stents were used compared to patients without spontaneous reperfusion, but the target vessel/culprit did not differ. During intervention, more patients without spontaneous

reperfusion received glycoprotein IIb/IIIa inhibitor (GPI), but despite this, fewer patients had TIMI III flow at the end of the procedure (Table 2).

No differences were observed in the pain-to-angiography, pain-to-balloon, door-to-balloon, and dual antiplatelet therapy loading-to-balloon times (Table 2).

Pre-discharge, patients with spontaneous reperfusion had better left ventricular systolic function, with a greater number of patients with ejection fraction (EF) ≥55%, and fewer with EF ≤40% (Table 2).

Prognostic significance of spontaneous reperfusion

Significantly lower MACE rates were observed in patients with spontaneous reperfusion compared to those without spontaneous reperfusion, at 30 days, 1 year and at a mean follow-up of 629 days (4.1% vs. 10.6%, p=0.013) (Figure 3, Table 4), with 65% lower MACE rate in patients with spontaneous reperfusion at maximal follow-up (HR: 0.35 [0.14-0.97] p=0.024), driven by a lower incidence of cardiovascular death (HR: 0.38 [0.15-0.95], p=0.038) (Table 5).

Patients with both spontaneous reperfusion and complete ST-segment resolution on the ECG had the lowest MACE rate, compared to patients without spontaneous resolution (and either no or partial ST-segment resolution) (1.5% vs 10.1% p=0.029).

At 48 and 72 hours post-admission, there were no MACE events in patients with spontaneous reperfusion and complete ST-segment resolution on ECG, whereas there were 24 (3.3%) and 27 (3.7%) MACE in patients without spontaneous reperfusion (and either no or partial ST-segment resolution) respectively.

Prognostic significance of endogenous fibrinolysis and platelet reactivity

Using ROC curve analysis and Youden's index, independent OT and LT cut-offs were defined to optimally predict patients with MACE. An LT ≥1800 s predicted MACE with 67% sensitivity and 69% specificity and OT< 317s predicted MACE with 68% sensitivity and 69% specificity. Kaplan-Meier curves for MACE in patients with LT ≥1800s and LT< 1800 s, and OT <317 s and OT ≥317 s demonstrated a significant difference using the log-rank test ($p<0.001$) (Figure 4). Patients with LT ≥1800 s were more likely to experience a MACE event compared to those with LT <1800 s (HR: 3.9 [2.5-6.3], $p<0.001$) and patients with OT <317 s were more likely to experience MACE compared to those with OT ≥317 s (HR: 4.2 [2.6-6.8], $p<0.001$).

Kaplan Meier curves were also plotted for patients according to LT and OT criteria defined for optimal prediction of spontaneous reperfusion. Patients with OT ≥398 s and LT≤ 1402 s had the highest incidence of spontaneous reperfusion and lowest MACE rate (Table 3, Figure 5). The converse was seen for patients with OT< 398 s and LT >1402 s (HR 4.7 (2.0-11.1), $p<0.001$). However, the best predictor for MACE was both OT< 317 s and LT ≥1800 s, which were individually derived using ROC curve analysis. Patients with both OT< 317 s and LT ≥1800 s had the highest MACE rate (33%) and were at much higher risk compared with patients with OT ≥317 s and LT< 1800 s (HR 5.3, 95% CI: 3.4-8.2) (Figure 6).

DISCUSSION

Our study provides novel insights into the pathomechanism of STEMI, with a novel haematological signature in patients with spontaneous reperfusion, which is apparent on admission. In perhaps the largest study of the thrombotic profile of STEMI patients with spontaneous reperfusion, we show that patients with a patent IRA have longer thrombotic occlusion time, and more rapid/effective endogenous fibrinolysis *in vitro*, compared to those without spontaneous reperfusion. Furthermore, the longer the OT and the shorter the LT, the greater the chance of spontaneous reperfusion at baseline pre-PCI angiography. Even amongst patients with spontaneous reperfusion at angiography, those with concomitant ST-segment resolution on the ECG exhibited even more favourable thrombotic profiles, with longer OT and shorter LT, than those with spontaneous reperfusion without ECG resolution. We confirmed previously documented associations of spontaneous reperfusion with smaller infarct size, as detected by troponin level and left ventricular function, and a lower incidence of MACE, compared to patients without spontaneous reperfusion. Furthermore, the thrombotic status at baseline was highly predictive of MACE.

We did not observe differences in the results of conventional tests of coagulation between patients with and without spontaneous reperfusion. Our finding of longer OT, which likely reflects lower platelet reactivity, in patients with spontaneous reperfusion, is supported by some prior studies. Earlier, smaller studies assessed the relationship of platelet reactivity, using the VerifyNow assay, with spontaneous reperfusion in STEMI. One study assessed platelet function at the time of angiography in 164 patients with STEMI, of whom 65 patients had TIMI II or III flow pre-PCI.[20] High on-treatment platelet reactivity (HTPR) was associated with lower rates of pre-PCI TIMI II or III flow compared to that observed in patients with no-HTPR group (32.5% vs 51.1%, p = 0.04), although no difference was observed when considering just those patients with TIMI III flow. Another study of 50

STEMI patients receiving aspirin and 600-mg clopidogrel loading, showed that amongst patients with a patent IRA pre-PCI, the prevalence of HTPR to clopidogrel was lower than in those without spontaneous reperfusion pre-PCI (15.9% vs 66.7%; p=0.013).[21]

The finding of shorter (more efficient) endogenous fibrinolysis time in patients with spontaneous reperfusion is both mechanistically plausible, and supported by earlier studies showing that prolonged (less efficient) endogenous fibrinolysis *in vitro* identifies a high risk cohort of STEMI patients and is an independent, adverse prognostic biomarker.[12,14] The importance of fibrinolytic potential as a determinant of outcome in patients with acute coronary syndrome was also shown in a substudy of the PLATO trial.[22] Plasma clot lysis time was found to be an important determinant of cardiovascular mortality, regardless of whether patients were taking clopidogrel or ticagrelor, in addition to aspirin,[22] supporting the assumption that LT in whole blood, as measured in the present study, is an important determinant of outcome, regardless of the degree of platelet inhibition. Analysis of plasma clot lysis time in 60 patients, sampled 4-12 months after STEMI, showed that patients who had presented with transient STEMI had a significantly faster plasma clot lysis time, than those with persistent STEMI at presentation, with no difference in von Willebrand factor, plasmin-antiplasmin complex, d-dimer or thrombin generation.[23]

Limitations

Our study has notable limitations, that include the sample size and being a single-centre study. Thrombotic profile of patients was assessed only at a single timepoint pre-PCI, so we cannot comment whether this thrombotic profile persists over time and following established treatment with dual antiplatelet medications. Furthermore, the blood samples were taken (shortly) after dual antiplatelet therapy loading, and therefore, whilst the results reflect global

thrombotic status, it is not possible to determine how much this reflects a patient's pre-existing thrombotic status and how much it reflects the effect of the recently administered dual antiplatelet medication. On the other hand, whilst antiplatelet therapy may influence OT, prior data indicate that it has negligible effect on LT.[12,24] Whilst a particular strength is the "global" nature of the thrombotic status assessment used here, since we did not specifically measure response to aspirin or $P2Y_{12}$ inhibition with a specific platelet function test, we cannot further classify the impact of these medications, or their variable/differential effects, on the overall thrombotic state. Lastly, we cannot comment on the changes in thrombotic profile that may exist over time in STEMI patients. Our findings should therefore be regarded as exploratory and hypothesis generating.

Clinical relevance and future directions

Our observation that pre-PCI IRA patency is related to a more favourable global thrombotic profile, indicates a possible role for modulating the global thrombotic status rapidly following the onset of STEMI, to facilitate spontaneous reperfusion, reduction in infarct size and mortality.

A strength of our study is that the global test employed here to assess platelet reactivity employs high shear, rather than a specific agonist (such as ADP or thromboxane), to initiate platelet activation, aggregation and coagulation. Therefore, the results reflect the haematological response to a global stimulus rather than one specific to a particular agonist and better reflect *in vivo* pathological thrombus formation in a severely stenosed coronary artery.

