The role of global thrombosis in patients undergoing percutaneous coronary intervention (PCI)

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Abstract

Coronary artery disease (CAD) is a leading cause of mortality worldwide, despite great efforts in designing several risk stratification models for more effective prevention of related complications and creating more advanced treatment methods. Furthermore, the average age of affected patients observed recently, is significantly lower compared to previous decades and potential complications remain devastating. In recent years, the global thrombosis test (GTT), which is a novel test assessing clot formation/thrombosis and subsequent endogenous thrombolysis (ET) under physiological conditions, has been used in several studies of patients with acute coronary syndrome (ACS), providing us with valuable information. This test is unique, as it is currently the only test of its kind, able to assess individual ET, which according to these studies can predict cardiovascular death and non-fatal myocardial infarction (MI) in ACS patients. However, stable angina patients and patients with MI undergoing primary percutaneous coronary intervention (PPCI), the most critically affected CAD patients, had not been previously investigated, therefore this enabled us to design a study investigating fields never been previously explored, with the prospect of presenting data that could stimulate further research and enhance future management of patients with cardiovascular disease. We successfully demonstrated that individual ET status can predict major adverse cardiovascular events (MACE) following PPCI. Furthermore, we showed that increased individual platelet reactivity before PPCI, is related to non-fatal MI, which had not been shown in previous studies of global thrombosis. In the group of non-ST elevation myocardial infarction (NSTEMI) patients, we observed significant relationship between ET and peak Troponin level, which is a marker of the size of myocardial damage, resulting from lasting coronary thrombus. Finally, we demonstrated that the medication vorapaxar, which is a direct thrombin inhibitor, can enhance ET and could be proven to be beneficial when administered in certain patients, for the prevention of future adverse cardiovascular events. The main limitation identified in all sub-studies, was the small patient number, therefore in view of the positive results demonstrated in

our study, larger scale studies should be carried out, aiming to reinforce the vital role of global thrombosis assessment in patients with CAD.

Background

Introduction/ Study aims

Ischaemic heart disease (IHD) is often considered as an exclusive problem of wealthy western countries, in which stressful lifestyle and careless diet promote coronary artery disease (CAD). According to recent epidemiological studies though, IHD is the leading cause of death in developing countries as well, where it accounts for nearly 30% of all deaths (Finegold, Asaria, & Francis, 2013). Furthermore in spite of the fact that it has always been regarded as the disease of old age, recently the average age of patients with cardiac events has been decreasing dramatically (Finegold et al., 2013).

The most worrying feature though, is the fact that it is still the leading cause of death worldwide, despite great advances demonstrated in both prevention and treatment. The role of endogenous thrombolysis (ET) has recently been shown to be an important predictor of major adverse cardiovascular event (MACE) in patients with ACS and end-stage renal failure, according to studies of global thrombosis, using a novel platelet function test (PFT), called the global thrombosis test (GTT)(Saraf, Christopoulos, Salha, Stott, & Gorog, 2010)(Sharma et al., 2012), which is valuable information and the stimulus for designing our study.

The primary purpose of our study is to investigate whether global thrombosis status assessed by the GTT, can predict future adverse events following PCI, which is the main treatment of CAD. Despite great efforts for development of better stent structure and stent implantation techniques, as well as for optimization of pharmacotherapy following PCI with antiplatelet therapy and CVD risk factor modification, adverse events still occur, they are life threatening and can cause psychological impact to patient who survive them and their families. Furthermore, for each adverse event, there is a significant financial charge to the NHS, which is currently under a lot of pressure. The prospect of identifying high risk patients for adverse events following PCI by routinely using the GTT, would revolutionize future management of CAD.

Following a literature review on the timing of PPCI in STEMI patients, which is a very important topic, as it refers to the most clinically unstable patients with CAD, we believe that investigation of the relationship between delay in PPCI coronary revascularization and enhanced thrombogenicity or delayed ET, could potentially provide us with valuable information that could reinforce our results supporting the main purpose of the study.

Antiplatelet agent resistance and consequent stent thrombosis is the reason for development of more pharmacotherapy, aiming for more efficient prevention of thrombosis. However, development of medication enhancing ET is something that has never been previously proposed and definitely something worth investigating. Vorapaxar is an antiplatelet medication recently investigated in two large trials (Leonardi et al., 2013) (Cavender et al., 2015), including the UK and specifically our cardiology centre. We obtained permission from the trial organisers to investigate the effect of this medication on global thrombosis and particularly the effect on ET using the GTT. Results of this sub-study have been included and discussed in the final chapter of thesis.

Coagulation cascade

Coagulation cascade is a protective measure in response to blood vessel damage. It consists of two pathways, the extrinsic or tissue factor pathway and the intrinsic or contact activation pathway leading to the final common pathway resulting in a stable fibrin clot (Lefkowitz, n.d.).

The role of the extrinsic pathway is rapid production of thrombin. Initially factor VII comes into contact with tissue factor (TF) following blood vessel damage, forming TF-VIIa complex. Factor VII is itself activated by thrombin and factors XIa, XII, Xa.

TF-VIIa complex then activates factors IX and X.

Factor Xa with the aid of its co-factor Va form the prothrombinase complex, which activates prothrombin to thrombin (Lefkowitz, n.d.).

Intrinsic pathway

The intrinsic pathway begins with the formation of the primary complex on collagen, pre-kallikrein and factor XII or Hageman factor. Then pre-kallikrein is converted into kallikrein and factor XII into XIIa. Subsequently factor XIIa converts factor XI into XIa which in turn activates factor IX to form the tenase complex with the aid of the co-factor VIIIa. Eventually tenase complex activates factor X to Xa (Lefkowitz, 2006).

In the final common pathway thrombin serves several tasks. It primarily converts fibrinogen to fibrin monomer, which is the main building element of the blood clot. Furthermore it activates factors VIII, V and finally factor XIII to form fibrin polymers from activated monomers (Lefkowitz, 2006). For example, cross-linking by activated factor XIII, enhances fibrin elasticity, as well as promotes resistance to fibrinolysis. Factor XIIIa also cross-links a2-antiplasmin, plasminogen activator inhibitor-2 and thrombin-activatable fibrinolysis inhibitor (TAFI) to fibrin, further enhancing resistance to fibrinolysis (Undas & Ariëns, 2011).

This process is facilitated by different co-factors and regulators. One regulator is Protein C, which is a physiological anticoagulant. It is activated by thrombin into activated Protein C, which in combination with Protein S degrade factors Va and VIIIa (Lefkowitz, 2006).

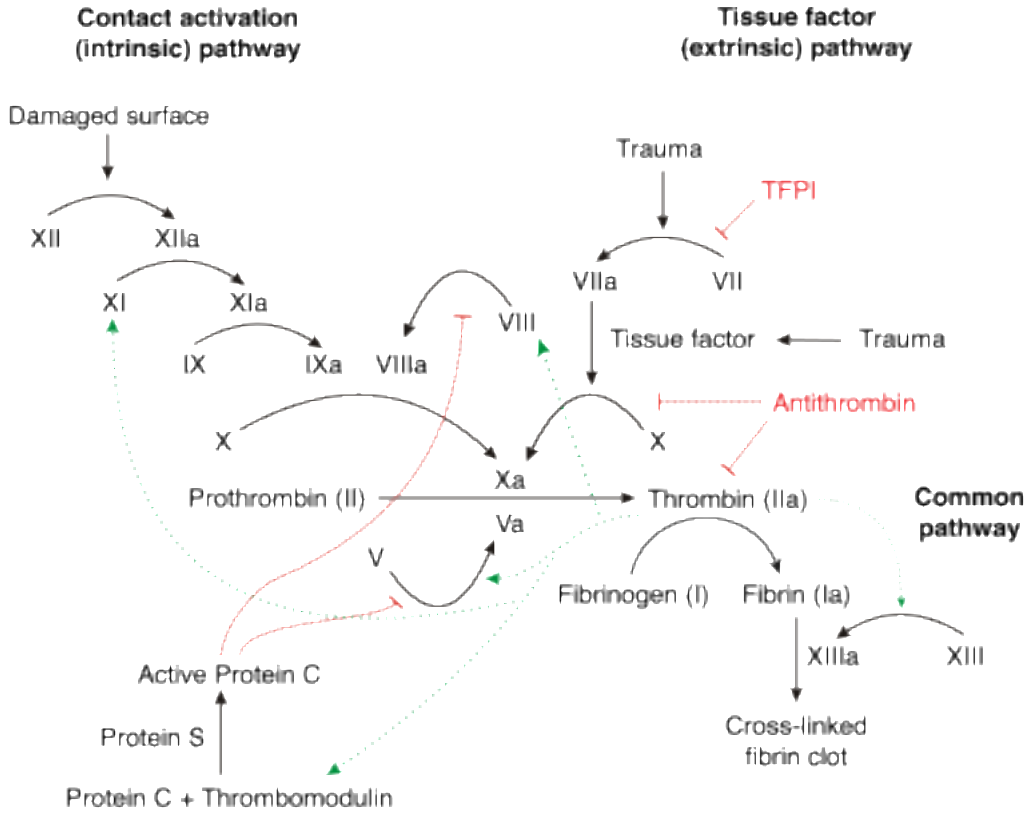
Deficiency in any of these factors and co-factors results in coagulation problems or bleeding disorders. Furthermore most of the pharmacological agents used nowadays, target different parts of the cascade in order to prevent thrombosis (Lefkowitz, 2006).

Extrinsic pathway

The extrinsic pathway is the first step in plasma mediated haemostasis and it is activated by TF, which is expressed in the sub-endothelial tissue. Following a vascular insult, exposed TF binds with factor VIIa and calcium, in order to trigger the conversion of factor X to Xa, which then changes factor II into

thrombin. Thrombin promotes coagulation in two ways. Firstly, it changes fibrinogen to fibrin and secondly turns factor XIII into XIIIa, which in turn forms fibrin polymer into cross-linked clot (Lefkowitz, 2006).

Figure 1. Coagulation pathways



Thrombosis

Endothelial dysfunction in thrombosis

Thrombosis plays a crucial role in the pathogenesis of ACS. Following many years of research it is believed that it is a multifactorial phenomenon that manifests according to changing physiological conditions (Chung et al., 2003).

Since atherosclerotic plaques arise from the coronary artery endothelium, one should first investigate the potential involvement of **endothelial dysfunction** in atherothrombotic events. Healthy endothelium should promote vasodilation and exhibit anti-platelet as well as pro-fibrinolytic properties, unlike abnormal endothelium in ACS, in which we observe the exact opposite pathophysiological features. Several factors have been suggested as possible contributors to endothelial dysfunction, such as the presence of higher levels of circulating von Willebrand factor (vWF) and lower levels of tissue factor pathway inhibitor in ACS patients. vWF is a specific marker for endothelial cells and it is known to promote platelet adhesion to diseased vessel wall and to activated endothelial cells, thus favoring thrombogenesis (Chung et al., 2003).

Factors promoting thrombosis

Oxidative stress is another factor eventually promoting thrombosis. It is achieved by both reducing nitric oxide bioavailability and promoting prostacyclin production by endothelial cells, with resulting inability to inhibit inflammatory and subsequently prothrombotic response within an atherosclerotic lesion, thus increasing ACS risk (Libby & Theroux, 2005).

Platelets play a key role in the pathogenesis of ACS. Exposure of the sub-endothelial matrix resulting from plaque rupture is a trigger for platelet adhesion at first, followed by platelet activation which is facilitated by inflammatory cells, metalloproteinases, thrombin, thromboxane A2 (TXA2), platelet activating factor (PAF), adenosine 5-diphosphate (ADP), epinephrine and serotonin. Thrombogenesis can also be induced by shear forces within the unstable plaque due to stimulating prostaglandin release (Libby & Theroux, 2005).

The bond though between a ruptured plaque and platelets is through the complex interaction between vWF, collagen, platelet glycoproteins Ib and after activation of the platelet surface IIb/IIIa receptor, which is the a binding site for fibrinogen binding. Finally b3 integrins are responsible for the definite adhesion (Libby & Theroux, 2005).

Lately there has been emerging evidence of the crucial role of ADP in thrombosis by acting on P2X1, P2Y1 and P2Y12 receptors which are situated

on the platelet surface, eventually triggering fibrinogen binding on the platelet surface glycoprotein IIb/IIa receptor, causing platelet adhesion and aggregation (Libby & Theroux, 2005).

It has recently been suggested that PCI patients are at risk of future ischaemic events from stent thrombosis, due to procedure-related platelet activation, especially following ACS, when platelet hyper-reactivity and enhanced thrombin formation is observed (Libby & Theroux, 2005).

Tissue factor (TF) also triggers thrombosis within coronary lesions, where TF concentrations have been found to be very high. Furthermore its levels are also increased in circulating blood of patients with unstable angina, as are the levels of thrombin, promoting the adverse thrombotic events. Under normal circumstances tissue plasminogen activator (t-PA) and urokinase are inhibited by plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2, whereas plasmin is inhibited by circulating alpha-2-antiplasmin. These two mechanism are interrupted during the thrombotic process, resulting in lower levels of t-PA and higher levels of PAI-1, thus impairing endogenous thrombolysis (Libby & Theroux, 2005).

Inflammation The role of inflammation is extremely important in the pathogenesis of atherosclerosis, from development and progression of atheroma to plaque instability, as well as in restenosis following angioplasty.

This process is initiated by the adherence of circulating leucocytes to injured endothelium via several classes of adhesion molecules, such as vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and selectins, that co-operate with cytokines in order to enable adherence. Subsequently, chemoattractant cytokines promote transmigration of inflammatory cells into the sub-endothelial space (Libby, 2012)(Zakynthinos & Pappa, 2009).

At a later stage, low-density lipoproteins (LDL) bind to monocyte-derived macrophages forming lipid-laden foam cells, the main constituent of the fatty streak, which is a key feature of the advanced atherosclerotic plaque.

At the same time mononuclear cells release interleukin-1 (IL-1) and interleukin-6 (IL-6) attracting more inflammatory cells, resulting in even more uptake and oxidation of LDL (Libby, 2012)(Zakynthinos & Pappa, 2009).
Furthermore these cytokines stimulate smooth muscle cell proliferation and development of the collagenous fibrous cap. The synthesis and degradation of collagen and subsequently the stability of the fibrous cap is dynamically controlled by inflammation (Libby, 2012)(Zakynthinos & Pappa, 2009).

Inflammatory cells, such as activated T cells, produce interferon gamma (INFγ) which causes a decrease in the production of collagen by smooth muscle cells. Additionally activated macrophages produce matrix metalloproteinases (MMPs) that promote collagen destruction, thus promoting fibrous cap instability and subsequently plaque rupture.
Following plaque rupture there is direct contact between the pro-coagulant lipid core and the blood, favouring further inflammatory reactions leading to platelet activation, coagulation cascade activation, vasomotor dysfunction and coronary lumen occlusion leading to myocardial infarction (Libby, 2012)(Zakynthinos & Pappa, 2009).

Inflammatory response is also an important factor in the development of neo-intimal thickening and restenosis following PCI.
The injury caused by balloon angioplasty and stent insertion to the coronary wall can cause platelet activation and subsequent leukocyte attraction to the site. There is evidence from published research, demonstrating that PCI causes activation and up-regulation of Mac-1 on the surface of neutrophils, promoting in-stent restenosis within 48 hours of stenting (Libby, 2012)(Zakynthinos & Pappa, 2009).

Since inflammation plays such an important role in atherogenesis in coronary arteries, as well as in restenosis post-PCI, several research studies have investigated potential inflammatory markers as biomarkers for cardiovascular risk assessment.

C-reactive protein (CRP) is the most investigated inflammatory marker of cardiovascular disease. It has been suggested that CRP levels correlate with the presence of plaque rupture as evidenced by intravascular ultrasound (Sadeghi, Pourmoghaddas, Tavasoli, & Roohafza, 2010). Furthermore, increased temperature, a feature of unstable coronary plaque, detected by invasive thermogenetic catheter, was associated with higher levels of CRP (Sadeghi et al., 2010). Finally several studies have suggested that this marker could be a predictor of adverse outcomes following MI (Zakynthinos & Pappa, 2009) (Ridker, 2003). The THROMBO study, one of the biggest studies assessing the role of CRP, recruiting 1045 post-MI patients, despite demonstrating association of the marker with recurrent coronary events, it failed to prove that CRP is an independent factor of events (Zakynthinos & Pappa, 2009).

Procedural determinants of stent thrombosis

Since their introduction, stents have been widely used to treat patients with coronary artery disease, with around 2.5 million being implanted yearly. Despite improvements in stent design and implantation techniques and more effective anti-platelet regimes, stent thrombosis remains an issue, due to its devastating consequences (short-term mortality up to 25%). The wide variability observed in its estimated incidence shows that stent thrombosis should be regarded as a multifactorial phenomenon.

Several studies have identified important clinical, angiographic and procedural determinants of stent thrombosis, however most of these studies had limitations, such as their small sample size, variation in the definition of stent thrombosis and overlooking less frequently occurring risk factors that could not be explored. Additionally the majority of them did not focus on differences in underlying pathophysiological mechanisms between various indications of PCI (stable vs ACS patients and early vs late thrombosis) (D'Ascenzo et al., 2013) (Neville Kukreja et al., 2009).

The metal stent material, the polymer and the eluted drug are potential stent-related causes of thrombosis. The purpose of the eluted drugs (sirolimus, paclitaxel, zotarolimus and everolimus), is to inhibit cell cycle, thus delaying neo-intimal growth, with migration and proliferation of vascular smooth muscle cells. Since PCI is known to cause endothelial destruction and cell cycle inhibitors prevent re-endothelialisation, these can predispose to thrombosis (Neville Kukreja et al., 2009).