Currently available antiplatelet therapies block very specific pathways of platelet activation, but platelet activation may still occur via other agonists. For example, $P2Y_{12}$ inhibitors block

ADP-induced amplification of platelet activation, but do not directly inhibit the effect of other platelet agonists such as, for example thrombin, thromboxane and von Willebrand factor. Similarly, whilst the use of GPI can prevent the cross-linking of platelets, it does not impact on platelet adhesion, pro-coagulant activity or platelet-leukocyte aggregate formation. The target-specific effect of routinely administered antithrombotic medications in the setting of acute myocardial infarction may, in part, explain the lack of significant impact of these agents on reperfusion. Although the more potent $P2Y_{12}$ inhibitors prasugrel and ticagrelor achieve more rapid and more profound inhibition of platelet reactivity than clopidogrel,[25] the ATLANTIC study showed that pre-hospital administration of ticagrelor did not improve pre-PCI reperfusion, compared to in-hospital administration.[26] In the PLATO trial, there was no difference between patients receiving clopidogrel or ticagrelor with respect to pre-PCI coronary flow or myocardial perfusion.[27]

However, the lack of benefit with early or more potent oral $P2Y_{12}$ inhibition on IRA patency may also reflect the slow onset of action of these agents due to delayed absorption in the setting of STEMI, particularly when opiates are used.[25,28]

Whether earlier and more profound platelet inhibition could enhance reperfusion is unknown. In 50 STEMI patients receiving crushed oral ticagrelor loading, the additional administration of the intravenous $P2Y_{12}$ inhibitor cangrelor significantly reduced platelet reactivity as early as 5 minutes after bolus injection, compared to placebo.[29] Selatogrel, a novel, selective, subcutaneous $P2Y_{12}$ inhibitor, can achieve profound (>80%) inhibition of ADP-induced platelet aggregation within 15 minutes,[30] but whether this can enhance early coronary reperfusion will be tested in the ongoing Selatogrel Outcome Study in Suspected Acute Myocardial Infarction (SOS-AMI) trial (ClinicalTrials.gov Identifier: NCT04957719). While earlier studies showed that upstream administration of GPI, compared to placebo, in STEMI patients was associated with rapid platelet inhibition, more frequent pre-PCI TIMI II-III flow

and ST-segment resolution, overall routine prehospital GPI administration did not improve outcomes and caused excess bleeding.[31] Zalunfiban, a novel subcutaneous GPI developed for prehospital administration in STEMI,[32] achieved similar rapid, potent platelet inhibition as selatogrel in phase 2 studies. Its effects on coronary flow pre-PCI and ST-segment resolution will be assessed in the CELEBRATE trial (NCT04825743).

Potent intravenous $P2Y_{12}$ inhibition may enhance endogenous fibrinolysis, particularly under conditions of high shear.[24] Modulation of the coagulation pathway may also improve thrombotic profile. Recent data from the Swedish Coronary Angiography and Angioplasty Registry of 41,631 patients with STEMI undergoing primary PCI, showed that pre-hospital treatment with unfractionated heparin compared to no heparin was associated with an 11% relative risk reduction in patients presenting to the catheterisation laboratory with an occluded coronary artery.[33] There are also data in patients with atrial fibrillation that non-vitamin K antagonist oral anticoagulants may enhance endogenous fibrinolysis.[34] Whether these approaches can modulate endogenous fibrinolysis in the acute STEMI setting, including with novel factor XIa inhibitors,[35] and whether that translates into improved spontaneous reperfusion and clinical outcomes, requires future studies.

Conclusion

We demonstrate that in the setting of STEMI, spontaneous reperfusion is related to a more favourable global thrombotic profile, with reduced platelet reactivity and more effective endogenous fibrinolysis, which in turn is related to smaller infarct size, shorter hospitalisation and improved survival. This indicates a possible role for modulating global thrombotic status early following the onset of STEMI, to facilitate spontaneous reperfusion, reduction in infarct size and mortality, which will need to be assessed in future trials.

PERSPECTIVES

Competency in Medical Knowledge 1: Spontaneous reperfusion, seen in ~20% of patients with ST-segment elevation myocardial infarction, manifests as normal flow in the infarct-related artery, with or without ST-segment resolution on the ECG, before percutaneous coronary intervention. The drivers mediating this are unknown.

Competency in Medical Knowledge 2: Patients with a heart attack who exhibit spontaneous reperfusion have smaller infarcts and better long-term outcomes than those without spontaneous reperfusion.

Translational Outlook 1: Our study shows that patients who have spontaneous reperfusion have a more favourable *in vitro* thrombotic profile, with reduced platelet reactivity and more efficient endogenous fibrinolysis, compared to patients without spontaneous reperfusion.

Translational Outlook 2: The relationship between spontaneous reperfusion and favourable thrombotic profile indicates a possible role for modulating the global thrombotic status rapidly following the onset of myocardial infarction, to facilitate spontaneous reperfusion, and improve clinical outcomes.

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Figure Legends

Central Illustration. Spontaneous reperfusion is related to reduced platelet reactivity and rapid endogenous fibrinolysis

In patients with STEMI, spontaneous reperfusion of the IRA is related to reduced platelet reactivity (long occlusion time) and rapid endogenous fibrinolysis (short lysis time). These in vitro findings are related to pre-PCI TIMI III flow, ST-segment resolution, infarct size and cardiovascular outcomes. Thus, reduced platelet reactivity and rapid endogenous fibrinolysis appear to be key to the pathomechanism of spontaneous reperfusion in STEMI. Modulating thrombotic status early following STEMI onset may facilitate spontaneous reperfusion and improve outcomes.

Figure 1. Relationship between incidence of spontaneous reperfusion and occlusion time

The prevalence of spontaneous reperfusion (defined by initial thrombolysis in myocardial infarction [TIMI] flow during emergency angiography) is directly related to occlusion time (OT), with patients with the longest OT (lowest platelet reactivity) having the highest prevalence of spontaneous reperfusion.

* $p<0.05$ and ** $p<0.01$

Figure 2. Relationship between incidence of spontaneous reperfusion and endogenous fibrinolysis

The prevalence of spontaneous reperfusion (defined by initial thrombolysis in myocardial infarction [TIMI] flow during emergency angiography) is inversely related to lysis time (LT), with patients with the shortest LT (most rapid endogenous fibrinolysis) having the highest prevalence of spontaneous reperfusion.

** p<0.01

Figure 3. Relationship between spontaneous reperfusion and event-free survival

Kaplan-Meier curves showing event-free survival in patients with and without spontaneous reperfusion. Major adverse cardiovascular events were significantly lower in patients with spontaneous reperfusion, as defined by initial thrombolysis in myocardial infarction (TIMI) flow during emergency angiography, compared to those without spontaneous reperfusion. Log-rank p=0.013.

Figure 4. Relationship between event-free survival and occlusion time or fibrinolysis time

(A) Kaplan-Meier curves showing event-free survival in relation to lysis time (LT). Patients with a LT ≥1800 s had a significantly higher rate of major adverse cardiovascular events than patients with shorter LT (log rank p<0.001, HR: 3.9 [2.5-6.3], p<0.001), cut-offs defined by ROC curve analysis.

(B) Kaplan-Meier curves showing event-free survival in relation to occlusion time (OT). Patients with OT <317 s had a significantly higher rate of major adverse cardiovascular events than patients with longer OT (log rank p<0.001, HR: 4.2 [2.6-6.8], p<0.001), cut-offs defined by ROC curve analysis.

Figure 5. Relationship between thrombotic status (combined occlusion and lysis times) and major adverse cardiovascular events

Kaplan-Meier curves showing event-free survival in patients, in relation to both occlusion time (OT) and lysis time (LT), with cut-offs derived from the prediction of spontaneous reperfusion. Major adverse cardiovascular events occurred more frequently in those patients

with both LT >1402 s and OT <398 s, while the lowest adverse event rate was observed in patients with both short LT ≤1402 s and longer OT ≥398 s.