The presence of a polymer in the vessel wall could induce local hypersensitivity which could be thrombogenic. Likewise exposure of stent struts, which is considered as pro-inflammatory, can cause eventual thrombosis. It is thought that this phenomenon is more often seen with drug eluting stents (DES) due to the delay in endothelial re-growth and consequently coverage of struts. Furthermore poor procedural techniques such as stent under-deployment can lead to thrombosis. Finally thrombosis is commoner in longer stent implantation and stented bifurcations, in which use of DES is preferable (Neville Kukreja et al., 2009).

Antiplatelet resistance causing stent thrombosis

Apart from stent-related causes of thrombosis there are also patient-related causes, such as anti-platelet therapy resistance (Aspirin+/- Clopidogrel resistance), malabsorption and non-compliance to pharmacotherapy (Casterella, n.d.).

Anti-platelet duration following PCI has been a hot topic for discussion, hence the American College of Cardiology as well as the American Heart Association recommended use of dual anti-platelet therapy for one year in patients without major risk of bleeding, as a measure for adequate decrease in thrombosis incidence (Casterella, n.d.) (O'Gara et al., 2013).

Resistance to either one or both anti-platelet agents following PCI implantation, is an issue that has recently emerged and it is regarded as very serious. According to published data among the patients treated with coronary stents, 15% are resistant to Aspirin and 24% are resistant to Clopidogrel, with 50% of Aspirin-resistant patients exhibiting reduced response to Clopidogrel as well

(Dupont, Gabriel, & Cohen, 2009).

Clopidogrel's target is the P2Y12 receptor on the platelet surface, which it binds irreversibly. Variation in the drug's response depends on polymorphism of the hepatic CYP3A4 pathway that can affect the production of active drug, thus reducing its effect on binding the platelet receptor and inhibiting platelet function. Apart from genetic variability, there are also other reasons for reduced drug activity, such as interaction with other drugs, low intestinal absorption and variation in loading and maintenance doses (Dupont et al., 2009).

Aspirin targets platelet cyclooxygenase-1 (COX-1), which binds irreversibly in order to exhibit its anti-platelet purpose. Resistance to Aspirin is a multifactorial phenomenon and probably the most important contributory factor is poor medication adherence. Another potential cause for resistance is the adverse effect of underlying inflammatory process that can promote faster regeneration of platelets and COX-1 at a rate that a single dose of Aspirin is not effective. The full mechanism of Aspirin resistance phenomenon though is not fully understood and it is a subject of ongoing research (Dupont et al., 2009).

Stent thrombosis incidence

A large study in the Netherlands (N Kukreja et al., 2009) investigated the incidence of stent thrombosis in 5816 patients undergoing PCI, amongst whom 2248 had a bare metal stent (BMS) and the remaining paclitaxel or sirolimus DES. Additionally these patients were categorized according to their presentation into patients with stable angina, unstable angina (UA)/non-ST elevation MI (NSTEMI) and ST elevation MI (STEMI).

The primary end point was stent thrombosis, defined as angiographically documented thrombus with Thrombolysis In Myocardial Infarction flow grade 0 or 1, accompanied by acute symptoms (consistent with the Academic Research Consortium classification of definite stent thrombosis)

The timing of stent thrombosis was classified as follows (Press, 2015):

1. Early (within a month from implantation)
2. Late (between one month and a year from implantation)
3. Very late (more than one year from implantation)

The results showed lower incidence in overall thrombosis in stable angina patients, regardless of the type stent inserted, compared to ACS patients. Amongst ACS patients, there was no significant difference observed between UA/NSTEMI and STEMI patients. In ACS patients with DES there was a non-significant trend towards very late thrombosis, however the incidence of early and late thrombosis did not reach statistical significant according to stent type. Surprisingly, higher mortality rates were observed in stable patients rather than in ACS patients. Additionally, while in the ACS group mortality was more common with early stent thrombosis, in stable patients mortality was independent of timing of thrombosis (N Kukreja et al., 2009).

The Dutch stent thrombosis registry has identified all the previously mentioned risk factors for stent thrombosis with the addition of two independent factors, the presence of malignancy, an intermediate lesion >50% and <70% proximal to the treated culprit lesion during index procedure (van Werkum et al., 2009).

New antiplatelet agents

Prasugrel is rapidly absorbed following oral administration and converted to its active metabolite, reaching peak concentrations within 30 minutes. It has more rapid onset of action and achieves more consistent and complete inhibition of ADP-induced platelet aggregation compared to Clopidogrel, mainly due to hepatic conversion into its active metabolite in one step rather than two-step hepatic conversion seen in Clopidogrel's metabolism (Cheng, 2013).

Clinical outcomes data for Prasugrel comes mainly from phase III TRITON-TIMI 38 (Matte-martyn et al., 2011), where 13608 patients with ACS and planned PCI were randomized to receive either Clopidogrel 300mg loading dose followed by 75mg daily or Prasugrel 60mg loading followed by 10mg daily and treated for a median of 14.5 months.

Patients randomized to Prasugrel had fewer primary end-point events compared with Clopidogrel. There was reduction in clinical ischaemic events as there was notable reduction in MI and urgent target vessel revascularisation. However prasugrel-randomised patients showed significantly higher tendency for major bleeding requiring transfusion, as well as higher tendency for minor

bleed.

A sub-analysis of the trial showed one of the most interesting qualities of Prasugrel which is the significant reduction in in-stent restenosis (ISR), regardless of the type of stent (Cheng, 2013).

Ticagrelor is a cyclopenthyl-triazolopyrimidine, a new class of anti-platelet agents, which is a selective antagonist of the P2Y12 receptor and inhibits ADP-mediated platelet response. Unlike Clopidogrel and Prasugrel its inhibitory activity does not require metabolic activation, despite the fact that its active metabolite also demonstrates P2Y12 receptor antagonism activity (Cheng, 2013).

The PLATO study (Lindholm et al., 2014) demonstrates the efficacy and tolerability of the use of Ticagrelor in ACS compared to that of Clopidogrel. In this study, 18624 patients admitted to hospital within 24h of an ACS were randomized to either receive Ticagrelor 180mg loading dose followed by 90mg twice daily or Clopidogrel 300 to 600mg loading dose followed by 75mg daily thereafter.

The outcome was that treatment with Ticagrelor significantly reduced the rate of death from vascular causes, MI or stroke, without an increase in the rate of overall major bleeding, however there was an increase in the rate of non-procedure related major bleeding (Cheng, 2013).

Future strategies for reducing thrombotic risk

Prevention of stent thrombosis should be guided by focusing on the main determinants of this serious complication. New stents in respects to both design and drug elution, have significantly contributed towards a dramatic reduction in stent thrombosis. The four-year follow-up results of the ABSORB trial looking at ischaemia driven MACE following bioabsorbable everolimus-eluting stent in patients with CAD, were very optimistic as no stent thrombosis was reported during the study period (Press, 2015).

Simultaneously, accompanying advances in pharmacotherapy, as well as

increase in its duration following PCI, have also reduced the incidence of thrombosis. Attention should not only focus on the efficacy of medications, but also on side effects such as bleeding, the result of which can be devastating for the affected patients.

Complexity of lesions and subsequent difficulties in performing PCI, is another risk factor for thrombosis. As mentioned earlier, stent malapposition can predispose to thrombosis, therefore it was suggested that surveillance for high risk cases is indicated with the aid of intravascular ultrasound or optical coherence tomography (Press, 2015). Published results of a study investigating bioabsorbable scaffold thrombosis via angiography and optical coherence tomography, prove that suboptimal stent insertion, under-expansion and malapposition are responsible for both early and late thrombosis. Furthermore, early antiplatelet agent discontinuation seems to be another contributor, particularly in late events. However, stent thrombosis for the above reasons are highly preventable (Karanasos, 2015).

The need for assessing and identifying patients following PCI at risk of thrombosis, is of great importance.

Finally, regular follow-up of post-PCI patients with view to promote drug compliance and risk factor modification as part of secondary prevention should be considered as an issue of equal importance.

Endogenous thrombolysis

Endogenous Thrombolysis mechanism

Tissue plasminogen activator is stored in large amounts intra-cellularly as well as in steady amounts intra-vascularly, where it circulates in 2 forms, one active and one in complex with PAI-1 and PAI-2. It is estimated that the active form of t-PA makes up about 20% of the total (Kovacs, Gorog, & Yamamoto, 2006) .

Release of t-PA can be stimulated by several factors involved in the process thrombosis like thrombin and PAF. Therefore endogenous release of t-PA is a mechanism of vital clinical importance as it is the basis of ET promoting spontaneous re-canalisation in occluded coronary arteries. However, apart from t-PA, PAI-1 and PAI-2 are also released from endothelial cells in response to ischaemia and it is still not known what drives the balance between fibrinolytic stimulators and inhibitors in this situation, as ET status differs among different individuals (Kovacs, Gorog, & Yamamoto, 2006).

Thrombosis vs Fibrinolysis

Several mechanisms have been identified from experimental studies to influence shift of this balance towards either fibrinolysis or inhibition.

The first mechanism described involves polymorphonuclear leucocytes which tend to accumulate within thrombi at an early stage of thrombogenesis. These monocytes can attack fibrin via plasminogen dependent ways (plasminogen activator release) and independent ways (serine protease release), thus favoring fibrinolysis (Kovacs et al., 2006).

On the other hand arterial thrombi are rich in platelets and more resistant to lysis due to release of PAI-1 upon platelet activation. It is estimated that around 90% of total PAI-1 in the blood is contained in platelets. Conversely, the levels of t-PA and t-PA/PAI-1 complex have been found to be low in thrombi, therefore one could postulate that this could be attributed to the imbalance between PAI-1 levels against t-PA and t-PA/PAI-1 complex levels during ischaemia (Kovacs et al., 2006).

MI is the outcome of an imbalance between the mechanism of thrombosis against that of spontaneous thrombolysis (Kovacs, Gorog, & Yamamoto, 2006), which is a potentially life saving process, but unfortunately often neglected when discussing the causes of atherothrombotic events. The majority of STEMI patients undergoing PPCI are found to have TIMI 0 flow in their culprit vessel, which is associated with larger myocardial damage and consequently with

higher morbidity and mortality rates, especially when pain-to-balloon time (PTB) is prolonged (Blankenship et al., 2011).

However in the case of spontaneous lysis due to ET, even the small improvement in TIMI flow, could be enough to result in better clinical outcomes following PPCI.

With the exception of pharmacological thrombolysis, which nowadays is not the mode of treatment in STEMI due to its high risk of complications (intracranial bleeding, reperfusion arrhythmias) and need for longer in-patient stay, current pharmacotherapy for both primary and secondary prevention for ACS focuses exclusively on the anti-thrombotic properties of medication. Currently there is no known pharmacological agent promoting ET, therefore one could question the efficiency of current medical treatment since not every determinant of ACS is targeted.

Platelet function test (PFT)

Traditional Platelet function tests

Platelet hyper-reactivity has been identified as a potential mechanism of acute MI and identification of individuals exhibiting this should contribute towards more effective primary as well as secondary prevention. Therefore the need for a novel test that could accurately assess platelet activity is crucial in our search for strategies to reduce the incidence of IHD. However none of the currently used PFTs stands out as superior to others for its purpose (Gorog & Fuster, 2013).

Over the last decades several PFTs have been introduced, however they have all shown limitations, such as complexity in their use (both to perform and to evaluate) and delay in providing us with results. Also some of the tests designed to assess platelet hyper-reactivity, failed to fulfill their task due to lack of standardization and inability to define ‘normal’ and ‘hyper-reactive’ ranges as well as to identify patients at risk (Gorog & Fuster, 2013). Tests employed to measure Aspirin resistance have provided us with conflicting results regarding the actual percentage of this phenomenon, up to a point where it has been

suggested that inadequate information may mislead physicians (Gorog & Fuster, 2013).

Clopidogrel resistance is a major issue nowadays, because this medication is the commonest agent used along with Aspirin in patients undergoing PCI. Several tests have been used in order to identify Clopidogrel non-responsiveness, however significant inconsistencies have been observed in the outcomes among different assays. This could be explained by the fact that these tests assess different aspects of platelet function. At the moment there is no PFT to be regarded as superior compared to others in detecting clopidogrel resistance (Gorog & Fuster, 2013).

Since the usefulness of Clopidogrel in reducing the risk of MACE following ACS has been statistically proven, regardless of the magnitude of resistance, one would question the usefulness of PFTs in assessing non-responsiveness (Gorog & Fuster, 2013). However due to the fact that its prevalence has been estimated by some tests up to 33%, it would not be unreasonable to search for the ideal PFT that would offer accurate assessment of resistance prevalence as well as its involvement in post-PCI event risk. This latter task is not easy to be performed due to the low frequency of MACE following PCI. Therefore in order to demonstrate relationship between PFT and events, larger studies should be designed (Gorog & Fuster, 2013).

Bleeding in response to anti-platelet agent use is another issue PFTs have attempted to assess. This can be achieved by assessing platelet reactivity as well as the number of bleeding events in response to different agonists. However this task can become more challenging due to attempted combination of anti-platelet agents for prevention of coronary events, requiring the assessment of response to a greater number of agonists by PFTs (Gorog & Fuster, 2013).

Use of citrate-anticoagulated blood instead of native blood could be the cause of inconsistencies observed in the results of various tests, because different citrate concentrations can influence results as platelet response to agonists can vary.

Additionally, use of anti-coagulants such as heparin can alter platelet reactivity and impair measurement of the effect of thrombin (Gorog & Fuster, 2013).

Novel platelet function tests

The ideal PFT should demonstrate some qualities not been observed so far by any other test on the whole. Firstly, this test should be simple in its use and fast in providing us with results. Complex tests can be time consuming and hence unsuitable, especially in the acute setting, such as in STEMI situation where testing should not interfere with PPCI process and results are required in a short time. Secondly it should demonstrate accuracy and consistency. This can enable researchers to define ranges for risk and safety, thus promoting safety in future patient management. Furthermore it should be cost effective. As larger trials are necessary for providing statistical power, for example in obtaining greater number of post-PCI events, a greater number for tests will be needed, so we should aim for lower costs without altering test quality. This issue is also important as more tests are necessary for each recruited patient in an attempt to assess thrombosis through a patient's hospitalization from initial event day until outpatient follow-up in order to investigate possible variations thrombosis in response to different risk factors, altering pharmacotherapy, inflammation, drug resistance (Gorog & Fuster, 2013).

The global thrombosis test (GTT)

Endogenous Thrombolysis and PFTs

The catastrophic consequences of thrombosis following the sequence of atherosclerotic plaque rupture, platelet adhesion and activation of coagulation cascade, are a major cause for morbidity and mortality worldwide.

ET, a natural and potentially life saving defense mechanism whose purpose is to balance thrombosis, is unfortunately a process that has been overlooked by researchers, hence the absence of pharmacotherapy aiming to promote fibrinolysis, with the exception of fibrinolytic agents used acutely in STEMI. Even this treatment though has been recently overshadowed by PPCI, which according to current evidence has been proven to be superior than fibrinolysis due to a better profile demonstrated in terms of successful outcomes, reduced mortality and hospitalisation times. Spontaneous fibrinolysis has never been assessed previously, but its relevance to STEMI and its future outcomes has been highlighted in several published papers. Specifically, the occurrence of this process was confirmed by both angiographical and electrocardigraphical evidence and was associated with lower mortality, lower heart failure incidence and better future prognosis in terms of lower risk for recurrent ACS.

A series of issues, such as the absence of a reliable PFT, as well as recognition of the great importance of ET in relation to IHD and its management, have led researchers in the manufacture of a new point-of-care test which is very simple in its use, aiming to assess both thrombosis and ET in the clinical setting, providing us with results in a short time (Saraf et al., 2010) (Sharma et al., 2012).

The Global Thrombosis Test

The Global Thrombosis Test (GTT) (Montrose Diagnostics, London, United Kingdom) (Yamamoto, Inoue, Otsui, Ishii, & Gorog, 2014) is a novel test designed to simultaneously measure platelet reactivity and endogenous thrombolysis using native, non-anticoagulated blood. The main device has 4 separate channels able to accommodate a single test tube each, placed in a vertical position. The lumens of these test tubes are designed in such a way in order to resemble the lumen of an atherosclerotic coronary vessel. Their distal end has a conical shape inside which two small ceramic balls are placed one on top of the other, just above the distal opening, through which blood can drip inside a reservoir that is a part of the plastic test tube. Optical sensors can detect blood flow as well as its rate by measuring the interval between consecutive falling blood drops into the reservoir.

Once blood is injected into the narrow lumen and flows through the gaps between the balls and the tube walls, platelet activation occurs due to shear forces exerted, leading to thrombin generation and subsequently thrombus formation, eventually obstructing blood flow completely. ‘Occlusion Time’ (OT) is the time between initial injection of blood in the tube until flow arrest and it is defined as duration between two consecutive drops exceeding 15 seconds.