Log-rank p<0.001

Figure 6. Relationship between thrombotic status (combined occlusion and lysis times) and major adverse cardiovascular events

Kaplan-Meier curves showing event-free survival in patients, in relation to both occlusion time (OT) and lysis time (LT), with cut-offs derived from the prediction of major adverse cardiovascular events. Major adverse cardiovascular events occurred significantly more frequently in those patients with both LT≥1800 s and OT <317 s, while the lowest adverse event rate was observed in patients with both short LT <1800 s and longer OT ≥317 s.

Log-rank p<0.001. HR 5.3 (95% CI: 3.4-8.2), p<0.001.

Table 1. Baseline patient characteristics

Abbreviations: APTT: activated partial thromboplastin time, BMI: body mass index, CABG: coronary artery bypass grafting, CAD: coronary artery disease, CKD: chronic kidney disease, CVA: cerebrovascular accident, FH: family history, Hs-CRP: high sensitivity C-reactive protein, INR: international normalised ratio, LT: lysis time, MI: myocardial infarction, NLR: neutrophil-to-lymphocyte ratio, OT: occlusion time, PAD: peripheral arterial disease, PCI: percutaneous coronary, PLR: platelet-to-lymphocyte ratio, PT: prothrombin time, SII: systemic immune-inflammation index, TIMI: thrombolysis in myocardial infarction.

	Patients with < TIMI III, n=653	**Patients with TIMI III (spontaneous reperfusion), n=148**	**p value**
Clinical characteristics			
Age, years (SD)	63.5 (13.0)	63.5 (12.4)	0.742
Male, n (%)	514 (79)	108 (73)	0.092
Caucasian, n (%)	579 (89)	132 (89)	0.663
BMI, kg/m2 (IQR)	26.8 (24.1-30.3)	26.7 (23.8-30.1)	0.769
Smoking, n (%)	207 (32)	50 (34)	0.838
Hypertension, n (%)	273 (42)	71 (48)	0.206
Hyperlipidaemia, n (%)	219 (34)	55 (37)	0.459
Diabetes, n (%)	111 (17)	32 (22)	0.208
FH premature CAD, n (%)	224 (37)	44 (30)	0.157
Angina, n (%)	39 (6)	11 (8)	0.530
Prior MI, n (%)	69 (11)	16 (11)	0.962
Prior PCI, n (%)	65 (10)	12 (8)	0.470
Prior CABG, n (%)	7 (1)	3 (2)	0.353
CKD, n (%)	20 (3)	7 (5)	0.332
PAD, n (%)	21 (3)	9 (6)	0.103
CVA, n (%)	22 (3)	6 (4)	0.701
Laboratory characteristics			
Peak Troponin T, ng/l (IQR)	2460 (1258 – 4519)	1070 (464 – 1925)	**<0.001**
Creatinine, µmol/L (IQR)	82 (71 – 96)	82 (66 – 100)	0.711
Hs-CRP, mg/l (IQR)	3 (1 – 7)	3 (2 – 7)	0.466
Total cholesterol, mmol/l (IQR)	5.0 (4.2 - 5.7)	4.6 (3.8 - 5.5)	0.345
LDL cholesterol, mmol/l (IQR)	2.9 (2.2 - 3.7)	2.6 (1.8 - 3.2)	**0.015**
HDL cholesterol, mmol/l (IQR)	1.2 (0.98 - 1.4)	1.2 (0.97 - 1.5)	0.244
Triglycerides, mmol/l (IQR)	1.6 (1.1 - 2.1)	1.6 (1.2 - 2.4)	0.598
Haemoglobin, g/dl (IQR)	141 (128 - 150)	141 (132 - 149)	0.217
Haematocrit, (IQR)	0.41 (0.38 - 0.44)	0.41 (0.39 - 0.44)	0.550
Platelets, $x10^9$/l (IQR)	243 (204 – 286)	248 (217 – 293)	0.988
Leucocyte count, (IQR)	11.3 (8.9 - 14.3)	10.6 (9.2 - 14.3)	**0.004**
Neutrophil count, (IQR)	8.7 (6.4 - 11.0)	8.2 (6.4 - 10.3)	**0.024**
Lymphocyte count (IQR)	1.74 (1.26 - 2.35)	1.71 (1.24 - 2.20)	**0.028**
PLR, (IQR)	136 (99 - 185)	153 (109 – 213)	**0.015**
NLR, (IQR)	4.5 (2.9 – 6.7)	4.6 (3.0 – 7.0)	0.682
SII, (IQR)	1084 (682 - 1660)	1077 (663 - 1748)	0.818
INR (IQR)	1 (1 – 1)	1 (1 – 1)	0.277
PT, s (IQR)	11.3 (10.9 – 11.9)	11.3 (10.7 – 12.0)	0.324
APTT, s (IQR)	26.2 (22.5 – 29.4)	26.5 (23.5 – 29.4)	0.061
Fibrinogen, g/l (IQR)	3.9 (3.3 – 4.8)	4.2 (3.7 – 5.0)	**0.010**
Thrombotic status			
OT, s (IQR)	366 (268 – 485)	435 (324–545)	**<0.001**
LT, s (IQR)	1616 (1315 – 2165)	1257 (1058 – 1571)	**<0.001**
Chronic antiplatelet therapy pre-admission			
Aspirin, n (%)	115 (18)	26 (18)	0.940
$P2Y_{12}$ inhibitor, n (%)	34 (5)	11 (8)	0.300
DAPT, n (%)	23 (4)	3 (2)	0.450
Loading $P2Y_{12}$ inhibitor pre-PPCI			
Clopidogrel loading (only), n (%)	428 (67)	104 (71)	0.314
Prasugrel loading, n (%)	12 (2)	0 (0)	0.137
Ticagrelor loading, n (%)	192 (30)	41 (28)	0.681

Table 2. Angiographic, revascularisation and left ventricular systolic function data in patients with and without spontaneous reperfusion.

	Patients with < TIMI III flow, n=653	**Patients with TIMI III flow (spontaneous reperfusion) n=148**	**p value**
Coronary disease burden			
Unobstructed arteries, n (%)	7 (1)	5 (3)	**0.037**
1-Vessel disease	322 (49)	76 (51)	0.654
2-Vessel disease	187 (29)	45 (30)	0.668
3-Vessel disease	137 (21)	22 (19)	0.092
Revascularisation details			
CABG, n (%)	21 (3)	5 (3)	1.000
PCI, n (%)	625 (96)	125 (85)	**<0.001**
Culprit			
None identified, n (%)	13 (2)	17 (12)	**<0.001**
LAD, n (%)	269 (41)	61 (42)	0.996
Diagonal, n (%)	3 (0.5)	1 (1)	0.736
LCx, n (%)	98 (15)	15 (10)	0.124
OM, n (%)	10 (2)	5 (3)	0.134
IM, n (%)	4 (1)	1 (1)	1.000
RCA, n (%)	252 (39)	47 (32)	0.121
VG, n (%)	3 (0.5)	0 (0)	1.000
Stent thrombosis, n (%)	22 (4)	1 (1)	**0.042**
PCI outcome			
Number of stents, (SD)	1.3 (0.8)	1.1 (0.8)	**0.033**
Stent <3mm, n (%)	172 (29)	34 (28)	0.163
Final TIMI III flow, n (%)	595 (91)	147 (99)	**0.008**
Glycoprotein IIb/IIIa use	231 (36)	22 (15)	**<0.001**
Timeline			
Pain to angiography, min (IQR)	143 (105-254)	148 (112-274)	0.412
Pain to balloon time, min (IQR)	161 (118-231)	179 (130-266)	0.080
Call to balloon time, min (IQR)	105 (86-133)	114 (94-145)	**0.022**
Door to balloon time, min (IQR)	30 (23-41)	31 (24-44)	0.235
DAPT to balloon time, min (IQR)	48 (37-63)	46 (34-62)	0.307
Pre-discharge LV function			
Normal Ejection Fraction (EF≥55%), n (%)	204 (32)	72 (49)	**<0.001**
Mild LV impairment (40% < EF < 55%), n (%)	219 (34)	44 (30)	0.373
Moderate LV impairment (35% < EF ≤ 40%), n (%)	154 (24)	22 (15)	**0.021**
Severe LV impairment (EF ≤ 35%), n (%)	63 (10)	8 (6)	0.101

Abbreviations: CABG: coronary artery by-pass grafting, DAPT: dual antiplatelet therapy,EF: ejection fraction, IM: intermediate artery, IQR: inter-quartile range, LAD: left

anterior descending artery, LCx: Left circumflex artery, LV: left ventricle, OM: obtuse marginal, PCI: percutaneous coronary intervention, RCA: right coronary artery, TIMI: thrombolysis in myocardial infarction, VG: vein graft

Unobstructed arteries: All vessels contain < 50% luminal stenosis
1-Vessel disease: Only 1 vessel contains ≥ 50% luminal stenosis
2-Vessel disease: Only 2 vessels contain ≥ 50% luminal stenosis
3-Vessel disease: All 3 vessels contain ≥ 50% luminal stenosis

Table 3. Incidence of spontaneous reperfusion, infarct size and MACE according to OT and LT

	OT ≥ 398	**OT < 398**
LT ≤ 1402	Incidence of spontaneous reperfusion amongst patients with OT ≥ 398 and LT ≤ 1402: 64/161 (40%) Proportion of patients with spontaneous reperfusion who have an OT ≥ 398 and LT ≤ 1402: 44% Peak Troponin T: 1747 (835-3419)ng/l MACE: 4%	Incidence of spontaneous reperfusion amongst patients with OT < 398 and LT ≤ 1402: 32/152 (21%) Proportion of patients with spontaneous reperfusion who have an OT < 398 and LT ≤ 1402: 22% Peak Troponin T: 1843 (770-3914)ng/l MACE: 12%
LT > 1402	Incidence of spontaneous reperfusion amongst patients with OT ≥ 398 and LT > 1402: 29/202 (14%) Proportion of patients with spontaneous reperfusion who have an OT ≥ 398 and LT > 1402: 20% Peak Troponin T: 1867 (840-3992)ng/l MACE: 8%	Incidence of spontaneous reperfusion amongst patients with OT < 398 and LT > 1402: 21/272 (8%) Proportion of patients with spontaneous reperfusion who have an OT < 398 and LT > 1402: 14% Peak Troponin T: 2488 (1425-4735)ng/l MACE: 20%

Abbreviations: LT: lysis time, MACE: major adverse cardiovascular events, OT: occlusion time.
Cut-offs defined by ROC curve analysis and Youden's statistic.

Table 4. Length of stay and MACE in relation to spontaneous reperfusion.
(A) Length of stay and MACE in counts and Kaplan-Meier estimates for patients with and without spontaneous reperfusion (TIMI III), and with and without spontaneous reperfusion and complete ST-segment resolution on ECG. (B) Adjusted hazard ratios (and 95% confidence intervals) for the risk of MACE at presented time points for patients with and without spontaneous reperfusion (TIMI III), and with and without spontaneous reperfusion (TIMI III) and complete ST-segment resolution on ECG.
*Insufficient events for statistical analysis

(A)

	Whole group (n=801)	TIMI<III (n=653)	TIMI III (n=148)	p value	No/ pECG resolution (n=735)	TIMI III & cECG resolution (n=66)	p value
Length of stay, days (SD)	3.6 (4.4)	3.8 (4.7)	2.9 (2.0)	**<0.001**	3.7 (4.5)	2.9 (1.9)	**0.019**
MACE 48 hours, n (%)	24 (3)	22 (3.4)	2 (1.4)	0.195	24 (3.3)	0 (0)	0.137
MACE 72 hours, n (%)	27 (3.4)	25 (3.8)	2 (1.4)	0.133	27 (3.7)	0 (0)	0.115
MACE 30 days, n (%)	51 (6.4)	48 (7.4)	3 (2)	**0.018**	51 (6.9)	0 (0)	**0.029**
MACE 1 year, n (%)	68 (8.5)	63 (9.6)	5 (3.4)	**0.014**	67 (9.1)	1 (1.5)	**0.035**
MACE 4 years, n (%)	75 (9.4)	69 (10.6)	6 (4.1)	**0.013**	74 (10.1)	1 (1.5)	**0.029**

Abbreviations: cECG: complete ST-segment resolution on electrocardiogram, MACE: major adverse cardiovascular events, pECG: partial ST-segment resolution on electrocardiogram, SD: standard deviation, TIMI: thrombolysis in myocardial infarction

(B)

	TIMI III HR, (95% CI)	p value	TIMI III & cECG resolution HR, (95% CI)	p value
MACE 48 hours, n (%)	0.24 (0.03-1.9)	0.242	*	*
MACE 72 hours, n (%)	0.22 (0.03-1.7)	0.144	*	*
MACE 30 days, n (%)	0.22 (0.05-0.90)	**0.035**	*	*
MACE 1 year, n (%)	0.33 (0.12-0.91)	**0.032**	0.19 (0.03-1.4)	0.101
MACE 4 years, n (%)	0.35 (0.14-0.87)	**0.024**	0.17 (0.02-1.2)	0.077

Abbreviations: cECG: complete ST-segment resolution on electrocardiogram, CI: confidence interval, HR: hazard ratio, MACE: major adverse cardiovascular events, TIMI: thrombolysis in myocardial infarction

Table 5. Risk of MACE and its individual components, in patients with and without spontaneous reperfusion

Rate and hazard ratio for MACE and its individual components, in patients (A) with and without spontaneous reperfusion, (B) with and without spontaneous reperfusion and complete ST-segment resolution on ECG.

*Insufficient events for statistical analysis

(A)

	Whole group (n=801)	TIMI<III (n=653, 82%)	TIMI III (n=148, 18%)	P value	HR (95% CI)	P value
MACE 4 years	75 (9.4)	69 (10.6)	6 (4.1)	**0.013**	0.37 (0.16-0.84)	**0.018**
Acute ST	7 (0.9)	5 (0.8)	2 (1.4)	0.490	0.60 (0.07-4.8)	0.627
MI	22 (2.7)	19 (2.9)	3 (2)	0.505	0.66 (0.20-2.2)	0.509
CVA	13 (1.6)	12 (1.8)	1 (0.7)	0.299	0.36 (0.05-2.7)	0.321
CV death	40 (5)	38 (5.8)	2 (1.4)	**0.022**	0.22 (0.05-0.91)	**0.037**
All cause death	59 (7.4)	54 (8.3)	5 (3.4)	**0.031**	0.38 (0.15-0.95)	**0.038**

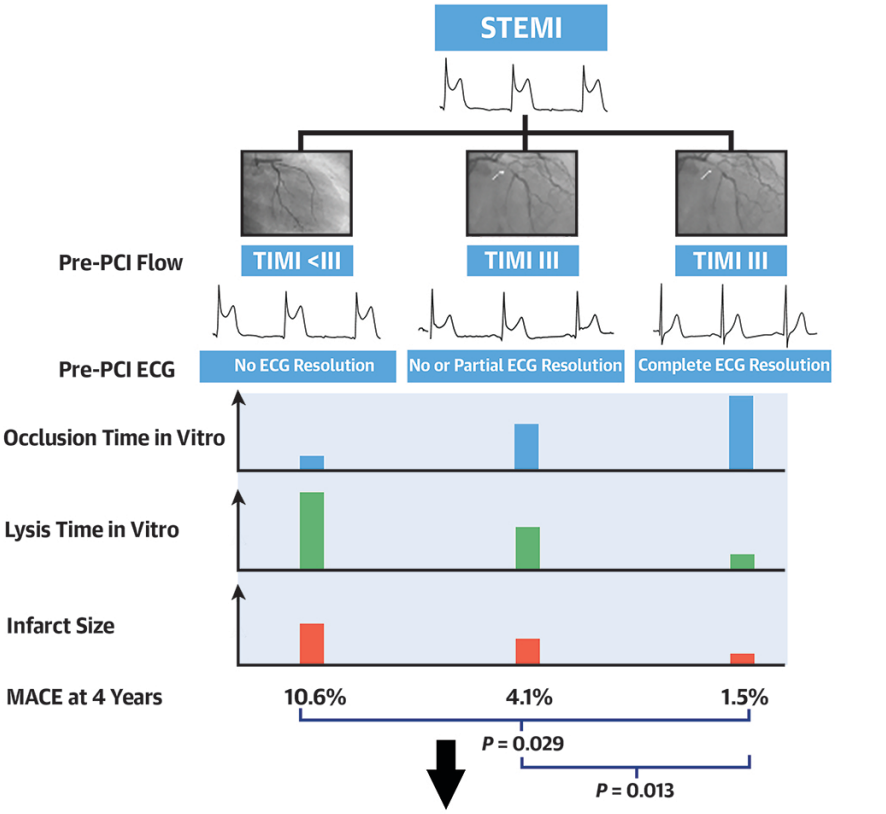
Abbreviations: CI: confidence interval, HR: hazard ratio, CV: cardiovascular, CVA: cerebrovascular accident, MACE: major adverse cardiovascular events, MI: myocardial infarction, ST: stent thrombosis, TIMI: thrombolysis in myocardial infarction