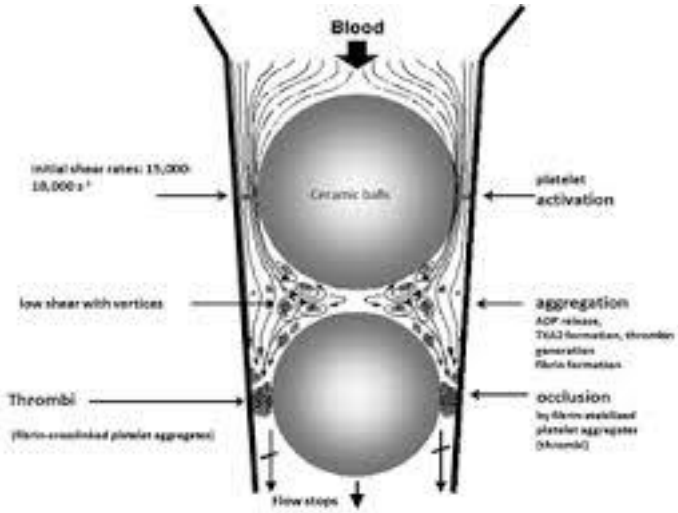
Apart from platelet reactivity that can be easily and reliably assessed in the clinical setting as explained above, this test demonstrates another unique quality, which is the ability to assess ET. The time elapsed between total occlusion and spontaneous flow restoration following thrombus lysis is called Lysis Time (LT) and it is measured in seconds.

The manufacturer has proposed OT/LT ranges for data interpretation, however more evidence has been recommended for confirmation of their accuracy and hence their reliability:

1. OT <80sec. (Unreliable test)
2. OT <300sec. (Platelet hyper-reactivity)
3. OT 300-500sec. (Normal platelet function)
4. OT 400-800sec. (Anti-platelet/anticoagulant effect or minor bleeding diathesis)
5. OT 500-700sec. (Optimal anti-platelet effect)
6. OT >900sec. (Possible bleeding risk)
7. LT <2000sec. (Normal spontaneous thrombolytic activity)
8. LT 2000-4000sec. (Reduced thrombolytic activity)
9. LT 4000-6000sec. (Markedly reduced thrombolytic activity)
10. LT >6000sec. (No detectable thrombolytic ability)

Figure 2. The global thrombosis test.

Figure 3. Platelet activation and thrombosis inside the GTT tube.



GTT and published data

Data using the GTT has been published in several papers. The two most important ones investigated the role of ET in ACS and End Stage Renal Failure (ESRF) patients respectively (Saraf et al., 2010) (Sharma et al., 2012) (Yamamoto et al., 2014).

The first one (Saraf et al., 2010) demonstrated for the first time the role of ET both as an independent risk factor for future thrombotic events. Furthermore, delayed ET was identified as a strong predictor of recurrent cardiovascular events (death and non-fatal MI) and an LT of 3000s was estimated as THE cut-

off value for prolongation. On the other hand no relationship was demonstrated between OT and MACE. The main limitations were identified and suggestions were proposed for future studies involving use of the GTT. In specific it was suggested that a bigger study should be designed, with more tests performed during index event hospitalization in order assess the influence of anti-platelet agents and inflammation on thrombosis, something that cannot be accurately measured with a single test. Additionally the effect of heparin was mentioned as a factor impairing quality of results due to its influence in the process of thrombosis.

The second study (Sharma et al., 2012) investigating ET in ESRF patients and showed coinciding results with the previous one. In specific impaired ET which is very common in ESRF patients was proven to be a strong predictor of MACE, unlike OT status which was not related to future outcomes. The conclusion from these two studies was that ET is a phenomenon needed to be further investigated in a greater depth and in more detail, taking into account several factors as with a potential to influence outcomes following STEMI that have been highlighted in current literature, but were not included in the previous two studies.

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CHAPTER 1

Can Global Thrombosis Test predict thrombotic complications and major adverse cardiac events (MACE) in STEMI patients undergoing PPCI?

Background

Primary percutaneous coronary intervention (PPCI) is currently the preferred option for coronary revascularization in patients with time from symptom (chest pain and/or dyspnea) onset of less than 12 hours, with persistent acute ST-segment elevation on electrocardiography (ECG) or with ST-segment elevation equivalents (Conference, Bcs, & Us, 2013). Coronary revascularization with PPCI, is also indicated in patients with time from symptom onset exceeding 12 hours, in the presence of continuing ischaemia, life-threatening arrhythmias or if pain and ECG changes have been stuttering (Steg et al., 2012).

Based on electrocardiographic criteria, acute STEMI, is defined as ST elevation >1mm in 2 or more contiguous limb leads or ST elevation >2mm in 2 or more chest leads. STEMI equivalents do not exhibit the classical ECG changes, but are associated with acute coronary occlusion and subsequently poorer outcomes and worse prognosis. The common STEMI equivalents are left main coronary occlusion with lead aVR ST-segment elevation, new or presumably new left bundle branch block (LBBB) and isolated posterior MI (Conference et al., 2013).

Furthermore, the duration from the time of symptoms onset until coronary revascularization, is of vital importance, as occlusive thrombus composition changes, from an initial platelet abundance to a gradual replacement by fibrin, making PPCI technically more difficult in achieving a final TIMI III flow in the culprit coronary artery. Additionally, prolonged coronary occlusion leading to extended myocardial necrosis, predisposes to unwanted life-threatening clinical complications, such as heart failure with cardiogenic shock, arrhythmias and death (Blankenship et al., 2011).

The choice of stent type during PPCI is of extreme importance. There are

currently two types of stents, the drug-eluding stent (DES) and the bare-metal stent (BMS). The former has three components, the metallic stent platform, the polymer coating and the anti-proliferative agent (Stefanini & Holmes, 2013). Available stent platforms are made of cobalt-chrome, platinum-chrome or stainless steel. Cobalt-chrome, as well as platinum-chrome alloys, provide good radial strength and increased radio-opacity, allowing for engineering of thinner struts that enable better stent delivery. Thinner struts in stent platforms, reduce the risk of coronary injury and consequently the risk of stent thrombosis (Stefanini & Holmes, 2013). The polymer coating acts as a drug carrier and allows controlled release of the drug (Stefanini & Holmes, 2013) .

Anti-proliferative agents are highly lipophilic molecules that are distributed into the coronary wall, exerting either immunosuppressive effects (inhibitors of mammalian target of rapamycin) or anti-proliferative effects (paclitaxel) on smooth-muscle cells (Stefanini & Holmes, 2013). In clinical practice, new generation stents releasing everolimus and zotarolimus and consisting of either cobalt-chrome or platinum-chrome platforms with thinner struts, have almost completely replaced paclitaxel-eluding stents and sirolimus-eluding stents, with the latter stopped being manufactured (Stefanini & Holmes, 2013). Drug-eluding stents allow physiological arterial healing with smooth endothelial coverage of all stent struts, but they prevent neo-intimal hyperplasia. Excessive anti-proliferative effects can lead to chronic inflammation and increasing thrombogenicity. Sirolimus and paclitaxel-eluding stents were associated with delayed arterial healing, featuring incomplete endothelialisation of stent struts, vessel remodelling, increasing platelet and fibrin deposition, as well as premature atherosclerosis. Usage of new-generation DES though, has resulted in better endothelial coverage, with consequent reduction in the occurrence of the above features (Stefanini & Holmes, 2013).

Lately, the emergence of biodegradable polymers, aimed to offer the benefits of a conventional DES in the early phase and a BMS at later stages, by enabling both controlled drug release and polymer biodegradation initially, leaving only the stent platform eventually. Another type of new DES is the polymer-free

DES, aiming to avoid potential adverse long-term effects of polymer presence. These two stent types are promising and currently being tested in clinical trials (Stefanini & Holmes, 2013).

In recent years, a third generation bioabsorbable drug-eluding vascular scaffold (BVS) has been released, aiming to provide transient vessel support with drug delivery capability, that in theory will not exhibit the long-term disadvantages of metallic DES, such as permanent vessel caging, possible malapposition, late stent thrombosis, local inflammation and neo-atherosclerosis. Furthermore, BVS can restore vascular physiology and anatomical integrity, as they provide only a temporary scaffold needed to maintain the patency of the vessel undergoing angioplasty. At the moment, there are four types of BVS, based on the material used for their manufacture. Lactide polymers are present in several devices and they are the most extensively investigated. Other materials include magnesium, polyanhydrides and polycarbonates (Stefanini & Holmes, 2013) .

Bare metal stents (BMS) were initially made of stainless steel, due to the fact that this material is biologically inert. Over the years though, cobalt-chromium and platinum-chromium alloys have replaced stainless steel as the material of choice for stents, due to their thinner struts that improve radial strength and enable corrosion resistance (Bangalore et al., 2013).

Choosing between BMS and DES is not always an easy task, and indeed there are some variations among different operators, as well as between regional and international guidelines. In general, it is acceptable that shorter lesions (≤15mm) in bigger vessels (≥3mm in diameter) in non-diabetic patients can be treated with BMS. On the other hand, diabetic patients, patients with lesions ≥15mm in length or patients with small diameter vessels (<3mm in diameter) should receive DES, provided that dual anti-platelet therapy (DAPT) is not contra-indicated. Also BMS should be inserted in patients not willing to receive DAPT or they have a medical condition in which prolonged DAPT is contra-indicated. Diabetes mellitus has been identified as an independent predictor of in-stent restenosis (ISR), therefore it is of no surprise that diabetic patients receiving DES have significantly lower rates of acute MI, death and repeat revascularisation, compared to those treated with BMS. However, diabetic

patients with multi-vessel coronary disease should be referred for coronary artery by-pass graft (CABG) operation, based on recent evidence (Iqbal, Gunn, & Serruys, 2013).

Data from 126 randomised trials (more than 250000 patient years of follow-up) has shown that biodegradable polymer DES are not superior to new generation durable polymer for both efficacy and safety outcomes. Also newer generation durable DES were the most effective stents as they exhibited the lowest rate of repeat revascularisation, no increase in very late stent thrombosis and significant decrease in the risk of MI. Additionally, a decrease in definite stent thrombosis, MI and death were observed when cobalt-chromium stents were compared with BMS (Bangalore et al., 2013).

Selection of the appropriate anti-thrombotic regimen to accompany stent selection is the next task when managing STEMI patients undergoing PPCI. These regimens include a combination of anti-platelet agents and procedural anticoagulants, such as unfractionated heparin with glycoprotein IIb/IIIa inhibitor (GPI)(reopro) or bivalirudin (direct thrombin inhibitor). The 3-year report from the HORIZONS-AMI trial states that use of bivalirudin in STEMI patients undergoing PPCI, significantly reduces non-CABG-related bleeding, re-infarction and all cause mortality, when compared with treatment with unfractionated heparin and GPI. However, no difference was observed between groups in the rates of stent thrombosis, target vessel revascularisation, stroke and net adverse clinical events (Stone et al., 2011). Current guidance for secondary prevention for cardiovascular disease, suggests that a combination of two different anti-platelet agents should be administered for a period of twelve months, irrespective of the type of the stent implanted. Once patients complete this twelve-month dual anti-platelet agent course, one agent should be discontinued and they should continue taking the other one, which is usually aspirin, for life. The anti-platelet agent which is most commonly prescribed nowadays in combination with aspirin is ticagrelor, however very often clopidogrel is also prescribed in many cardiac centres (Roffi et al., 2015).

Once decision has been made about suitability of a patient for PPCI, immediate administration of a loading dose of Aspirin (300mg orally) and Copidogrel (600mg orally) or Ticagrelor (180mg orally) should follow, before initiation of the procedure. The purpose of this is to prevent further thrombus formation, that not only can establish total coronary occlusion, but it can also prolong the process of revascularisation during PPCI, leading to extended myocardial necrosis and consequently life threatening peri-procedural complications and long-term heart failure issues (Roffi et al., 2015).

Following successful angioplasty, maintenance dose of aspirin (75mg daily) and clopidogrel (75mg daily) or ticargelor (90mg twice a day) is recommended, with the medical team emphasising the importance of drug compliance, in order to avoid stent thrombosis and further MI, which is expected upon premature discontinuation of dual anti-platelet treatment (Roffi et al., 2015).

Based on the latest guidance, it is acceptable to discontinue the second anti-platelet agent three to six months following DES implantation and one to three months following BMS or new generation DES implantation, in case of very high bleeding risk or need for non-cardiac surgical operation that cannot be postponed (Roffi et al., 2015).

Identification of patients at high risk of major adverse cardiac events (MACE) post-PPCI has always been a major challenge, and currently no risk stratification model can be fully applied to these patients, due to lack of adequate number of subjects when some studies were carried out, as well as due to failure to include some very important parameters that determinate future outcomes post-PPCI, such as door-to-balloon time (DTB time), initial TIMI flow prior to mechanical revascularisation, type of anti-platelet agents and whether a loading dose has been used, level of left ventricular function impairment assessed by echocardiography (Mrdovic et al., 2013).
MACE is the composite of end points, reflecting both the safety and effectiveness of various MI treatment approaches. In literature, three definitions of MACE have been described, including a definition postulated to relate primarily to safety (death, MI, or stent thrombosis), and two definitions postulated to relate to both safety and effectiveness (death, MI, stent

thrombosis, or target vessel revascularisation and death, MI, stent thrombosis, or any repeat revascularisation) (Kip, Hollabaugh, Marroquin, & Williams, 2008). Occurrence of adverse outcomes in STEMI patients is higher within the first 30 days after infarction and despite their low incidence, their potentially catastrophic consequences still create great concern (Mrdovic et al., 2013).

Stent thrombosis, is a serious complication of treatment with both BMS and DES. According to the Academic Research Consortium, stent thrombosis has been given a definition based on the time of occurrence following angioplasty and the degree of diagnostic certainty. Early thrombosis occurs ≤1 month, late occurs between 1 month and ≤1 year and very late >1 year, and the degree of diagnostic certainty as definite, probable and possible (Stefanini & Holmes, 2013). It is related to procedural factors, as well as to inadequate platelet inhibition during the early post-implantation period and chronic inflammation leading to delayed arterial healing. Furthermore, aspirin and/or clopidogrel resistance has recently emerged as another cause of stent thrombosis. Aspirin resistance, the incidence of which varies between 5-60% among patients with cardiovascular disease, has a multi-factorial aetiology (Casterella, n.d.). Non-absorption, insufficient suppression of COX-1, over-suppression of COX-2 mRNA, erythrocyte-induced platelet activation, GP IIb/IIIa receptor polymorphism, collagen receptor polymorphism, vWF receptor polymorphism, P2W1 single nucleotide polymorphism are the major potential factors leading to aspirin resistance, as described in literature (Casterella, n.d.). Clopidogrel resistance varies from 4-30% in patients with coronary artery disease (CAD), depending upon the clinical scenario, test assay, and clopidogrel dose prescribed (Casterella, n.d.). Several contributing factors leading to this complication have been identified and are divided into intrinsic and extrinsic. Extrinsic factors include drug interactions, CYP3A4 activity, variable pro-drug absorption, variable active metabolite clearance and intrinsic factors include P2Y12 receptor variability, increased intrinsic ADP levels and up-regulation of other platelet pathways (Casterella, n.d.).

Several strategies have been suggested in order to tackle occurrence of anti-platelet resistance. Firstly, there is need for standardised methods of platelet

function testing and interpretation, along with integration of point-of-service testing, that may assist in individualising anti-platelet therapy and subsequently to improve outcomes in patients with aspirin/clopidogrel resistance. Usage of new agents with a more reliable pharmacologic effect and/or reduced incidence of resistance, may further assist in dealing with the unwanted effects of antiplatelet therapy resistance (Casterella, n.d.).

Although many platelet function tests (PFT) are currently available, none of them has reliably predicted the risk of thrombosis yet and there are several reasons to explain this. Firstly, all currently used PFTs measure the response of platelets to specific agonists, even though in the pathogenesis of thrombosis, lots of factors, such as high shear stress and thrombin generation are implicated. Additionally, these tests do not assess the role of thrombin formation, which is now recognized as a key factor in the process of arterial thrombosis, as they are performed on citrate-anticoagulated blood that prevents the in-vitro assessment of thrombin generation by activated platelets. Furthermore, currently used PFTs do not assess spontaneous endogenous thrombolysis (ET), which is an important, yet under-investigated, component of defense against lasting arterial occlusion, as MI is the result of failure of timely spontaneous thrombolysis (Gorog & Fuster, 2013). The role of global thrombosis has never been mentioned in any of the current risk stratification models, and based on data from previous studies using the global thrombosis test (GTT), proving the role of impaired ET as a predictor of MACE in acute coronary syndrome (ACS) and end-stage renal impairment (Saraf et al., 2010) (Sharma et al., 2012), it is fair to conclude that the need of more advanced risk stratification models is necessary, in order for high risk patients to be accurately identified and treated accordingly. For example, identified patients with impaired endogenous thrombolysis could be treated more aggressively. Additionally, patients demonstrating enhanced thrombotic tendency, a possible sign of anti-platelet medication resistance, could be either treated with higher doses of anti-platelet agents or have one or both their anti-platelet agents switched to different ones. Also, in patients exhibiting delayed thrombotic and/or enhanced thrombolytic tendency, in whom bleeding risk is potentially higher, different strategies could be followed in prescribing anti-thrombotic

medication, in order to balance the risk of stent thrombosis against that of bleeding.

Bleeding is a potential complication in STEMI patients, resulting due to patient's bleeding diathesis, as well as secondary to anti-platelet agent use. It can occur at any time from the onset of STEMI management onwards and it could be life threatening in some occasions. For example, a major bleeding event on its own, is a life threatening situation to manage, but dealing with it in a very early stage post-PPCI with DES implantation, is an extremely challenging situation for the team managing the case, as any decision made regarding treatment can be proven to be a double-edge sward.

The above statement highlights the great importance in usage of accurate bleeding risk stratification scoring systems when managing STEMI patients.

The CRUSADE, ACUITY-HORIZONS, and ACTION risk scores demonstrated good calibration and discrimination for major bleeding prediction in STEMI patients undergoing PPCI, however the CRUSADE and ACTION risk models showed greater predictive capacity for major bleeding than the ACUITY-HORIZONS risk score (Flores-Ríos et al., 2013). CRUSADE and ACTION models distinguished five risk categories of bleeding (very low, low, moderate, high, and very high risk) (Flores-Ríos et al., 2013).