(B)

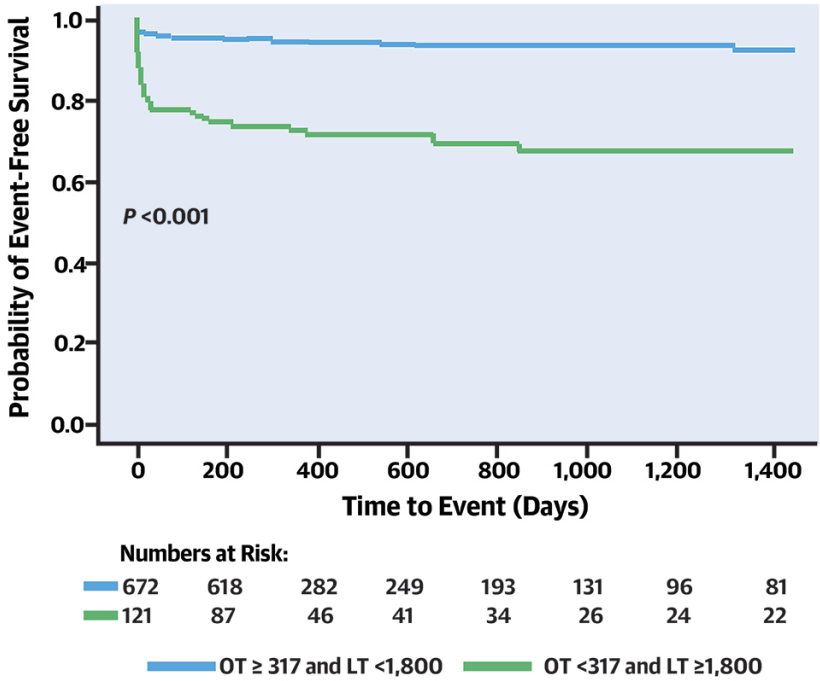
	Whole group (n = 801)	No/ pECG resolution (n=735, 92%)	TIMI III & cECG resolution (n = 66, 8%)	P value	HR (95% CI)	p-value
MACE 4 years	75 (9.4)	74 (10.1)	1 (1.5)	**0.029**	0.15 (0.02-1.1)	0.059
Acute ST	7 (0.9)	7 (1)	0 (0)	0.426	*	*
MI*	22 (2.7)	22 (3)	0 (0)	0.165	*	*
CVA	13 (1.6)	13 (1.8)	0 (0)	0.267	*	*
CV death	40 (5)	39 (5.3)	1 (1.5)	0.184	0.28 (0.04-2.1)	0.214
All cause death	59 (7.4)	57 (7.8)	2 (3)	0.194	0.41 (0.10-1.7)	0.210

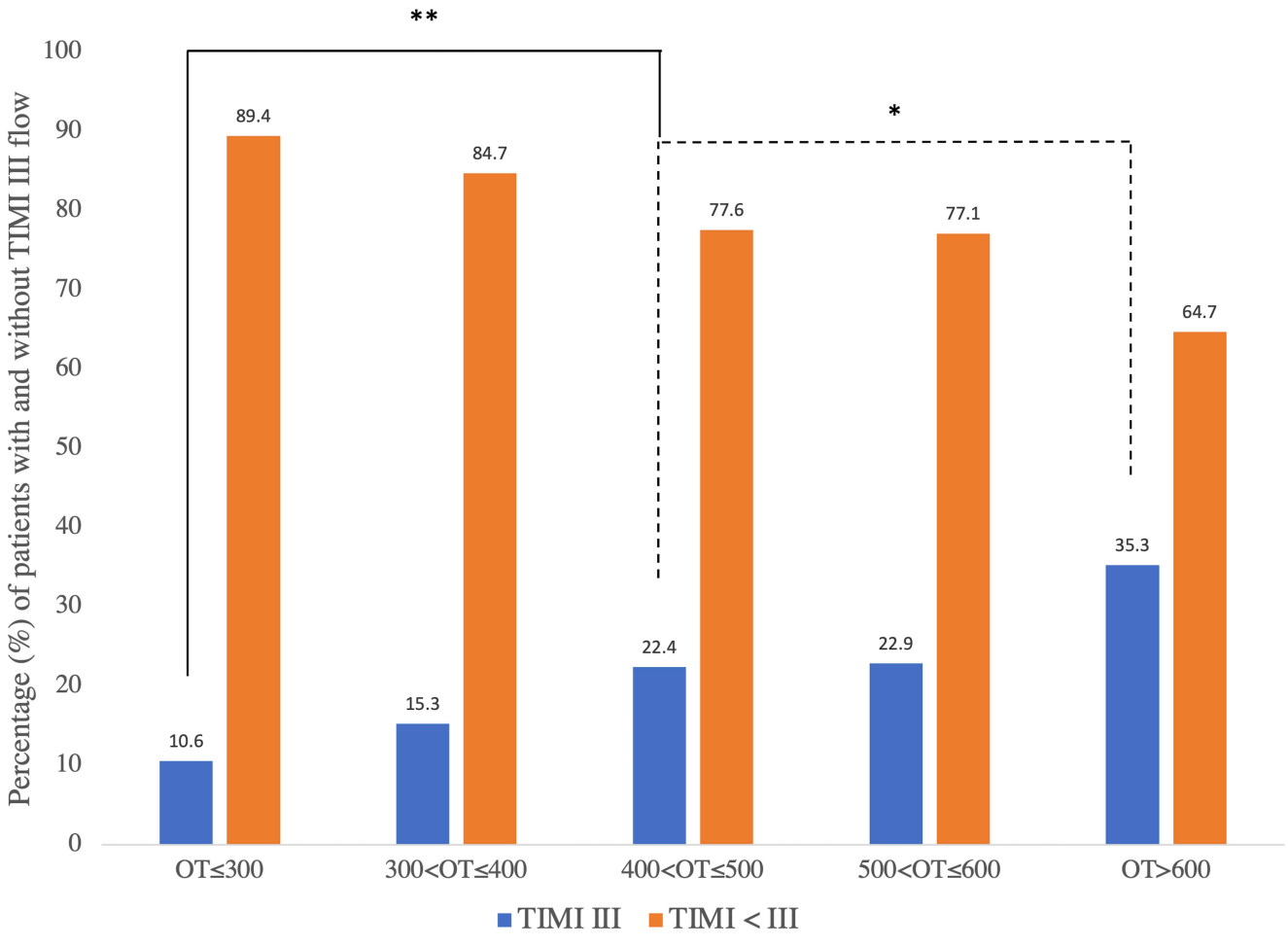
Abbreviations: cECG: complete ST-segment resolution on electrocardiogram, CI: confidence interval, HR: hazard ratio, CV: cardiovascular, CVA: cerebrovascular accident, MACE: major adverse cardiovascular events, MI: myocardial infarction, pECG: partial ST-segment resolution on electrocardiogram, ST: stent thrombosis, TIMI: thrombolysis in myocardial infarction