Aims

This study is unique, because it aims to investigate for first time the role of global thrombosis test (GTT) in identifying STEMI patients undergoing PPCI, that remain at risk of thrombotic complications and MACE, despite being on dual anti-platelet therapy. Accurate identification of high risk patients will enable clinicians to adjust pharmacotherapy in such a way that the incidence of acute thrombosis can be significantly reduced. On the other hand, by assessing patients' global thrombosis status, another complication such as bleeding can also be tackled. In this way, future management of patients with ischaemic heart disease (IHD) will be tailored to their needs, based on evidence from the GTT, aiming to reduce all possible complications to the minimum.

Methods

Study design This was a prospective, observational, single-centre study, it was approved by the local research ethics committee and all subjects gave written informed consent in order to participate.

Eligibility criteria Patients admitted to our unit with a presumed diagnosis of ST-elevation MI were included. Acute STEMI is defined as ST-elevation >1mm in 2 or more contiguous limb leads or ST-elevation >2mm in 2 or more chest leads. Common STEMI equivalents are left main coronary occlusion with lead aVR ST-segment elevation, new or presumably new left bundle branch block (LBBB) and isolated posterior MI (Conference et al., 2013), and were also included in our study. Exclusion criteria are listed in table 1:

Table 1. Exclusion criteria

Age below 18 years
Refusal to provide informed consent
Life expectancy of less than 1 year due to known non-cardiovascular condition
Administration of thrombolytic agent or glycoprotein IIb/IIIa before PPCI
Heparinisation of inserted arterial sheath or administration of intra-arterial heparin before obtaining initial GTT sample.

Blood sampling Blood samples were taken at 4 time-points: 1) upon presentation, before PPCI, 2) after the administration of reopro, 3) prior to hospital discharge and 4) at 4-6 weeks follow-up. The choice of the timing of samples was made for the following reasons: The first sample reflects an individual's global thrombosis status. We would not expect that aspirin and clopidogrel loading doses given within at most the previous 30 minutes to the patient, would have affected the GTT results much. With the second and third samples, we would like to investigate for possible changes in the global thrombosis status on and off reopro. With the last sample we aimed to investigate global thrombosis status once the patient is clinically stable and whether we can detect for possible antiplatelet resistance. The first samples were taken through the radial or femoral sheath. Before their insertion in the artery, sheathes were only flushed with normal saline, the first 15ml of arterial blood obtained following insertion were used for routine blood tests and the next 5ml sample was used for thrombotic status assessment. The second sample was again taken via the sheath, having discarded the first 5ml and used the next 5ml for thrombotic status assessment. The 3rd and 4th samples were

taken using a 16G butterfly cannula via an antecubital vein. Again the first 5ml was used for routine blood tests and the next 5ml used for thrombotic status assessment.

PPCI protocol All patients had received loading doses of Aspirin (300mg) and Clopidogrel (600mg) in the ambulance or the emergency department upon diagnosis of STEMI, either orally or via a nasogastric tube. The PPCI procedure was performed either via the radial or the femoral routes and according to conventional practice. At the time, it was standard protocol in our unit to use reopro for almost all cases where PPCI was undertaken.

Assessment of thrombotic and thrombolytic status Thrombotic status and endogenous thrombolytic activity were assessed using the Global Thrombosis Test, GTT (Montrose Diagnostics Ltd., UK). This novel, point-of-care assay employs native blood to assess the time taken to create a shear-induced thrombus under physiological conditions (occlusion time OT; seconds) and in the second phase of the test, measures the time to achieve endogenous thrombolysis of the thrombus created during the first phase (lysis time, LT; seconds). If lysis does not occur until 6000 seconds following OT (LT cut-off time), “No lysis” is displayed and recorded. The coefficient of variation (cv) was assessed by testing 10 healthy volunteers twice, at 48-hour intervals.

Study endpoints The primary end point of the study was occurrence of a major adverse cardiovascular event (MACE) at 1 year, defined as the composite of cardiovascular death, non-fatal MI or stroke. Secondary endpoints included major bleeding as defined by GUSTO/TIMI Classification, not related to index procedure or non-cardiovascular death.

New cardiovascular events were diagnosed in the presence of (1) cardiovascular death, defined as death in the presence of ACS, significant arrhythmia, or refractory congestive heart failure or death attributed to cardiovascular cause at post-mortem; confirmed from death certificates as well as medical records and observers’ accounts. Sudden death was included as cardiovascular events, or (2) non-fatal MI, defined as a rise in serum Troponin I or Creatine Kinase-MB isoenzyme ≥x2 upper normal limit, with at least one of

the following: acute onset ≥20 minutes typical ischaemic chest pain; ST-segment elevation ≥1mm or ST-segment depression ≥0.5mm in ≥2 contiguous leads; or T wave inversion >1mm in leads with predominant R waves.

New-onset cerebrovascular event was suspected with recent onset of neurological symptoms or signs, e.g., aphasia, focal deficits, or unilateral paresis, thought to be vascular in origin and confirmed by computerised-tomography or MRI.

New onset major bleeding was defined based on TIMI and GUSTO bleeding classifications (see tables 2 and 3):

Table 2. TIMI bleeding classification (Rao et al., 2006)

TIMI Bleeding Classification	
Major	Intracranial haemorrhage or a ≥5 g/dl decrease in the haemoglobin concentration or a ≥15% absolute decrease in the haematocrit
Minor	Observed blood loss: ≥3 g/dl decrease in the haemoglobin concentration or ≥10% decrease in the haematocrit
Requiring Medical Admission	No observed blood loss: ≥4 g/dl decrease in the haemoglobin concentration or ≥12% decrease in the haematocrit
Minimal	Any clinically overt sign of haemorrhage (including imaging) that is associated with a <3 g/dl decrease in the haemoglobin concentration or <9% decrease in the haematocrit

Table 3. GUSTO Bleeding classification (Rao et al., 2006)

GUSTO Bleeding Classification	
Severe or life-threatening	Either intracranial haemorrhage or bleeding that causes haemodynamic compromise and requires intervention
Moderate	Bleeding that requires blood transfusion, but does not result in haemodynamic compromise
Mild	Bleeding that does not meet criteria for either severe or moderate bleeding

Data collection and follow-up Basic demographics were obtained during index admission from patients' medical notes and if necessary from their primary care records. Follow-up was done either in person, or over the telephone at 1, 3, 6 and 12 months. Collection and adjudication of events was performed blinded to GTT results. Source documents for all adverse events were obtained.

Statistical analysis Analysis was performed with Stata/IC version 11.2. Paired and unpaired t-tests were used for comparison of normally distributed variables and Mann-Whitney U-test was used for non-normally distributed variables. Chi-squared test with continuity correction or Fisher's exact test were used to compare dichotomous variables. With Univariate linear regression model we assessed the relationship between a continuous variable (dependent) given a dichotomised variable (independent). Spearman's rank test was used for correlations' analysis and (ROC) curve analysis for discrimination between patients with and without MACE. In order to compare survival curves we used Kaplan-Meier survival methods with log-rank tests. For investigation of the relationship between increasing LT and MACE we performed Univariate Cox proportional hazard regression on LT divided into bands of 1000s. In order to adjust for potential confounders that are associated with clinical end-points on univariate analysis, we used Multivariate hazard regression Cox model. Finally, for assessment of the added predictive ability of LT≥3000 for MACE, we used Net reclassification improvement (NRI).

Baseline OT/LT were separately related to OT/LT at other pre-specified time points and OT/LT at the 4 pre-specified time points were separately related to study end-points, in order to establish whether any of the time points can be predictive of MACE or major bleeding.

Results

Table 4. Baseline patient characteristics

	Overall group (n=82)	Baseline LT<3000s (n=71)	Baseline LT LT≥3000s (n=11)	P value
Age, years	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

Table 5. Medications on discharge

Medications on discharge	Overall group (n=82)	Baseline LT<3000s (n=71)	Baseline LT LT≥3000s (n=11)	P value
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 agonists	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

For tables 4 and 5: Values are mean±standard deviation or n(%). Renal insufficiency is defined by creatinine levels>177µmol/L. Prior aspirin use was defined as use of the drug before hospitalisation. ACE: angiotensin-converting enzyme, CAD: coronary artery disease, CVA: cerebrovascular accident, PVD: peripheral vascular disease, VKA: vitamin K antagonist.

Table 6. Blood tests on admission

Biochemistry	Overall group	Baseline LT<3000s	Baseline LT LT≥3000s	P value

	(n=82)	(n=71)	(n=11)	
Haemoglobin (g/L)	139±20	140±19	130±27	0.115
Haematocrit (%)	42±6	42±6	39±6	0.077
Platelet count (x10^9/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 I (ng/ml), peak at 12 hours	20.2±27.7	21.3±28.5	9.8±16.7	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 is 130-180 g/L in adult males and 115-165 g/L in adult females; haematocrit is 40-52% in adult males and 36-47% in adult females; platelet count is 150-400x10^9/L; creatinine is 60-110 µmol/L in adult males and 45-90 µmol/L in adult females; C-reactive protein is 0-5 mg/L; troponin I is <0.04 ng/ml; total cholesterol is ≤4.0mmol.

Table 7. Angiographic, interventional and echocardiographic patient characteristics

	Overall group (n=82)	Baseline LT<3000s (n=71)	Baseline LT LT≥3000s (n=11)	P value
1-vessel disease (VD)	43(52.4)	39(54.9)	4(36.4)	0.260
2VD	22(26.8)	17(23.9)	5(45.5)	0.141
3VD	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 predilation	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 (36-44%)	13(17.1)	13(18.3)	0	0.264
Severely impaired (≤35%)	1(1.3)	1(1.4)	0	0.673

Values are mean±standard deviation or n (%). BMS: bare-metal stent, DES: drug-eluding stent, EF: ejection fraction, GPI: glycoprotein IIb/IIIa inhibitors, LAD: left anterior descending artery, LCA: left circumflex coronary artery, LT: lysis time, LV: left ventricle, OM: obtuse marginal artery, PCI: percutaneous coronary intervention, RCA: right coronary artery, TIMI: Thrombolysis in myocardial infarction.

Table 8. Clinical outcome at 12 months of follow-up

	Overall group (n=82)	**Baseline LT<3000s (n=71)**	**Baseline LT LT≥3000s (n=11)**	HR (95% CI)	P value
Cardiovascular death, non-fatal 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)	0.051
Non-fatal MI	3(3.7)	2(2.8)	1(9.1)	3.96(0.36-	0.263

				43.94)	
Ischaemic 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 available, cardiovascular death: death in the presence od acute coronary syndrome, significant arrhythmia or refractory congestive heart failure or death attributed to cardiovascular cause at post-mortem.

We recruited 82 patients for this sub-study. Their clinical characteristics are demonstrated in tables 4-6, and their angiographic, procedural and echocardiographic characteristics are shown in table 7. Out of all variables interrogated for relation to baseline OT and LT, only smoking was found to be related to OT, as demonstrated by shorter (more thrombotic) OT in smokers compared to OT of non-smokers (294±105s vs. 365±153s, p=0.026). Furthermore, there was weak negative correlation between baseline OT and admission creatinine levels (r= -0.4, p=0.057) and weak positive correlation between baseline OT and pre-PPCI TIMI-3 flow (r= 0.2, p=0.063). On the other hand, baseline LT≥3000s was significantly associated with female sex, and admission creatinine levels.

Univariate analysis suggested that the following clinical and interventional characteristics were related to MACE outcome: increasing age (p=0.008), statin therapy (p=0.012), prior aspirin use (p=0.001), beta blocker use (p=0.024), angiotensin-converting enzyme (ACE) inhibitor use (p=0.0008), calcium antagonist use (p=0.001), history of renal insufficiency (p=0.002), elevated admission serum creatinine (p=0.001) and culprit vessel being the right coronary artery (p=0.02). There was weak relation between bare metal stent insertion and MACE (p=0.077) and finally there was no relation between any of the variables and major bleeding outcome, however the number of events was small.

Certain variables were entered into the final baseline multi-variate Cox proportional hazard model (age with HR: 1.07, 95%CI: 1.02-1.13, p=0.005,

statin therapy with HR: 6.39, 95% CI: 1.95-20.99, p=0.002, previous diabetes mellitus diagnosis with HR: 0.51, 95% CI: 0.15-1.71, p=0.276 and previous diagnosis of hypertension with HR: 0.51, 95% CI: 0.16-1.6, p=0.246), however none of these covariates was found to be correlated, either with LT or its dichotomised version. Once more, baseline LT≥3000s was shown to be strongly associated with MACE (Figure 6), after adjustment for the baseline risk factors (HR: 4.26, 95% CI: 1.13-16.01, p=0.032). Finally, net reclassification improvement (NRI) demonstrated that inclusion of baseline LT≥3000s in a model containing three baseline predictors, such as age, previous diagnosis of diabetes mellitus and statin use, significantly added to the model effectiveness (NRI estimate: 0.347, p=0.018).

OT was normally distributed at baseline, however shortly after treatment with GPI (65% of patients received the medication), OT was markedly prolonged compared to baseline (743±157s vs. 330±136s, p=0.00001. OT remained significantly prolonged prior to hospital discharge (536±195s vs. 331±129s, p=0.00001) compared to baseline and there was change between hospital discharge and at 1 month follow-up (529±195s vs. 528±144, p=0.995) (Figures 2 and 3).

At baseline, LT was positively skewed and many patients exhibited prolonged lysis times. Following treatment with GPI, LT was significantly reduced compared to baseline LT (709±149s vs.140±210s, p=0.00001). No difference was observed in LT at hospital discharge (1523±224s vs. 1332±206s, p=0.365) or at 1 month compared to baseline LT (1132±173s vs. 1239±194s, p=0.354). Also, at 1 month, LT was significantly reduced compared to baseline LT (1238±162s vs. 1598±221s, p=0.034) (Figure 4).

A total of 13 MACE events were documented in 13 different patients. In specific, we recorded 8 cardiovascular deaths, 3 non-fatal MI (1 due to acute stent thrombosis within 24 hours from insertion and 1 due to late thrombosis, 76 hours following insertion), 2 ischaemic strokes not related to atrial fibrillation and 2 major bleeding events. No issues were reported regarding anti-platelet agent compliance in any of these patients. MACE outcome was found to be strongly related to platelet reactivity at presentation, manifested as shorter

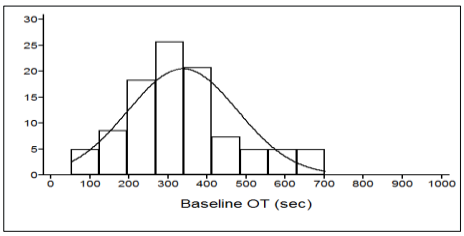
baseline OT (more thrombotic) in patients with adverse events compared to baseline OT in patients without MACE (253±150s vs. 354±134s, p=0.017) and particularly in the occurrence of non-fatal MI (OT 135±45s vs. 346±137s, p=0.009) and ischaemic stroke (133±31s vs. 343±38s, p=0.036) (Figure 7). There was no difference in baseline OT between patients with major bleeding and those without bleeding (297±121s vs. 339±141s, p=0.682). OT at other pre-specified time points was not related to MACE or major bleeding (Figure 7).

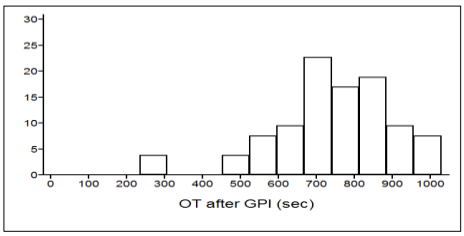
Despite demonstrating the relationship between short baseline OT and MACE occurrence, no threshold was found in baseline OT, as a discriminator of MACE risk, as per ROC curve analysis (Figure 8). On the other hand, LT significantly discriminated between patients with and without MACE, with an area under the curve of 0.59 (95% confidence interval (CI): 0.471-0.693, $p<0.05$. LT≥2901s, was identified as the cut-off point to predict MACE (sensitivity 31% and specificity 89%) (Figure 9). However, we rounded this to 3000s for clinical ease, aided by the fact that no LT readings between 2900 and 3000s could have been wrongly classified based on this rounding.

Survival analysis did not demonstrate any relationship between OT and MACE (hazard ratio [HR]: 0.99, 95% CI:0.989-0.999) or major bleeding at any pre-specified time point. However, MACE was found to be strongly related to baseline LT, particularly when LT was ≥3000s (HR: 3.31, 95% CI:1.02-10.78, p=0.045), driven predominantly by cardiovascular death (HR: 4.17, 95% CI: 0.99-17.51, p=0.051) (Figure 5). Baseline LT was not associated with an increase in the risk of major bleeding, however the number of events was small. Furthermore, no relationship between LT and MACE or major bleeding was observed at other pre-specified points.

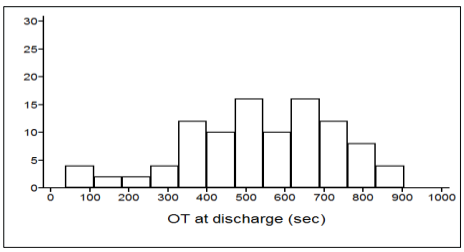
Figure 1. Distribution of OT at the 4 pre-specified time points.

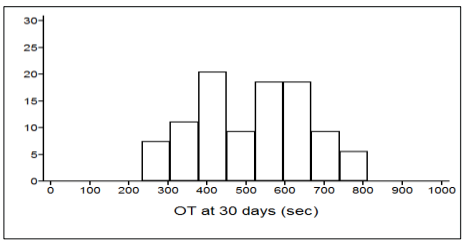
(A) (B)





(C) (D)

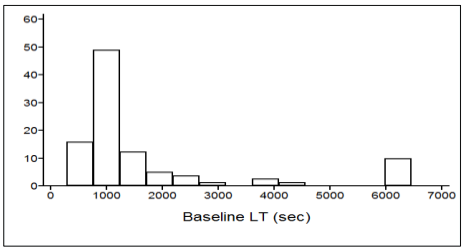


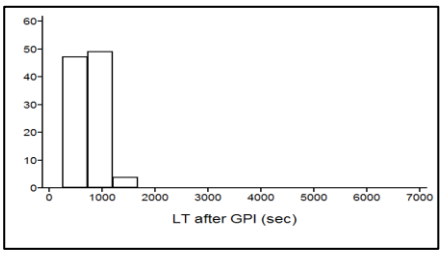


(A): baseline, before PPCI, (B): after administration of GPI, (C): prior hospital discharge, (D): 1 month follow-up. GPI: glycoprotein IIb/IIIa inhibitor, OT: occlusion time. There is a significant shift of OT towards higher values due to the strong antithrombotic effect of aspirin/clopidogrel and reopro, as shown in diagram B. As the anti-thrombotic effect of reopro gradually wears off upon its discontinuation, average OT is slightly displaced towards the left (diagram C) and finally in the 1-month appointment, average OT lies in a position between the average value at baseline and the average value while on reopro.

Figure 2. Distribution of LT at the 4 pre-specified time points.

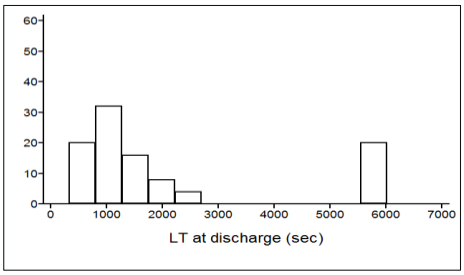
(A) (B)

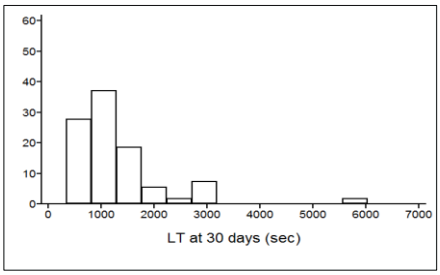




(C)

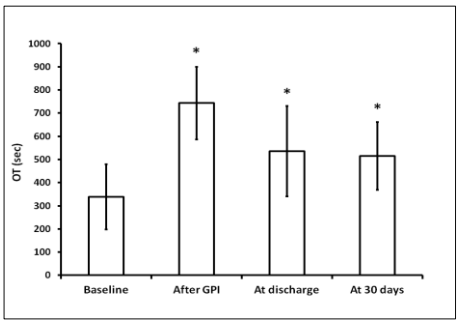
(D)





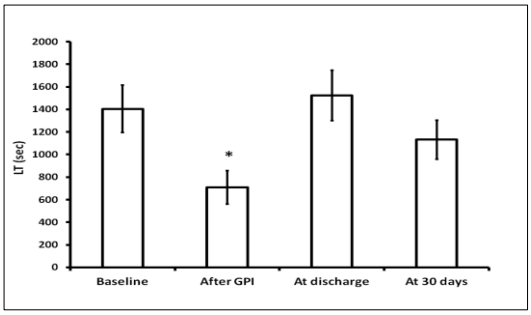
(A): baseline, before PPCI, (B): after administration of GPI, (C): prior hospital discharge, (D): 1 month follow-up. GPI: glycoprotein IIb/IIIa inhibitor, LT: lysis time. These diagrams demonstrate enhancement of ET by reopro, as evident by the lower LT values in diagram B. As the effects of reopro wear off upon discontinuation, LT values shift towards the right, as demonstrated in diagrams C and D. Absence of LT values above 2000s in diagram B, shows the strong enhancement of ET probably promoted by reopro.

Figure 3. OT values (mean±SD) at 4 pre-specified time points.



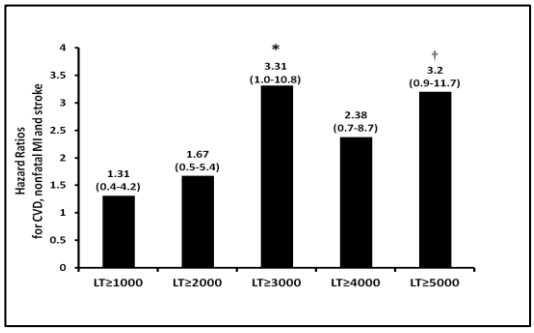
*p<0.05, GPI: glycoprotein IIb/IIIa inhibitor, OT: occlusion time. This diagram demonstrates the antithrombotic effect of dual anti-platelet regimen of aspirin and clopidogrel, if we compare average OT at baseline and 1-month post-hospital discharge tests. Additionally, it shows the strong anti-thrombotic properties of reopro, that in combination with anti-platelet agents, causes a huge increase in the average value of OT.

Figure 4. LT values (mean±SD) at 4 pre-specified time points.



*p<0.05, GPI: glycoprotein IIb/IIIa inhibitor, LT: lysis time. In this diagram we can first observe a sudden decrease in the average value of LT while on reopro. This could be explained by the enhancement of ET by reopro, causing LT reduction. Secondly, we can observe a significant increase in LT upon discontinuation, which shows that the pharmacological effect of reopro in enhancing ET is short-lasting and perhaps there is a rebound adverse effect on ET upon reopro upon discontinuation, which is not translated into any increase in event rate as per data analysis.

Figure 5. Hazard ratios (HR) for cardiovascular death, non-fatal MI, and stroke by baseline LT

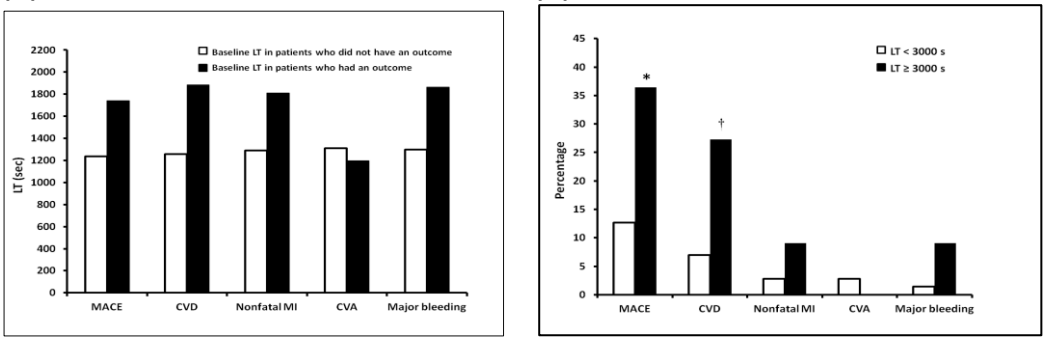


†p=0.077, * p<0.05, 95% confidence interval (CI) in brackets,

CVD: cardio-vascular death, LT: lysis time, MI: myocardial infarction. Hazard ratios for CVD, MI and stroke significantly increase for LT values equal to or higher than 3000s.

Figure 6. MACE and major bleeding according to baseline LT.

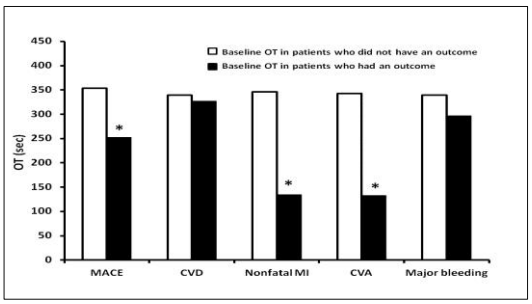
(A) (B)



*p<0.05, †p=0.051

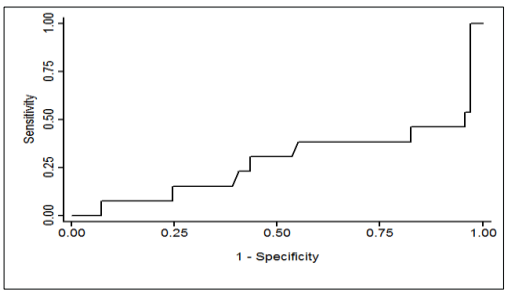
CVD: cardio-vascular death, CVA: cerebro-vascular accident, LT: lysis time, MACE: major adverse cardiac event, MI: myocardial infarction. In diagram A we can see that MACE and major bleed are related to high LT at baseline and in diagram B there is proof that LT≥3000s can predict adverse events.

Figure 7. Differences in baseline OT between patients with and without MACE and major bleeding.



* p<0.05, CVD: cardio-vascular death, CVA: cerebro-vascular accident, MACE: major adverse cardiac event, MI: myocardial infarction, OT: occlusion time. Patients with stroke and non-fatal MI had significantly lower baseline OT.

Figure 8. ROC curve for occlusion time at baseline



According to this diagram, baseline OT is not a discriminator of MACE.

Figure 9. ROC curve for lysis time at baseline.

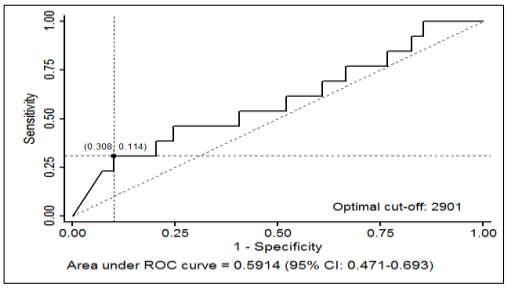
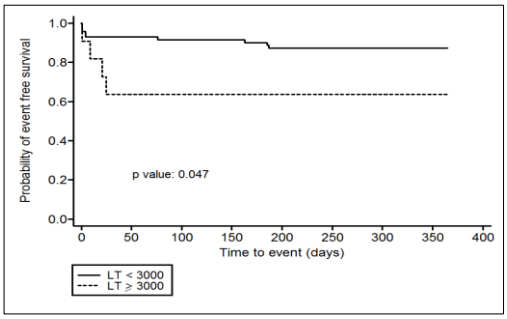


Figure 9, demonstrating that baseline LT is indeed a discriminator of MACE, with an optimal cut-off value of 2901s.

Figure 10. Probability of event-free survival in STEMI patients based on baseline LT.



Thrombolytic status was strongly predictive to MACE, with baseline LT≥3000s being associated with HR of 3.31. Solid line indicates LT<3000s and dotted line indicated LT≥3000s.

Discussion

Previous studies of global thrombosis have highlighted the role of impaired ET in predicting increased risk of adverse cardiovascular events in patients with ischaemic heart disease. Our study has proven this once more, and in particular that impaired ET is predictive of cardiovascular death within one year from PPCI. We also showed that cut-off point of LT that predicts MACE is a value equal or higher than 3000s, which coincides with the results of previous studies of global thrombosis. However, we have also proven that enhanced ET leading to spontaneous coronary reperfusion and featured by short LT, complete or partial ECG ST-segment resolution and TIMI-3 coronary flow prior to intervention, is predictive of good post-PPCI outcomes, as described more extensively in another chapter. Furthermore, enhanced thrombogenicity, in the form of short OT, was associated with recurrent adverse events, such as non-fatal MI and ischaemic stroke.

DAPT with aspirin and clopidogrel, a pharmacological regimen extensively used worldwide, including the UK, for the prevention of stent thrombosis, has no effect on endogenous thrombolysis. On the other hand, it causes a long-term reduction in thrombogenicity, as demonstrated by prolongation of OT during the 1-month follow-up testing.

Usage of GPI in the acute phase, results in both prolongation of OT and reduction in LT, however as the medication is only given once during the PPCI and its effect wears off with time, it is expected to observe OT to slightly shorten, because DAPT still offers some prolongation of OT, and LT to return to baseline, because DAPT does not cause any reduction in the LT.

Another interesting finding was the association of baseline LT≥3000s and female sex in STEMI patients, and given the fact that a previous assessment of global thrombosis status in healthy volunteers did not show any difference in LT between males and females, it is interesting for further studies to investigate possible tendency for delayed ET in women with STEMI, thus increasing their mortality risk compared to male patients.

Despite the relationship between creatinine and LT≥3000s, there is no evidence in this study that this blood marker is related to future adverse events. According to the results of previous studies of GTT, delayed ET predicts future adverse events in patients with cardiovascular disease and end-stage renal failure. However, in this study, creatinine levels were very high given the degree of renal impairment, which is not the case in our study.
Therefore, the validity of our finding regarding creatinine, is questionable.

The main limitation of the study is the small sample size. Also, due to the fact that several clinically unwell patients, such as patients with cardiogenic shock, were excluded from the study, as they were not able to provide us with an informed consent, we missed a great opportunity to assess global thrombosis in patients at potentially high risk of MACE. Although when in hospital patients' compliance with medication is documented on a daily basis, it is impossible to confirm this upon hospital discharge, therefore during the 1-month follow-up GTT, results may not necessarily reflect the actual effect of DAPT. Of course this issue should be alleviated statistically with a significantly greater number of recruited patients.

There are several other factors, potentially able to affect GTT results. The timing of aspirin/clopidogrel loading dose administration before GTT sampling was never documented, therefore it is not possible to know whether this had any effect on either OT/LT at baseline. Also we are unsure whether flushing of the arterial sheath with saline had any effect on the GTT results. Furthermore, in the absence of previous results of global thrombosis comparing samples from different vascular sites (arterial and venous), it is not possible to know about possible impact of this on the GTT results. In this study, with the exception of the baseline samples and in some occasions of the second sample (while on reopro), all the remaining samples were venous. Additionally, as variations in platelet reactivity between radial and femoral arterial blood has never been tested before, it is possible that not using the same arterial access for every study patient undergoing PPCI, may have affected GTT results as well. Finally, blood sample handling can affect the quality of the test, however

in this study there is not such an issue, as the majority of blood sample handling was performed by one operator.

More studies are necessary to be carried out in order to investigate for more pharmacological agents exhibiting significant effects on global thrombosis status, thus serving their purpose of secondary prevention of coronary thrombotic events. The role of PAR-1 inhibitor vorapaxar in enhancing endogenous thrombolysis will be discussed in another chapter, however there are plenty of other anti-thrombotic agents currently tested in patients with ischaemic heart disease, such as the novel oral anticoagulants. Finally, the GTT which has provided us with consistent results in several studies of global thrombosis, could also be used for risk stratification purposes, in order to target high risk patients, in whom more aggressive pharmacological treatment would be necessary and conversely to prevent us from administering unnecessary anticoagulation to low risk patients.

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CHAPTER 2

Investigation of the relationship between ST-segment resolution and global thrombosis

Background

According to the European Society of Cardiology/ACCF/AHA/
World Heart Federation Task Force for the Universal Definition of Myocardial Infarction, diagnostic ST-segment elevation, is defined as 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 (0.1mV) in other contiguous chest leads or the limb leads, in the absence of left ventricular hypertrophy or Left bundle branch block (LBBB) (O'Gara et al., 2013).

The diagnosis of acute myocardial infarction requiring PPCI is made upon detection of acute onset of symptoms such as chest pain, dyspnea, dizziness, sweating, in the presence of either new ST-segment elevation as described above or new LBBB documented on electrocardiogram (O'Gara et al., 2013). In the majority of cases ECGs are performed by paramedics out of hospital, who are adequately trained to activate the PPCI pathway once STEMI criteria are fulfilled. After successful coronary revascularization (or historically, thrombolysis) there is resolution of ST segment elevation and normalization of the ST segments.

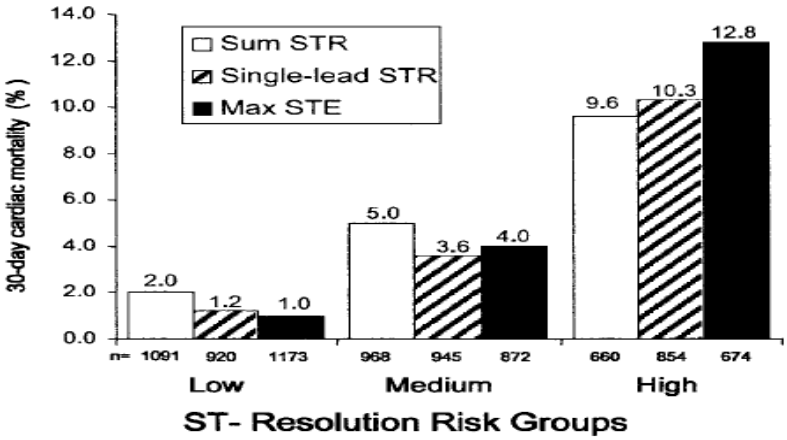
The importance of ST-segment resolution (STR) following coronary reperfusion with either thrombolysis or PPCI has been highlighted in several articles and the role of ST-monitoring in STEMI patients has been found to be very important for very early risk stratification in these patients (Johanson et al., 2003). Furthermore, the extent of STR has been linked to the size of infarct, as well as to the risk of early mortality. In specific, it was found that complete STR was associated with small infarcts and low early mortality rates, whereas no STR was associated with large infarcts and high early mortality rates. Partial STR was associated with large infarcts, but low early mortality rates (R Schröder, Wegscheider, Schröder, Dissmann, & Meyer-Sabellek, 1995).

In 2004, Rolf Schroder published a paper about prognosis in STEMI, based on the extent of early STR. According to this paper, there are three different ways

in assessing STR. The first method, sum STR, refers to resolution of the sum of ST-segment elevation, following reperfusion with thrombolysis or PPCI, and is used to predict infarct size, left ventricular function, epicardial vessel patency and mortality. Sum STR is calculated by measuring all ST-segment elevations in leads relevant to the infarct location. It is expressed as the percentage from baseline (Rolf Schröder, 2004).