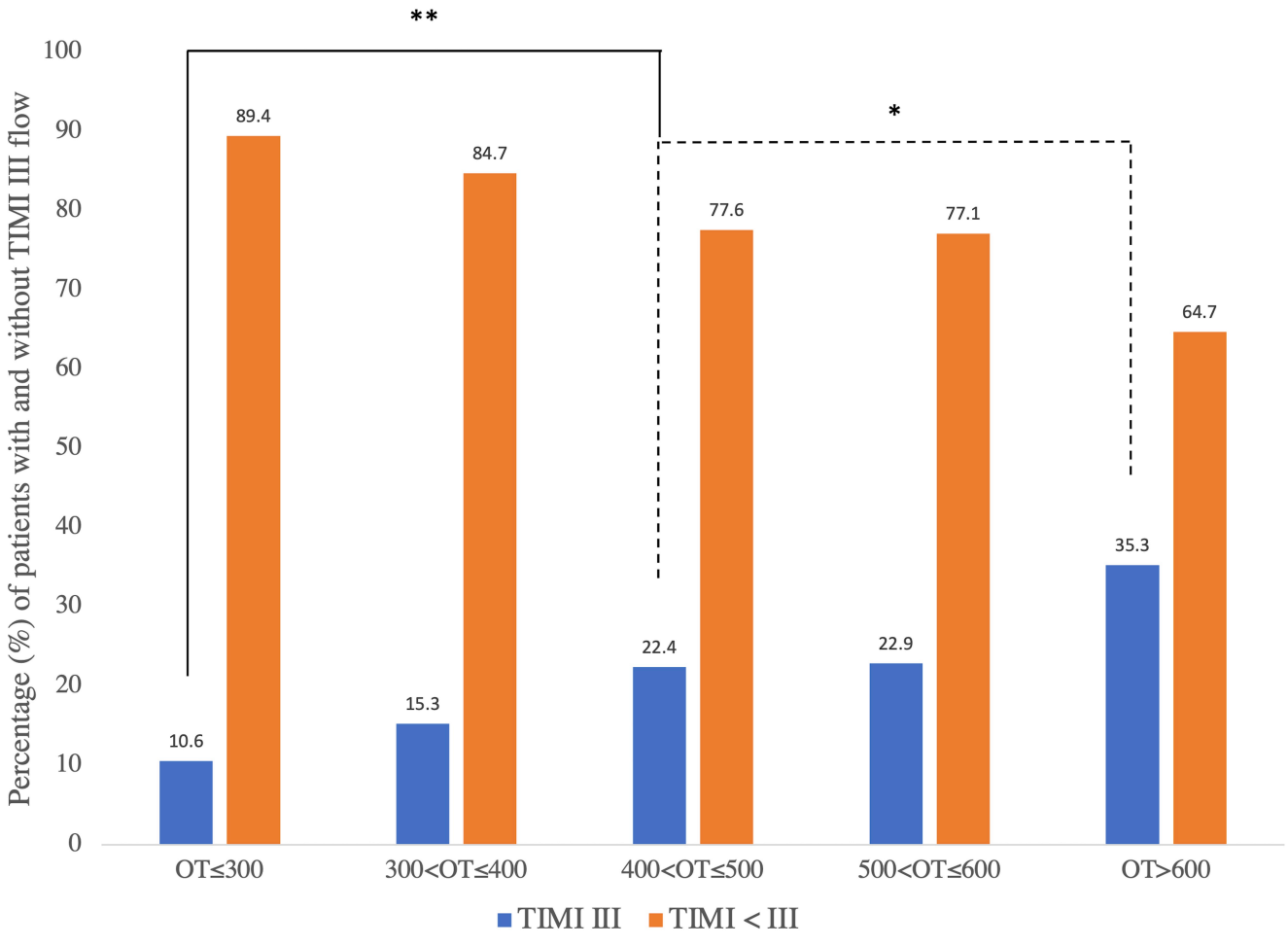
Hematological Signature of Spontaneous Reperfusion: Rapid Endogenous Fibrinolysis and Reduced Platelet Reactivity

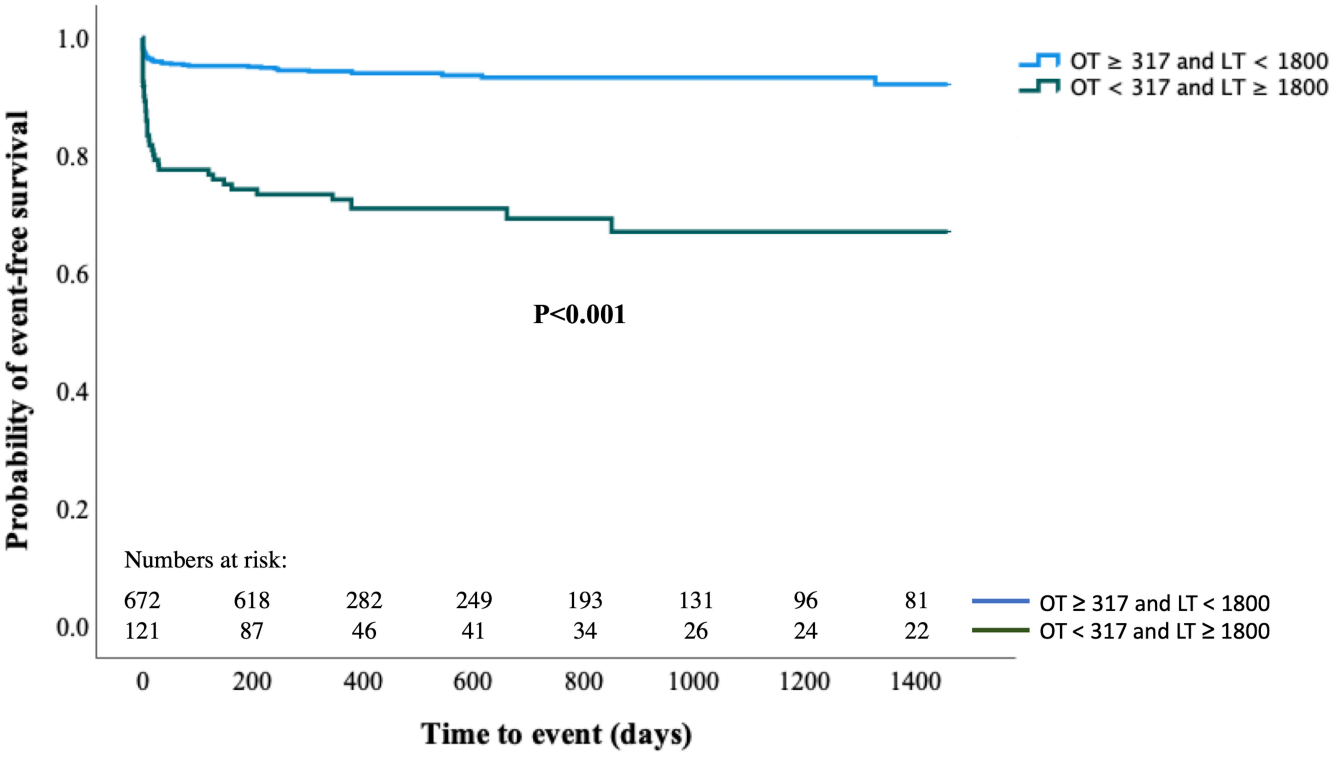


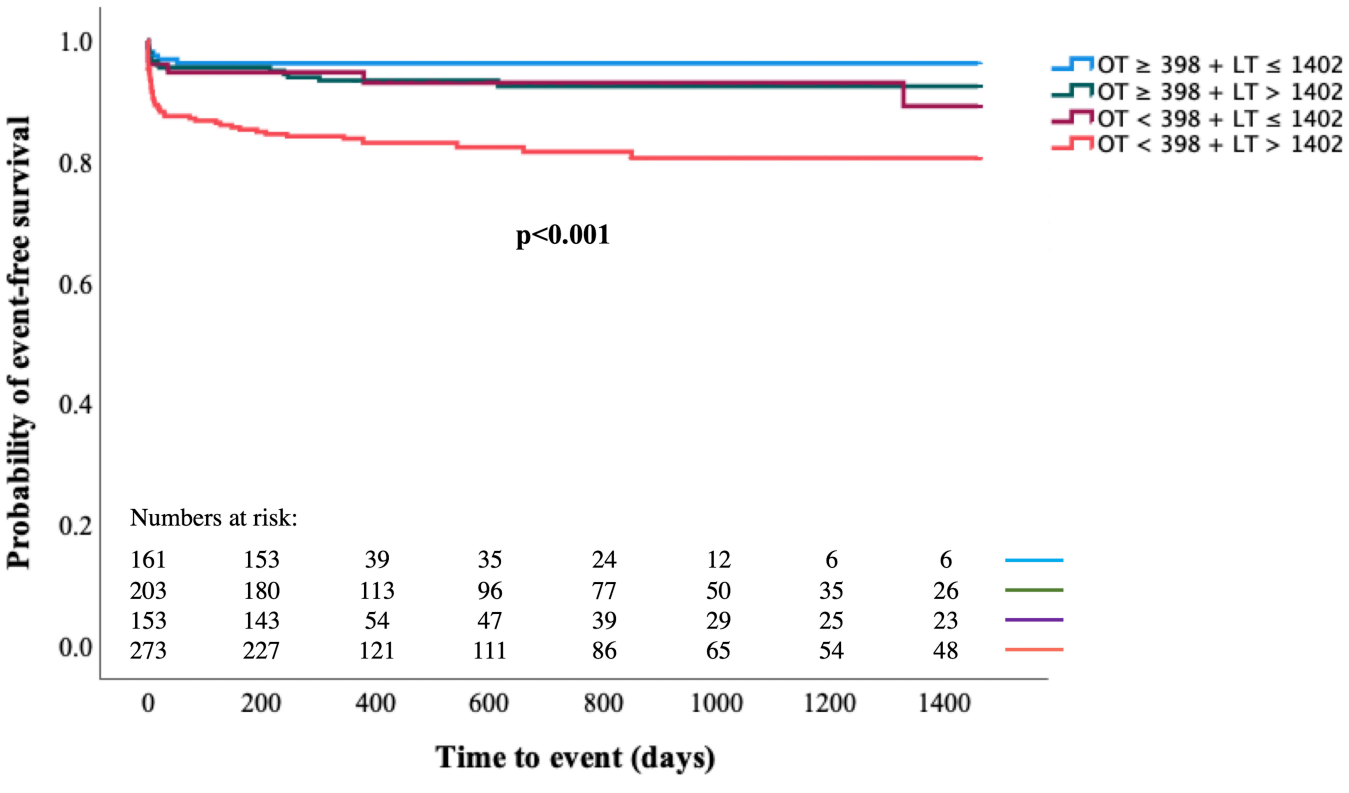
Relationship Between Thrombotic Profile, That Predicts Reperfusion, and Cardiovascular Outcomes