The second method is the Single-lead STR, which is measured by identifying the ECG lead with the most prominent ST-segment deviation between baseline and a given point of time following coronary reperfusion (Rolf Schröder, 2004). The final method of measuring STR is Max STE, which is the existing ST-segment deviation at a given time and it is measured as per Single-lead STR, but it is not compared with baseline ST-segment deviation (Rolf Schröder, 2004). According to the same paper, in order to define mortality risk groups, STR was categorised according to the extend of ST-resolution, namely as *complete* (>70%), *partial* (30-70%) and *no resolution* (<30%) (Rolf Schröder, 2004). In single-lead STR, the cutoff for complete STR was defined as resolution of >70% for both anterior and inferior STEMI, whereas no resolution was defined as resolution of <50% in anterior STEMIs and <20% in inferior STEMIs. The following table illustrates the relationship between 30-day mortality rates against STR groups, with ECG recorded 90 minutes in 2719 patients of the InTIME II Study (Rolf Schröder, 2004):

Figure 1. 30-day mortality vs STR groups (Rolf Schröder, 2004)



In literature, there is some evidence that STR can also occur spontaneously, which is usually associated with good future outcomes (Search et al. 2016)(Bainey et al. 2008). Even though it is believed that this phenomenon occurs due to spontaneous recanalization of an acutely occluded coronary artery, it is still unclear what the exact mechanism is.

Aims

We aimed to assess the relationship between global thrombosis status and spontaneous ST-segment resolution. Our hypothesis was that patients with spontaneous STR may have more effective endogenous thrombolytic pathways, enabling coronary thrombus lysis, with subsequent coronary reperfusion and STR. In addition to that, we also aimed to show that there is an association between spontaneous STR and low early mortality rates following STEMI.

Methods

For patients with evidence of STR, we compared the ECG on arrival at our unit with the ambulance ECG or the one done earlier in the A+E department. We then correlated this with OT and LT and angiographic findings.

Of the five cases with spontaneous ST-segment resolution, only four were found to be suitable for PPCI, because one of them was an elderly patient with terminal cancer, who was completely asymptomatic on arrival at the cath lab, with completely resolved electrocardiographic changes and not wishing to have

an invasive procedure. The remaining four patients underwent coronary angiography. TIMI flow was assessed during coronary angiography using TIMI flow classification, as illustrated in Table 1:

Table 1. Definition of TIMI flow (D a Morrow et al., 2000)

TIMI Grade	**Description**
TIMI 0- no perfusion	No anterograde flow beyond the point of occlusion
TIMI 1- penetration without perfusion	Faint anterograde coronary flow beyond the occlusion, with incomplete filling of the distal coronary bed
TIMI 2- partial perfusion	Delayed or sluggish anterograde flow with complete filling of the distal territory
TIMI 3- complete perfusion	Normal flow with complete filling of the distal territory

In order to assess ST-segment resolution we used the Sum STR method, measuring all ST-segment elevations in leads relevant to the infarct location. We elected to choose this method, as we felt that it would be more accurate to diagnose ST-segment resolution by taking into account ST-segment changes occurring in more than one leads.

Results

Overall we had five patients with ST-segment resolution upon arrival at our cardiac centre, all of them associated with either complete or partial clinical symptom resolution. However, the most interesting finding was the short LT (<1000s), observed in all five cases.

In patients with spontaneous ST-segment resolution, OT was no different to patients without spontaneous coronary reperfusion (306+/-156s vs 340+/-140s, p=0.609). Lysis time was inversely correlated with spontaneous coronary reperfusion before PPCI (r=-0.3, p=0.022).

The following four images illustrate ST-segment elevation seen initially in two

of our study patients with STEMI, followed by spontaneous resolution of electrocardiographic changes on both occasions:

Figure 2. Ambulance ECG (patient 1)

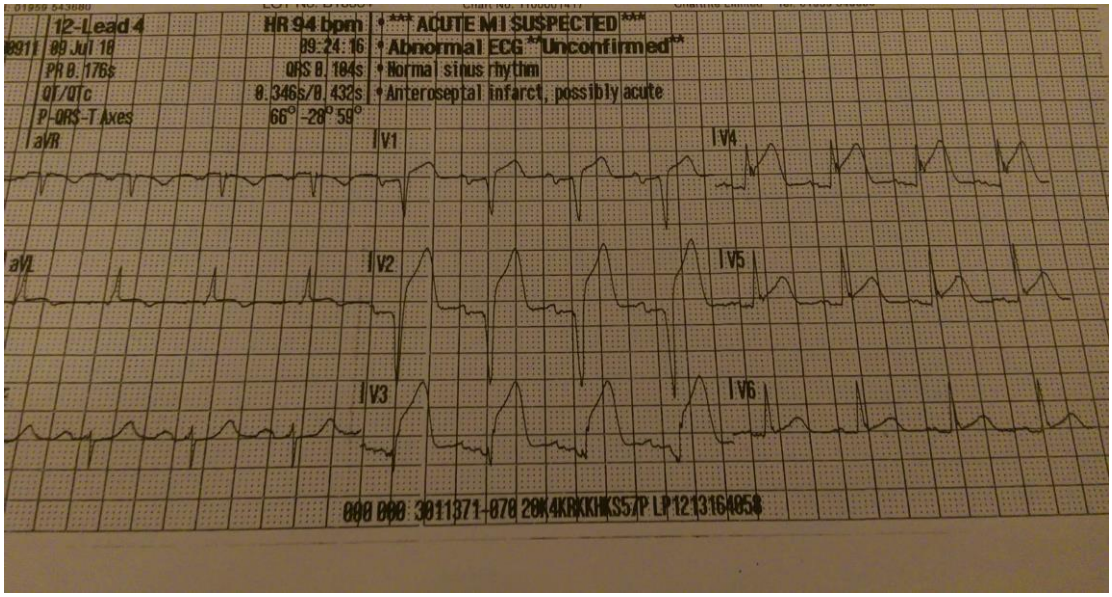


Figure 3. Pre-PPCI ECG in the cath lab, 34 minutes after initial ambulance ECG (patient 1).

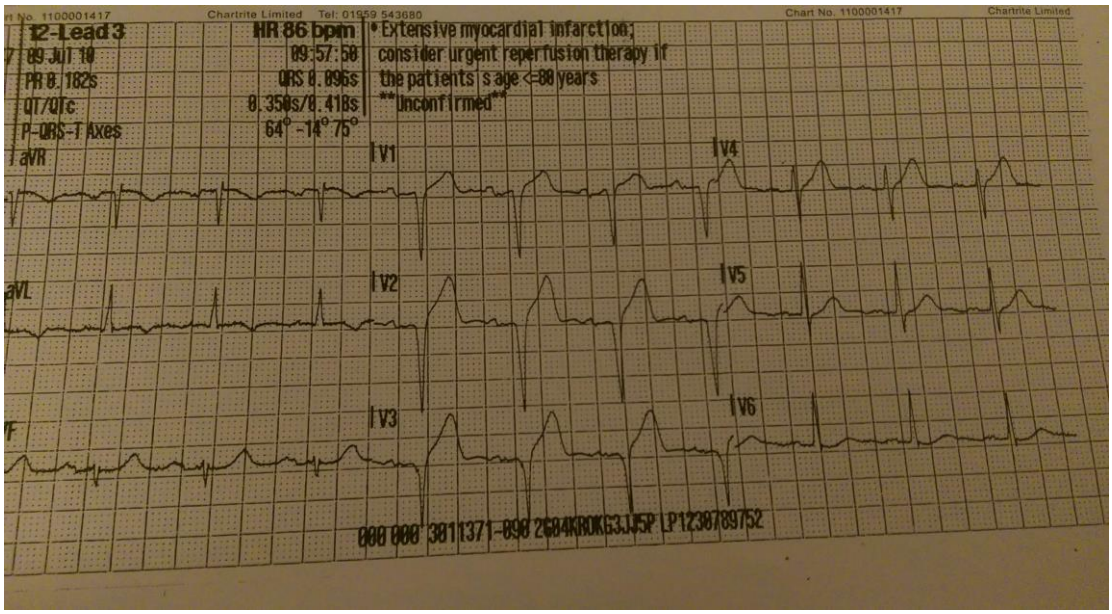


Figure 4. Ambulance ECG (patient 2)

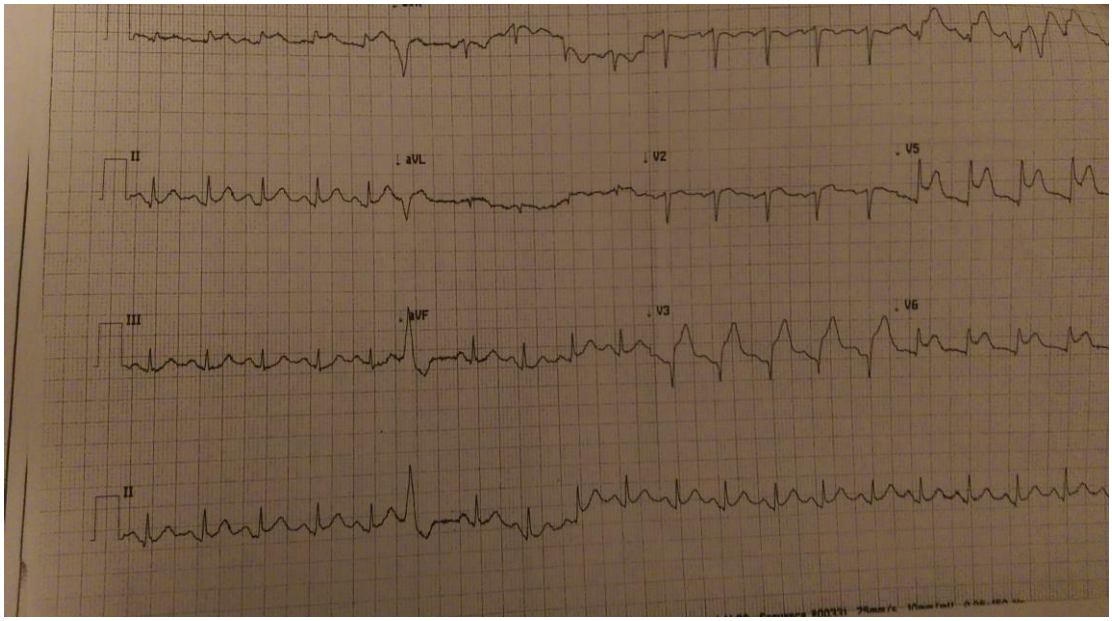
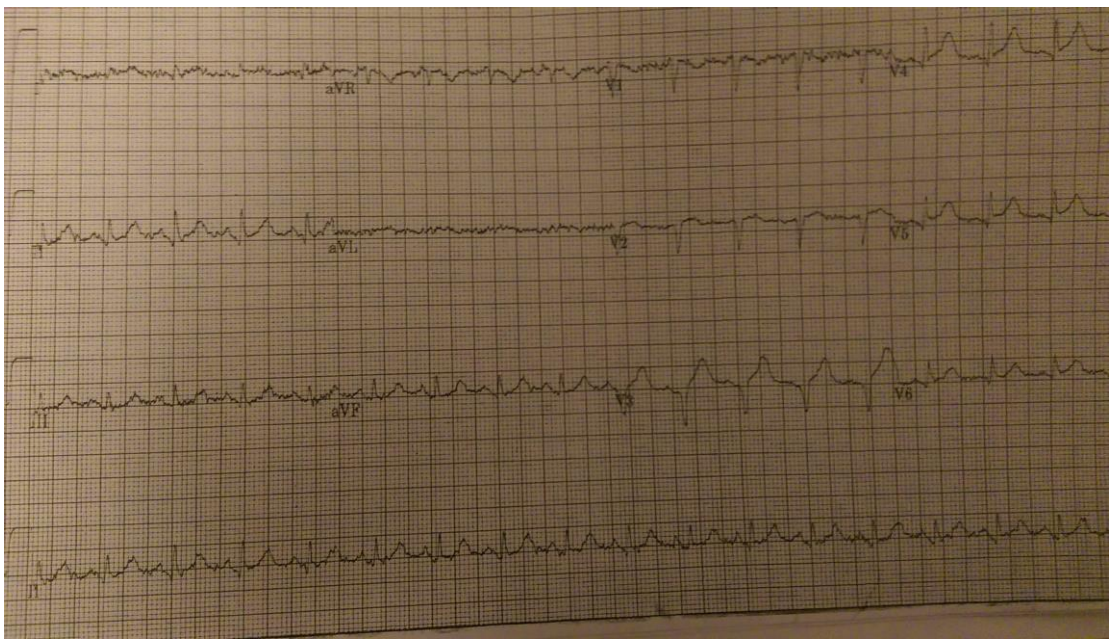


Figure 5. Pre-PPCI ECG in the cath lab, 65 minutes after initial ambulance ECG (patient 2).



Three out of four of our study patients with spontaneous STR, had TIMI 3 flow in their culprit vessel and one of them had TIMI 0 flow in the culprit vessel at angiography, before any coronary intervention. In comparison, in the patients from the PPCI group (discussed in chapter 1) without spontaneous STR, 14% had TIMI 3 flow, 4% had TIMI 2 flow, 11% had TIMI 1 flow and 70% had TIMI 0 flow at presentation.

Out of these four patients, one had unobstructed coronaries and did not require intervention. The remaining three patients had successful PPCI of their culprit vessel with DES.

All five patients had uncomplicated hospitalization and had no events during the 1-year follow-up of our protocol.
There was a total of 28 other patients who had LT<1000s, but without spontaneous ST-segment resolution and of these patients, 14% had TIMI 3 flow, 0% had TIMI 2 flow, 14% had TIMI 1 flow and 71% had TIMI 0 flow at presentation.

Discussion

Three out of four patients had TIMI 3 flow at presentation, probably due to spontaneous coronary reperfusion, however this could well be coincidental due to the very small number of patients demonstrating STR. Based on the history and ECG changes, they clearly had transient coronary occlusion, which had resolved by the time of arrival and angiography. Although coronary spasm could be a possible explanation, acute coronary thrombosis is a more likely cause of the initial ST-segment elevations.
All five patients had prolonged OT. This reflects reduced platelet activation. This in combination with the short LT, reflecting enhanced endogenous thrombolysis (ET) observed in the same patients, may be the reason for spontaneous coronary reperfusion and subsequently ST-segment/symptom resolution. Previous studies have shown no relationship between OT and major adverse cardiac event (MACE), even in cases of short OT (<200s). Furthermore, there has been no previous association between prolonged LT/short OT and MACE either. However, previous studies of ET (Saraf et al.,

2010) (Sharma et al., 2012) did not investigate clinically unstable patients with potentially life threatening acute arterial thrombosis, but they concentrated on stable patients with angina and NSTEMI, in whom extremes of OT/LT may have not been clinically significant. Perhaps future GTT studies in PPCI patients should investigate whether a combination of short LT/prolonged OT at baseline significantly enhances thrombus lysis, leading to spontaneous coronary reperfusion with TIMI 3 flow and subsequent STR on these patients ECGs.

In the acute setting, the role of enhanced ET in STR should not directly influence management, as there is no guarantee that TIMI III flow can be established, according to our study results. Furthermore there is data from other studies, also suggesting that angiography with a view to proceeding to PPCI should be immediately undertaken in STEMI without any delay, even if symptoms as well as ST-segment elevation have completely resolved (Sim, 2015). Therefore, we do not suggest in any way that angiography and PPCI should be delayed or avoided in case of spontaneous STR related to enhanced ET (this paragraph also answers examiner's comment regarding limitation of using STR as surrogate for TIMI 3 flow).

The main limitation of this sub-study is the very small number of patients with spontaneous STR, therefore there is still no clear explanation about this phenomenon. Global thrombosis status may indeed contribute, however other parameters not considered in this study, may also favor STR.
For example, the role of coronary collateralization has been recently suggested as a possible contributing factor to STR, in a study of 389 STEMI patients (Shen et al., n.d.). The majority of these patients had previous angina and hence CAD, leading to collateral vessel formation to support perfusion to the culprit vessel territory.

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CHAPTER 3

Does delayed ET increase the chance of peri-procedural complications in PPCI patients with prolonged door-to-balloon time (DTB)?

In early ST-elevation MI (STEMI), the formation of occlusive thrombus composed of platelets and fibrin is a fast evolving process. Specifically, there is a dramatic change in the percentage of these two main constituents, from an initial significantly higher percentage of platelet compared to fibrin, to a later lowering of platelet content and simultaneous abundance in fibrin, making the thrombus more difficult to dissolve (Blankenship et al., 2011). This process is powerful enough to determine the outcome following STEMI, and the effectiveness of treatment with coronary revascularisation with primary percutaneous coronary intervention (PPCI) and in some rare occasions nowadays with thrombolysis. In the year 2000, the National Service Framework for Coronary Heart disease, emphasised the importance of pre-hospital thrombolysis (PHT) and the standard set out at the time was that STEMI patients ought to have thrombolytic agent administered intravenously within 60 minutes of calling for professional help. Alternatively, thrombolysis could be given soon after admission to the accident and emergency department (De Belder et al., 2008). In 2002 some cardiac units in the UK set up a PPCI service and since then this has developed to an extent, that it has now completely replaced PHT, since data from several studies worldwide has shown superiority of PPCI over thrombolysis. Firstly, PPCI is associated with reduced mortality and improved long-term outcome compared to thrombolysis, even when both are undertaken within a similar timeframe. Secondly, PPCI is associated with a lower risk of bleeding, stroke and coronary thrombosis, than thrombolysis. Importantly, after thrombolysis, the atherosclerotic lesion that predisposed the patient to thrombosis, still persists and thus the risk of recurrent infarction continues. In contrast, PPCI alleviates the coronary stenosis, allowing normal flow and reducing markedly the risk of re-occlusion due to thrombosis. Also more patients are suitable for PPCI than they are for thrombolysis, as there is a list of several major contraindications for the latter:

Table 1. Major contraindications for thrombolysis in STEMI (De Belder et al., 2008).