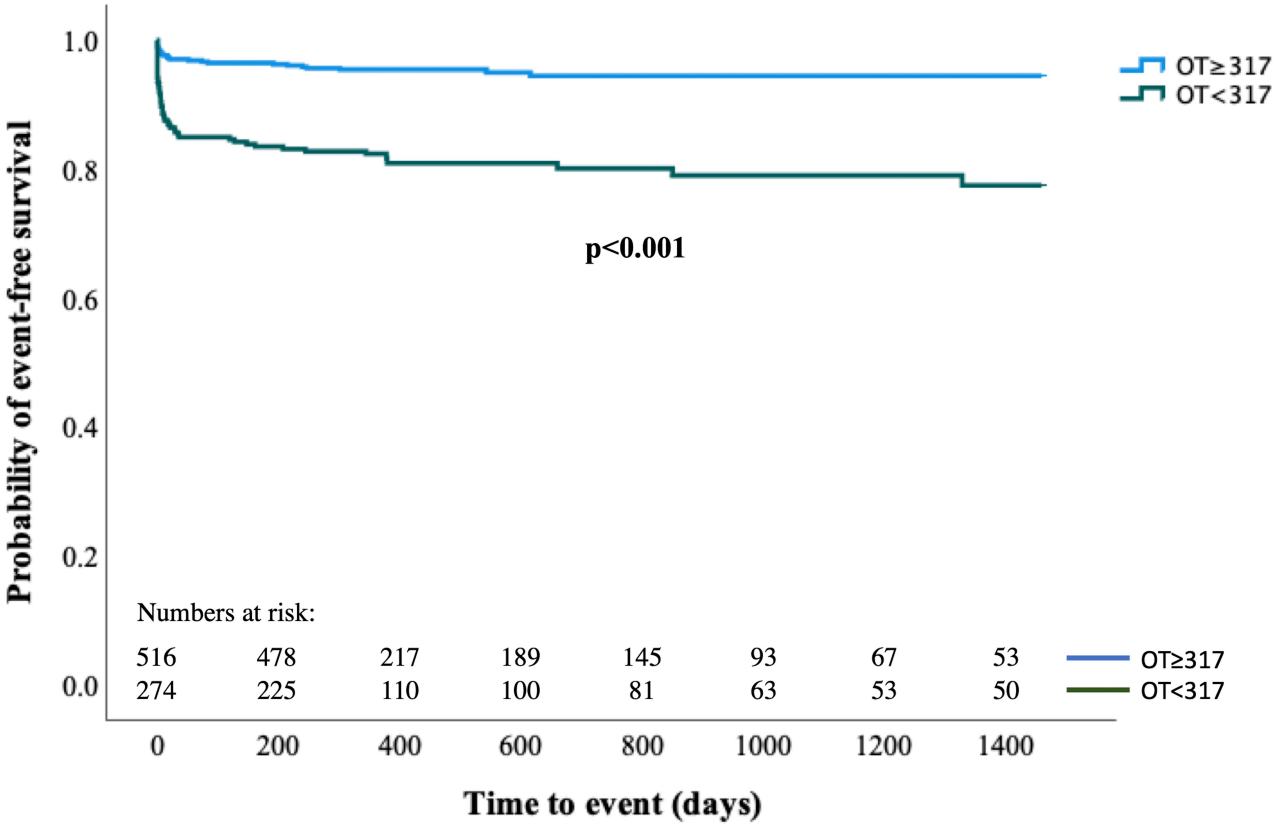


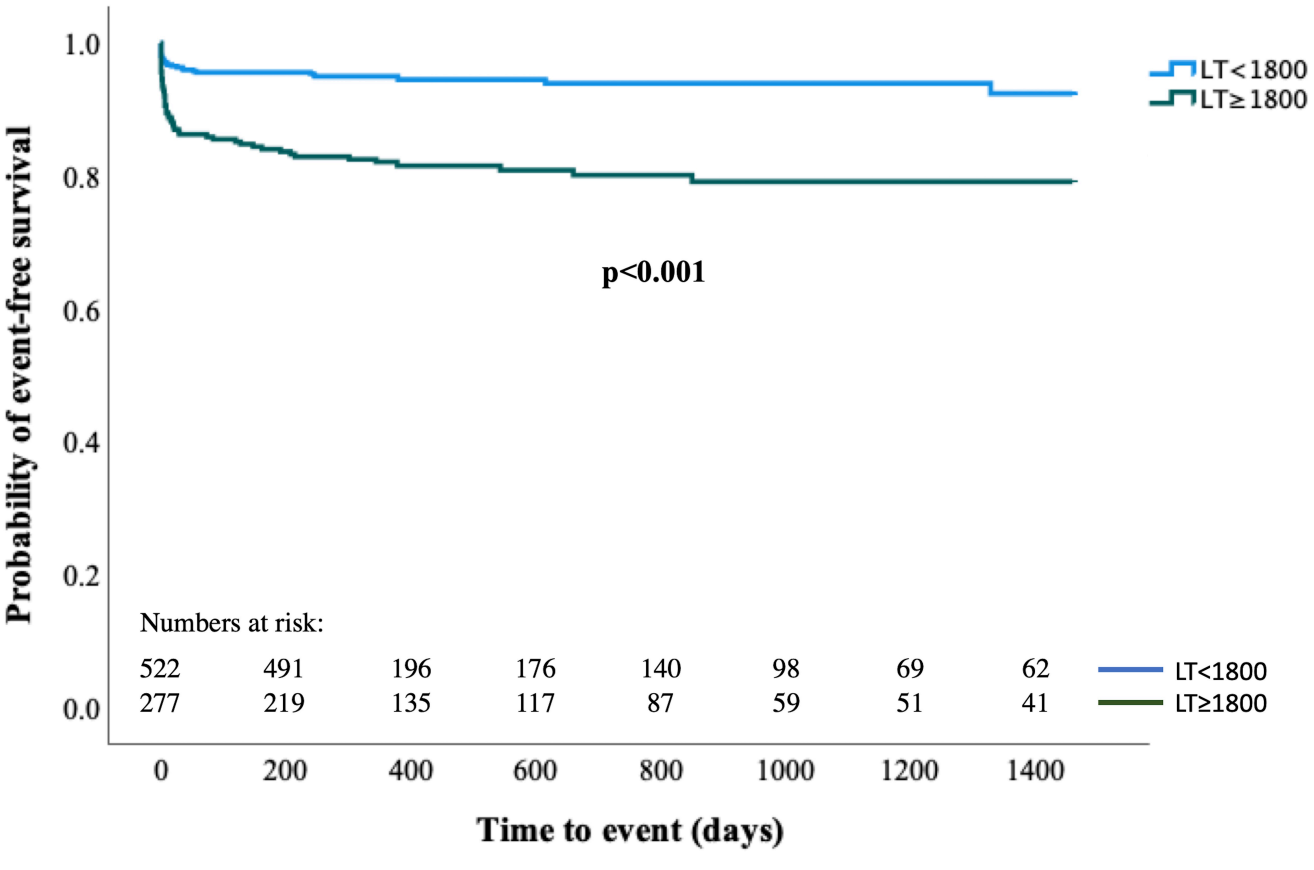

**
*
100
90
80
70
60
50
40
30
20
10
0
Percentage (%) of patients with and without TIMI III flow
89.4
84.7
77.6
77.1
64.7
35.3
22.9
22.4
15.3
10.6
OT≤300
300<OT≤400
400<OT≤500
500<OT≤600
OT>600
TIMI III
TIMI < III

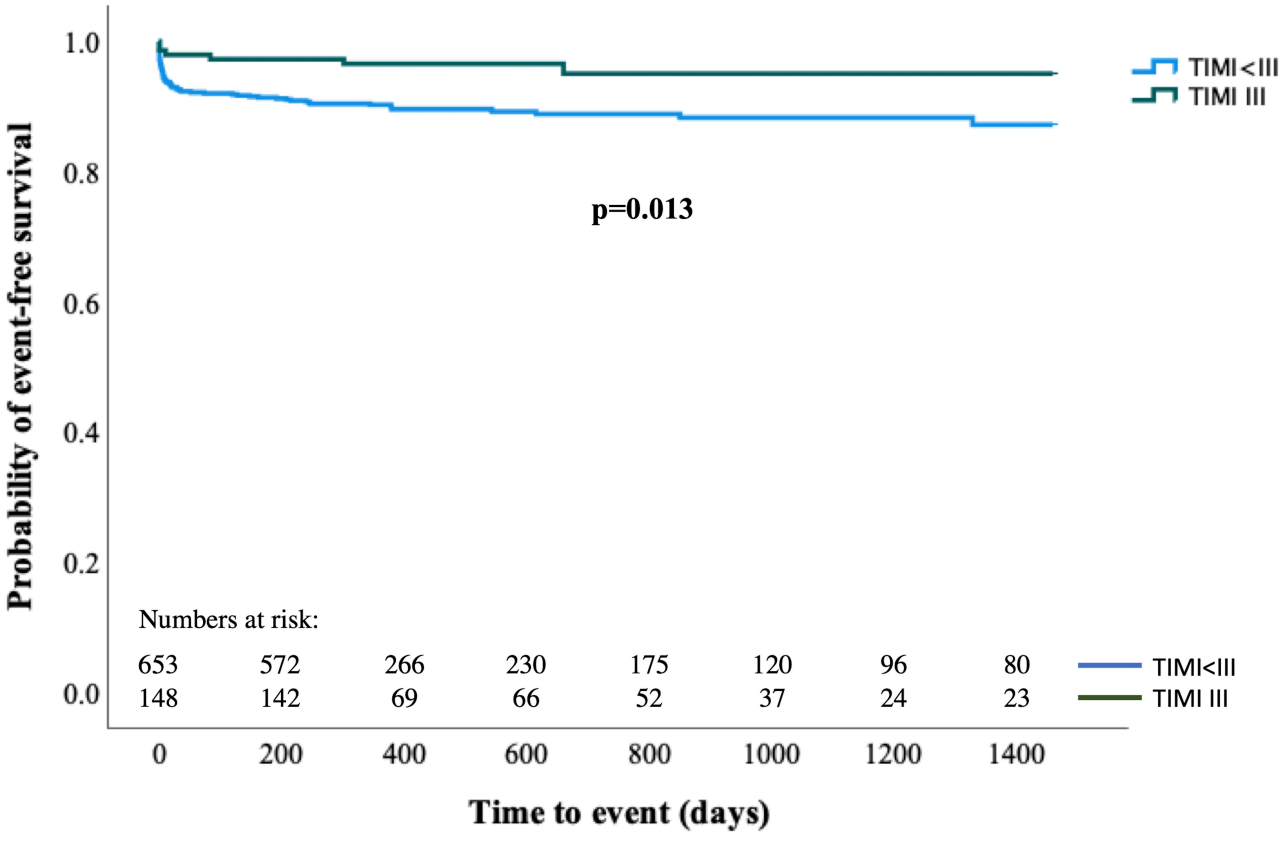

**
*
100
90
80
70
60
50
40
30
20
10
0
Percentage (%) of patients with and without TIMI III flow
89.4
84.7
77.6
77.1
64.7
35.3
22.9
22.4
15.3
10.6
OT≤300
300<OT≤400
400<OT≤500
500<OT≤600
OT>600
TIMI III
TIMI < III


Probability of event-free survival
1.0
0.8
0.6
0.4
0.2
0.0
OT ≥ 317 and LT < 1800
OT < 317 and LT ≥ 1800
P<0.001
Numbers at risk:
672 618 282 249 193 131 96 81
121 87 46 41 34 26 24 22
OT ≥ 317 and LT < 1800
OT < 317 and LT ≥ 1800
0 200 400 600 800 1000 1200 1400
Time to event (days)


Probability of event-free survival
1.0
0.8
0.6
0.4
0.2
0.0
OT ≥ 398 + LT ≤ 1402
OT ≥ 398 + LT > 1402
OT < 398 + LT ≤ 1402
OT < 398 + LT > 1402
p<0.001
Numbers at risk:
161 153 39 35 24 12 6 6
203 180 113 96 77 50 35 26
153 143 54 47 39 29 25 23
273 227 121 111 86 65 54 48
0 200 400 600 800 1000 1200 1400
Time to event (days)


Probability of event-free survival
1.0
0.8
0.6
0.4
0.2
0.0
OT≥317
OT<317
p<0.001
Numbers at risk:
516 478 217 189 145 93 67 53
274 225 110 100 81 63 53 50
OT≥317
OT<317
0 200 400 600 800 1000 1200 1400
Time to event (days)


Probability of event-free survival
1.0
0.8
0.6
0.4
0.2
0.0
LT<1800
LT≥1800
p<0.001
Numbers at risk:
522 491 196 176 140 98 69 62
277 219 135 117 87 59 51 41
LT<1800
LT≥1800
0 200 400 600 800 1000 1200 1400
Time to event (days)


1.0
0.8
0.6
0.4
0.2
0.0
Probability of event-free survival
TIMI<III
TIMI III
p=0.013
Numbers at risk:
653 572 266 230 175 120 96 80
148 142 69 66 52 37 24 23
TIMI<III
TIMI III
0 200 400 600 800 1000 1200 1400
Time to event (days)