Any previous history of haemorrhagic stroke
History of stroke, dementia or central nervous system damage within 1 year
Head trauma or brain surgery within 6 months
Known intracranial neoplasm
Suspected aortic dissection
Internal bleeding within 6 weeks
Active bleeding or known bleeding disorder
Major surgery, trauma or bleeding within 6 weeks
Traumatic cardiopulmonary resuscitation within 3 weeks

Finally, PPCI enables patients to have early treatment of the culprit coronary lesion and hospital discharge within 2-3 days from hospital admission, which is beneficial for both the patient from a psychological point of view, as well as for the NHS from a financial point of view. Supporting data for call-to-balloon times (CTB) in relation to patient follow-up firstly showed that mortality at all periods was lower when CTB time was shorter. Additionally for the cohort of patients with CTB time less than an hour, there was no later mortality. Finally it was noticed that as CTB time exceeded 120 minutes, mortality was noticeably diverged (De Belder et al., 2008).

Table 2. Demonstrating mortality related to CTB time (De Belder et al., 2008)

CTB time (minutes)	**In-hospital mortality (%)**	**30 days mortality (%)**	**1 year mortality (%)**
60-120	2.7	2.9	5.1
120-180	4.5	4.9	8.7
>180	11.4	12.2	15.9

According to the national guidance for the treatment of myocardial infarction, it is crucial for STEMI patients to undergo urgent PPCI, as randomised trials and registries have shown increasing mortality as times to treatment lengthen (De Belder et al., 2008).

Cardiac centres in the UK ought to achieve CTB times within 120 minutes and DTB times within 90 minutes, irrespective of the time or the day and regardless of their geographical location. Furthermore their performance should be audited

on a yearly basis to confirm national standards are achieved (De Belder et al., 2008).

If a thrombus is formed in a coronary artery, the natural defenses of enzymes which are responsible for endogenous thrombolysis, in particular t-PA released from activated platelets, should help dissolve that thrombus. If this natural defense mechanism is either overwhelmed or inefficient, the thrombus will persist in blocking the vessel and will worsen the outcome in patients with STEMI, since the persisting and stable thrombus will continue impairing myocardial perfusion. Relatively higher percentage of fibrin content within the thrombus and delay in coronary revascularization, could lead to prolongation of myocardial ischaemia time, reducing chance of myocardial salvage, with consequently more myocardial loss through infarction, heart failure, higher risk of arrhythmias and death. Inability to establish good distal flow and myocardial reperfusion, could predispose to a higher incidence of peri-procedural complications such as bleeding, coronary dissection, acute renal failure due to higher dose of contrast administration, exposure to higher doses of radiation (rare occasions of burns have been reported) (Blankenship et al., 2011).

Previous studies have investigated the relationship between DTB/CTB times and future outcomes in STEMI patients undergoing PPCI, however it is not known how time from onset of infarction or delay in treatment would be reflected in thrombotic status. Therefore, demonstrating that short DTB/CTB times, as well as short LT and/or prolonged OT favor good future outcomes and vice versa, would be an exciting finding, as it would be additional evidence supporting the great importance of ET in ischaemic heart disease (IHD).

Aims

We aimed to assess the relationship between time from onset of infarction and onset of treatment and relate this to thrombotic status.

Methods

In order to investigate the role of endogenous thrombolysis in the clinical outcomes of STEMI patients, in combination with the timing of PPCI, we documented PTB time, CTB time and DTB time for all patients participating in our study.

Call-to-balloon time refers to the time between the call for ambulance help or medical assistance until balloon dilation (or other device use, such as thrombus aspiration device) achieving coronary reperfusion, and it is measured in minutes (De Belder et al., 2008).

Door-to-balloon time is the time elapsing from arrival to the cardiac centre until balloon dilation (or other device use, such as thrombus aspiration device) achieving coronary reperfusion, and it is measured in minutes (De Belder et al., 2008).

Pain-to-balloon time (PTB time) is the time from the onset of symptoms until balloon dilation (or other device use, such as thrombus aspiration device) achieving coronary reperfusion, and it is measured in minutes.

We assessed the relationship between thrombotic status (OT and LT) and CTB, DTB and PTB times using the following statistical methods. We also assessed the relationship between these times and outcomes (major adverse events).

Results

The total number of our PPCI patients was 82, with 71 of them having LT≤3000s and 11 of them LT>3000s. Neither DTB nor CTB times were related to lysis times. DTB was not related to MACE, even in the group with prolonged LT.

Table 3. Comparing DTB and CTB times against LT

	Overall Group (n=82)	Baseline LT <3000s (n=71)	Baseline LT ≥3000s (n=11)	p Value
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

We then investigated the influence of CTB time on events. Again there was no relationship between CTB time and events in any of the two LT groups.

DTB and CTB times were not predictors of MACE in any OT group either, and there are several reasons to explain this. Firstly, due to the very short DTB and CTB times which reflect prompt coronary revascularisation, it was expected to observe very few complications/events, as previously discussed.

As CTB time does not always reflect the time from the onset of STEMI symptoms and because we observed a consistent delay in calling for professional help in several of our study patients, we introduced the parameter of PTB time.

Discussion

DTB was not related to MACE, even in the group with prolonged LT. This could be explained primarily by the fact that DTB time seen in every single case was much shorter than the target suggested in the paper mentioned earlier, as well as by the small number of subjects included in the study. It could also be explained by the fact that patients with widely varying durations of infarction (PTB, CTB), could have similar DTB times.

Therefore in our study with mean DTB time 23+/-16 minutes in the short LT group and 26+/-14 minutes in the prolonged LT group, it would be highly unlikely to observe statistically significant events.

The lack of relationship between CTB time and LT could in part be explained by the short CTB times seen in both groups (100+/-19 minutes and 98+/-14 minutes). Furthermore the small sample size is another reason for not observing a relationship with MACE, even in the prolonged LT group.

It is possible that some patients are markedly pro-thrombotic and refractory to endogenous lysis, and that this prolonged lysis time measured in our study, is not related to time from onset of pain/infarction. Although we would have

expected that as the time from infarction (and before PPCI) lengthens the fibrin content of the thrombus increases and this would be reflected in more prolonged lysis time, it is possible that the small sample size was responsible for the fact that we were not able to measure this.

Despite the fact that short DTB is required in order to reduce the risk of peri-procedural complications in PPCI as previously explained, PTB is far more important, as it measures the time from the onset of the coronary event until time of re-perfusion with balloon. Unfortunately, this cannot always be accurate, because we entirely rely on patient's memory of the event. Therefore, collateral history from patient's friends or relatives present during the onset of the symptoms is essential for obtaining a more precise documentation of the onset of symptoms.

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CHAPTER 4

Can Global thrombosis status predict future outcomes in patients undergoing elective or urgent PCI?

Background

Ischaemic heart disease (IHD) is the leading cause of death in the US and the UK, with one coronary event occurring every 25 seconds, from which 34% dying within the same year, that is one death every minute (Kones, 2011).

Furthermore, it is estimated that every year there are 6.2 million hospitalisations in the US for cardiovascular disease (CVD) and 7.2 million cardiac and vascular procedures are performed, consuming 17% of the national health budget (Kones, 2011). Although the death rate related to IHD has fallen over the last years due to prevention and treatment strategies being implemented, the prevalence of risk factors, such as obesity and diabetes has reached alarming levels, not favoring further improvement in related morbidity and mortality. Therefore, more efforts should be made for lowering the prevalence of risk factors, in order to achieve lower cardiovascular related mortality rates (Kones, 2011).

Risk factors are divided into modifiable and non-modifiable. Non-modifiable factors include age, gender and genetics. Modifiable factors comprise smoking, diabetes, hypertension, obesity and dyslipidemia. There is also emerging evidence, emphasizing the importance of novel risk factors of IHD, such as C-reactive protein (CRP), triglycerides, dense LDL, lipoprotein-associated phospholipase A2 (Lp-PLA2), interleukin-6 (IL-6) and others. Complex interactions between genetic and environmental factors, influence the process of atherosclerosis throughout life, determining the likelihood of adverse coronary events (Kones, 2011).

Primary prevention of CVD in patients with risk factors present, refers to preventing or delaying a first coronary event in these patients. This can be achieved with lifestyle changes, with or without administration of pharmacotherapy. However, in patients with diagnosed significant CAD, in whom the risk of adverse coronary event is high, treatment with primary percutaneous intervention (PCI) and additional pharmacotherapy is indicated,

in the form of statins for hypercholesterolemia, ACE inhibitors for hypertension and dual antiplatelet therapy for stent protection (Kones, 2011).

Secondary prevention, seeks to prevent or limit recurrence of ischaemic coronary events (Kones, 2011).

Between the two, the role of Primary prevention is far more important, yet more complex for implementation, because despite great efforts from different authorities in educating people regarding this issue, it is difficult to interfere and change social and cultural phenomena that prevent people from adopting healthy lifestyles. For example, coping with stress, smoking and dietary habits are not easy to be tackled in western societies, therefore the role of educating citizens about lifestyle modifications, should be undertaken by even more authorities, that should also try to discover different and more efficient strategies, in order to positively influence people. For instance, the role of educating public regarding non-healthy dietary habits, a major cause of obesity and subsequently diabetes and CVD, should be undertaken by even more authorities, such as education authorities (schools, universities), government departments and agencies, local authorities, NHS organisations, prison services and the armed forces.

The role of NHS primary care is very important, particularly with regards to primary prevention. General practitioners are responsible for identifying patients at risk of developing CVD and for taking appropriate measures, according to primary prevention strategies, as set out by the National institute of clinical excellence (NICE). The role of secondary care (hospital doctors), which is the focus of this chapter, is to investigate high risk patients referred by primary care, for presence of CAD in one hand, and treat patients with diagnosed coronary events on the other hand. Investigations of patients suffering with possible angina, includes non-invasive imaging with a variety of modalities, such as myocardial perfusion scan, stress echocardiography, CT coronary angiography and cardiac MRI scans or invasive investigation, such as coronary angiography, which is the gold standard of investigations for detection of coronary artery disease (Kones, 2011). With the aid of coronary angiography, clinicians can diagnose CAD and its severity, enabling them to decide about mode of management for each patient. The first option is to treat patients

conservatively with pharmacotherapy, targeting modifiable risk factors, not optimally treated by general practitioners. In this occasion, pharmacotherapy for diabetes, hypertension, hypercholesterolemia can be either initiated or up-titrated by clinicians. Furthermore, usage of anti-platelet agents in the form of aspirin is indicated, particularly in those with diagnosed CAD (Boon et al., 2014).

On the other hand, upon diagnosis of significant CAD, obstructing coronary flow and subsequently impairing myocardial perfusion, usage of pharmacotherapy, including agents previously mentioned, as well as anti-anginal agents (beta-blockers, calcium channel blockers, nitrates and nicorandil), should be adjuvant therapy to PCI (Boon et al. 2014). Following PCI, patients should initiate dual anti-platelet therapy, usually with aspirin and clopidogrel, among others targeting risk factors. DAPT should continue for 12 months, at which point clopidogrel should be stopped and aspirin to continue being administered for life (Cheng, 2013).

In a previous chapter we discussed the treatment in patients diagnosed with ST-elevation myocardial infarction (STEMI). In this chapter we will discuss the treatment of patients diagnosed with non ST-elevation MI (NSTEMI). A diagnosis of NSTEMI is accepted when, in the absence of ST-segment elevation, ischemic ST-segment or T-wave changes are present for at least 24 hours with positive cardiac enzymes (troponin) or a typical clinical presentation, or both (McManus et al., 2011). It represents partial obstruction of one of the main coronary arteries or complete occlusion of one of the minor coronary arteries. In the absence of ischaemic ECG changes, a diagnosis of NSTEMI is also acceptable, in the presence of positive cardiac enzymes and relevant clinical presentation (McManus et al., 2011). Upon making diagnosis of NSTEMI, patients should be treated with a loading dose of aspirin and a second anti-platelet agent, such as clopidogrel, ticagrelor or prasugrel (Roffi et al. 2015). Several trials have investigated the correct timing of coronary intervention (TIMACS/ABOARD/ISAR-COOL), with the general consensus being that this is better to be undertaken within 24 hours into hospitalization (Jneid et al., 2012). Following PCI, patients are advised to continue with DAPT

for 12 months and from then on with aspirin monotherapy for life (Roffi et al. 2015).

Despite introduction of new types of stents, improved stent insertion technologies and more effective anti-platelet regimens, stent thrombosis (ST) leading to MI is still possible, with estimated incidence between 1-5%. Based on the timing of its occurrence, ST can be classified as early when it occurs within 30 days from stent implantation, late when it occurs between 1 to 12 months post-implantation and very late when it occurs after 12 months post-implantation (Council & Vol, 2007). In a large Dutch study of 21,009 patients treated with either drug-eluding stent (DES) or bare-metal stent (BMS), it was shown that important determinants of this complication include anti-platelet non-compliance or early discontinuation, under-sizing of coronary stent during PCI, presence of moderate CAD proximal to the culprit lesion left untreated and co-existing malignant disease (van Werkum et al., 2009). Furthermore, the incidence of ST in patients undergoing elective PCI for angina, was lower than for those with ACS, undergoing urgent PCI and patients with STEMI undergoing PPCI demonstrated the highest incidence of ST. Also, STEMI patients experienced early ST more often than late or very late ST, compared to angina and ACS patients that demonstrated higher proportion of late and very late ST (van Werkum et al., 2009). In another paper, publishing the combined data from 9 studies investigating predictors of ST, including 153,350 patients with 2495 cases of ST, the commonest predictors of ST were the presence of diabetes mellitus, left ventricular dysfunction confirmed with echocardiography, ACS at admission, total stent number or stent length, anti-platelet therapy discontinuation before 30 days from stent implantation and extend of coronary disease (D'Ascenzo et al., 2013). However, even more risk factors are involved in the process of this catastrophic complication, that are listed in the following three tables:

Table 1. Procedure and lesion related parameters in relation to ST (Council & Vol, 2007).

1. Use of multiple stents
2. Small vessel diameter
3. Coronary dissection
4. Geographic miss
5. Slow coronary flow
6. Long coronary artery lesions
7. Stent malapposition
8. Stent underexpansion
9. Stent design (type of polymer and strut thickness)
10. Bifurcation lesions

Table 2. Patient characteristics in relation to ST (Council & Vol, 2007).

1. Diabetes mellitus
2. ACS (especially STEMI)
3. Left ventricular dysfunction (Heart failure)
4. Renal impairment
5. Advanced age
6. Platelet reactivity

Table 3. Antiplatelet therapy in relation to ST (Council & Vol, 2007).

1. Non-compliance
2. Premature discontinuation of antiplatelet therapy
3. Inadequate intensity of therapy (insufficient dose or antiplatelet monotherapy, instead of dual therapy)

High residual platelet reactivity (HRPR) or high on-treatment platelet reactivity (HTPR), which is one of the causes of ST, is defined as high platelet reactivity that is present within a few hours following loading dose of antiplatelet agents. According to literature, the risk of ischaemic events increases proportionally with higher values of platelet reactivity (Garabedian & Alam, 2013). However, there is still lack of an optimal method that could both accurately define HRPR and risk stratify patients.

Several studies have been carried out in order to find appropriate strategies against HRPR and subsequently ischaemic event prevention. For example, in the GRAVITAS study, the aim was to identify the effect of high dose clopidogrel

versus standard dose clopidogrel on platelet reactivity. This was a multi-center, double-blind study, enrolling 2214 patients with HTPR, 12 to 24 hours post-PCI with DES for stable CAD or following NSTEMI. In this study the Verify Now assay was used in order to assess platelet reactivity. Enrolled patients received high dose clopidogrel (loading dose of 600mg, followed by daily maintenance dose of 150mg/day) versus a placebo loading dose of clopidogrel, followed by maintenance dose of 75mg/day. Patients also received standard dose of aspirin on a daily basis. Furthermore, 586 patients without HTPR were also selected and followed-up throughout the study. Finally, all patients had two follow-up sessions (1 and 6 months from recruitment), with the VerifyNow assay being performed at each visit. The primary end-point for the study was the 6-month incidence of cardiovascular death, non-fatal MI and stent thrombosis (Garabedian & Alam, 2013). Despite the fact that this study did demonstrate significant reduction in platelet reactivity by using higher dose of clopidogrel, there was no evidence that this strategy reduces the primary end-point at 6 month follow-up. The main limitation of this study was the small number of events observed and the exclusion of STEMI patients, that represent a higher risk population (Garabedian & Alam, 2013).

Another study by Bonello et al, investigating platelet reactivity, aimed to show that tailored loading doses of clopidogrel could control residual platelet reactivity (Garabedian & Alam, 2013). This study confirmed a reduction in the rate of major adverse cardiac events and stent thrombosis in the short term, without increasing the risk of bleeding, however further studies need to be carried out in order to provide us with information regarding any long term benefits resulting from implementation of this strategy (Garabedian & Alam, 2013). Therefore more and larger-scale studies should be carried out in order to further investigate platelet reactivity, by exploring for more accurate ways in measuring it and identifying patients at high risk of thrombotic and bleeding events.

With enough evidence about the limitations of clopidogrel therapy due to platelet resistance, leading to recurrence of life-threatening ischaemic events, researchers moved their focus in developing new antiplatelet agents, aiming to achieve faster and more efficient platelet inhibition. In particular, ticagrelor, a

P2Y12 inhibitor that prevents ADP binding in order to prevent platelet aggregation, it has shown superiority compared to clopidogrel, when used in patients with stable CAD, as well as in patients with ACS, with or without ST-elevation, undergoing PCI (Garabedian & Alam, 2013). Firstly it does not require metabolic activation for manifesting its effects. Secondly it has shown greater inhibitory effect on platelets following 180mg loading dose, when compared to a 600mg loading dose administration of clopidogrel. Finally it has been shown that this medication is metabolized faster than clopidogrel, with its inhibitory effects being weaned off faster than that of clopidogrel (Garabedian & Alam, 2013). In the NSTEMI sub-group of the PLATO trial (18,624 participants), evaluating the efficacy and safety of ticagrelor against that of clopidogrel in NSTEMI patients, it was shown that usage of ticagrelor significantly reduces the rate of death related to vascular causes, MI or stroke, without increasing the overall risk of major bleeding. However, with ticagrelor therapy, higher rate of non-procedure related bleeding was observed (Lindholm et al., 2014). Additionally, in one of PLATO sub-studies, higher platelet reactivity was seen more frequently in the clopidogrel sub-group, following loading dose administration, as well as during maintenance dose, proving that greater platelet inhibition is achieved with ticagrelor (Garabedian & Alam, 2013).

Apart from searching for the best antiplatelet regimen following PCI, selection of an appropriate type of stent is also very important in reducing the risk of platelet resistance. New types of stents have been introduced in the market and more are still being tested, with a view to investigate for combinations of polymers and cytotoxic drug coatings that will affect vascular inflammation and tissue factor activity, which are determinants of thrombosis, as discussed in a previous chapter. For example stents with NO-donors could prevent platelet aggregation by decreasing platelet adhesion. Additionally, CD3 antibodies present in coatings, could prevent thrombosis by promoting stent endothelialisation via capturing circulating endothelial progenitor cells. Also, usage of biodegradable stents could reduce the incidence of late and very late stent thrombosis (Council & Vol, 2007).

Several platelet function tests (PFTs) have been used in clinical trials, however none of them has ever been very efficient in serving its purpose of accurately identifying patients at high risk of thrombosis or bleeding. Previously used PFTs have been designed to investigate platelet response to specific agonists, however thrombosis is a complex pathophysiological process, therefore other important determinants of thrombosis, such as shear stress and thrombin generation were not assessed at the time, thus failing to fully assess individual thrombosis status and its role in IHD (Gorog & Fuster, 2013). Apart from individual thrombosis status which has never been adequately investigated, the role of endogenous thrombolysis (ET) status had never been investigated either until recently, when published results from two studies showed consistent evidence that ET predicts future cardiac events in ACS and end-stage renal impairment patients respectively (Saraf et al., 2010) (Sharma et al., 2012). Assessment of both individual thrombosis and ET status (global thrombosis status) was performed using the global thrombosis test (GTT). Following discussion about the contribution of global thrombosis assessment in STEMI patients undergoing PPCI in a previous chapter, it would also be very important to investigate its role in patients with stable CAD and NSTEMI.

Aims

Primarily we aimed to demonstrate once more that ET predicts future adverse events following PCI, however we would also like to investigate which clinical or other patient characteristics influence individual ET status. We also aimed to similarly investigate individual thrombotic status, with particular interest in the possibility of this predicting future adverse events following PCI, something that was not demonstrated on previous studies of global thrombosis.

Methods

Study design This was a prospective, observational, single-centre study, that was approved by the local research ethics committee and all subjects gave written informed consent in order to participate. For this sub-study we randomly recruited patients suitable for non-emergency PCI, following diagnosis of CAD by coronary angiography. They were either patients with angina symptoms, scheduled for an elective outpatient PCI or patients with confirmed diagnosis of

NSTEMI, scheduled for urgent inpatient PCI. Diagnosis of NSTEMI was made based on symptoms of coronary ischaemia, ECG changes (absence of changes does not exclude ACS) and rise in blood troponin (biochemical marker of myocardial damage). ECG changes include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T-waves or pseudo-normalization of T- waves (Roffi et al., 2015).

Eligibility criteria All patients above the age of 18 years, with confirmed diagnosis of CAD and scheduled for PCI, able to provide us with written consent, were considered eligible for participation to our study. Patients with life expectancy of less than one year due to non-cardiac cause, patients with heparinised arterial sheath and patients administered intra-arterial heparin before obtaining initial GTT sample, were excluded for participation in the study.

Blood sampling Blood samples were taken from a 16G butterfly cannula via an antecubital vein or through the radial/femoral sheath used for PCI. When the sheath was inserted, it was initially only flushed with normal saline, the first 15ml of blood drawn were discarded, and the next 5ml sample was used for thrombotic status assessment.

Medications All patients participating had already been loaded with 300mg aspirin and 300mg of clopidogrel and were scheduled to receive 75mg of aspirin daily for life and 75mg of clopidogrel daily for 12 months. Additionally, NSTEMI patients were administered daily subcutaneous injections of low-molecular weight heparin until PCI, as part of the ACS protocol (Roffi et al., 2015).

Assessment of thrombotic and thrombolytic status Thrombotic status and endogenous thrombolytic activity were assessed using the Global Thrombosis Test, GTT (Montrose Diagnostics Ltd., UK). This novel, point-of-care assay employs native blood to assess the time taken to create a shear-induced thrombus under physiological conditions (occlusion time OT; seconds) and in the second phase of the test, measures the time to achieve endogenous thrombolysis of the thrombus created during the first phase (lysis time, LT; seconds). If lysis does not occur until 6000 seconds following OT (LT cut-off

time), “No lysis” is displayed and recorded. The coefficient of variation (cv) was assessed by testing 10 healthy volunteers twice, at 48-hour intervals.

Study end-points The primary end point of the study was occurrence of a major adverse cardiovascular event (MACE) at 1 year, defined as the composite of cardiovascular death, non-fatal MI or stroke. Secondary end-points included major bleeding as defined by GUSTO/TIMI Classification, not related to index procedure or non-cardiovascular death.

Data collection and follow-up Basic demographics were obtained during index admission from patients’ medical notes and if necessary from their primary care records. Follow-up was done either in person, or over the telephone at 1, 3, 6 and 12 months. Collection and adjudication of events was performed blinded to GTT results. Source documents for all adverse events were obtained.

Statistical analysis Analysis was performed using IBM SPSS statistics (version 23). Descriptive studies were used for calculation of standard deviation (SD) and means. Furthermore, we used this test to investigate the relationship between two linear values. Independent samples t-Test was used in order to investigate for statistically significant difference between unrelated groups and Crosstabs was used to describe the interaction between categorical values. ROC curve analysis was used to investigate for possibility of OT or LT to discriminate for events and Kaplan-Meier survival methods with log-rank tests to compare survival curves.

Results

Table 4. Baseline patient characteristics.

Risk factor	Yes/No	LT<3000s(%)	LT>3000s(%)	Pearson Chi-	P value

				Square (df)	
Diabetes mellitus	Yes	25 (19.2)	4 (18.2)	0.013	0.9
	No	105 (80.8)	18 (81.8)		
Hypertension	Yes	66 (51)	13 (59)	0.52	0.47
	No	64 (49)	9 (41)		
Smoking	Yes	29 (22.3)	2 (9.1)	2	0.155
	No	101 (77.7)	20 (90.9)		
Prior CAD	Yes	72 (55.4)	11 (50)	0.22	0.639
	No	58 (44.6)	11 (50)		
Renal insufficiency	Yes	12 (9.2)	2 (9.1)	0	0.983
	No	118 (90.8)	20 (90.9)		
PVD	Yes	2 (1.5)	1 (4.5)	0.879	0.348
	No	128 (98.5)	21 (95.5)		
Previous CVA	Yes	7 (5.4)	0 (0)	1.24	0.265
	No	123 (94.6)	22 (100)		
Previous bleeding	Yes	3 (2.3)	1 (4.5)	0.368	0.544
	No	127 (97.7)	21 (95.5)		

Significance was taken as $p<0.05$, CAD: coronary artery disease, CVA: cerebrovascular accident, PVD: peripheral vascular disease

Table 5. Medications on blood sampling.

Medication	Yes/No	LT<3000s(%)	LT>3000s(%)	Pearson Chi-Square (df)	P value
Aspirin	**Yes**	129 (99.2)	22 (100)	0.17	0.68
	No	1 (0.8)	0 (0)		
Clopidogrel	**Yes**	128 (98.5)	22 (100)	0.34	0.55

	No	2 (1.5)	0 (0)		
Statin	**Yes**	37 (84.1)	9 (100)	1.65	0.19
	No	7 (15.9)	0 (0)		
ACE inhibitor	**Yes**	99 (76.2)	17 (77.3)	0.01	0.90
	No	31 (23.8)	5 (22.7)		
Beta blocker	**Yes**	104 (80)	19 (86.4)	0.49	0.48
	No	26 (20)	3 (13.6)		
Oral nitrate	**Yes**	46 (35.4)	4 (18.2)	2.52	0.11
	No	84 (64.6)	18 (81.8)		
Warfarin	**Yes**	6 (4.6)	1 (4.5)	0	0.98
	No	124 (95.4)	21 (95.5)		
PPI	**Yes**	37 (28.5)	8 (36.4)	0.56	0.45
	No	93 (71.5)	14 (63.6)		
Nicorandil	**Yes**	11 (8.5)	1 (4.5)	0.39	0.52
	No	119 (91.5)	21 (95.5)		
CCB	**Yes**	30(23.1)	6(27.3)	0.18	0.66
	No	100(76.9)	16(72.7)		
Insulin	**Yes**	7(5.4)	1(4.5)	0.02	0.87
	No	123(94.6)	21(95.5)		
Metformin	**Yes**	11(8.5)	2(9.1)	0.01	0.92
	No	119(91.5)	20(90.9)		
Clexane	**Yes**	48(37.2)	13(59.1)	3.73	0.053
	No	81(62.8)	9(40.9)		

Significance was taken as p<0.05, ACE inhibitor: Angiotensin converting enzyme inhibitor, CCB: Calcium channel blocker

Table 6. Blood biochemistry on admission

Blood test	**LT<3000s N, mean+/-SD**	**LT>3000s N, mean+/-SD**	**t (df)**	**P**
Cholesterol (mmol/L)	(N=109), 4.25+/-1.93	(N=18), 4.34+/-1.4	-0.19 (125)	0.84
Creatinine (µmol/L)	(N=130), 112.72+/-96.72	(N=22), 100.5+/-25.1	0.58 (150)	0.55
Hb (g/L)	(N=130), 14.72+/-9.58	(N=21), 13.44+/-1.5	0.60 (149)	0.54
HCT (%)	(N=125), 0.43+/-0.25	(N=17), 0.38+/-0.5	0.76 (140)	0.44
Troponin I (ng/ml)	(N=129), 4.64+/-14.8	(N=22), 13.65+/-24.6	-2.35 (149)	0.02
Platelet count (x10^9/L)	(N=130), 236.59+/-57.5	(N=20), 253.0+/-180.2	-0.81 (148)	0.41
RBC	(N=128), 4.58+/-0.5	(N=21), 4.42+/-0.55	1.2 (147)	0.23
Urea (mmol/L)	(N=130), 6.56+/-3.2	(N=22), 6.64+/-1.9	-0.1 (150)	0.91

Significance was taken as p<0.05, SD: standard deviation, normal values: haemoglobin is 130-180 g/L in adult males and 115-165 g/L in adult females; haematocrit is 40-52% in adult males and 36-47% in adult females; platelet count is 150-400x10^9/L; creatinine is 60-110 µmol/L in adult males and 45-90 µmol/L in adult females; troponin I (at 12 hours from symptom onset) is <0.04 ng/ml; total cholesterol is ≤4.0mmol/L.

Table 7. Relationship between angiographic, interventional and echocardiographic characteristics with event occurrence.

Characteristic	**Event N (%)**	**No Event N (%)**

1-vessel disease on angiography	7 (7.9)	82 (92.1)
2-vessel disease on angiography	7 (15.6)	38 (84.4)
3-vessel disease on angiography	5 (27.8)	13 (72.2)
Stent type (BMS)	8 (15)	43 (85)
Stent type (DES)	9 (9)	88 (91)
LV function (Normal)	11 (11)	84 (89)
LV function (Mild impairment)	2 (13)	13 (87)
LV function (Moderate impairment)	3 (14)	19 (86)
LV function (Severe impairment)	3 (33)	6 (67)

Significance was taken as $p<0.05$, angiographic result: $x^2(2)=5.97$, $p=0.05$, stent type: $x^2(2)=1.48$, $p=0.47$, LV function: $x^2(3)=3.33$, $p=0.34$,
LV function: left ventricular function (on echocardiography)

In this sub-study we recruited 152 patients, the characteristics of which are outlined in tables 4-7. The extent to which patient characteristics varied by LT was evaluated. On the basis of previous publications, identifying the LT value of 3000s as cut-off value for future event prediction (Saraf et al., 2010) (Sharma et al., 2012), patients were divided into 2 groups (LT<3000s, and LT>3000s). There was unintentional male dominance, with 120 male and 32 female participants, however no statistical significance was demonstrated on estimating the proportion and number of male and female participants with regards to the two LT groups [$x^2(1)=0.128$, $p=0.721$]. Furthermore, no relationship was observed between age and LT (65+/-10 vs 64+/-11, $p=0.761$) or between any cardiovascular risk factor and LT. We then tested medications at the time of the baseline test, however no relationship was observed with LT. From all baseline biochemistry results, the only value showing statistically significant relationship with LT was the level of troponin. ACS patients with LT<3000s on GTT had a mean troponin I level of 4.6ng/ml, whereas patients with LT>3000s had mean troponin I level of 13.6 [$t(149)=-2.35$, $p=0.02$), which is a very important finding, confirming that delayed ET results in prolonged coronary flow occlusion by lasting thrombus, thus resulting in greater extend of myocardial damage.

Furthermore, no relationship was observed between LT and future adverse events [$x^2(1)=0.759$, $p=0.382$] or event type [$x^2(7)=7.37$, $p=0.391$]. Also no cut-off value for LT was found to be predictive of event occurrence [mean LT for

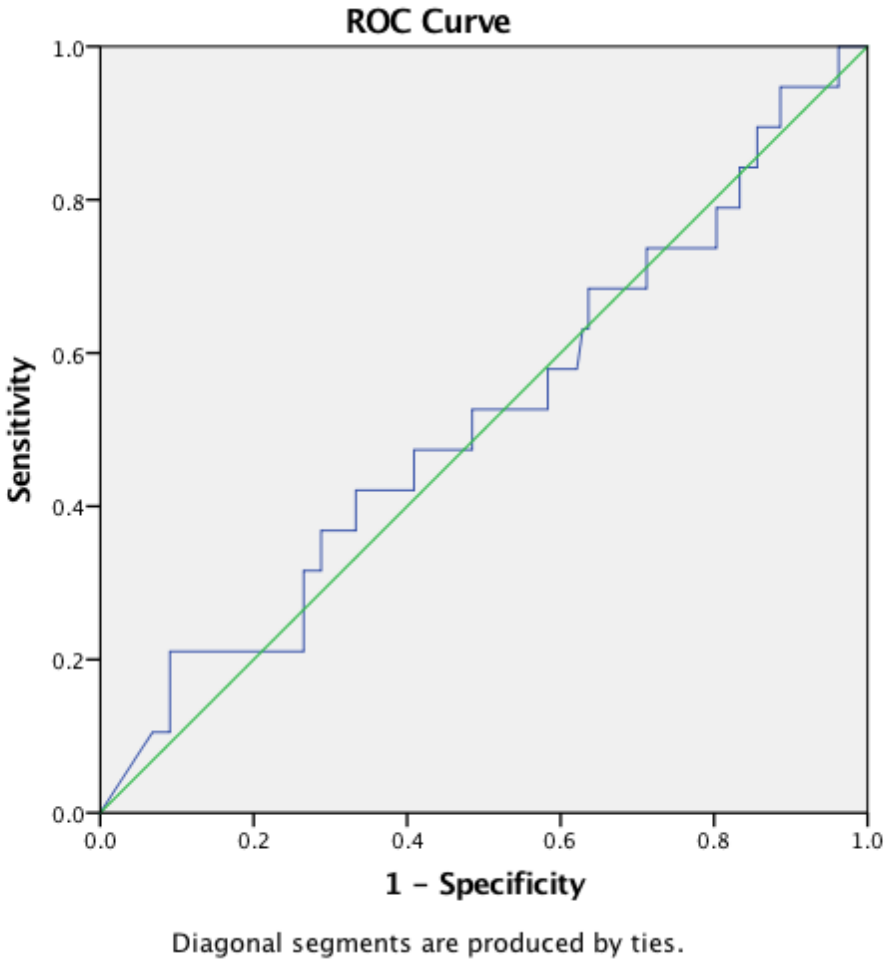
event 2018+/-1775 vs mean LT for no event 1738+/-1451, t(149)=0.765, p=0.446], but this can be explained by the small number of patients participating and subsequently the even smaller number of observed events, making extremely difficult to demonstrate statistically significant relationsip with LT. Finally, lysis time does not allow discrimination for events, as per ROC curve analysis (aoc=0.516, p=0.82).

Despite demonstrating higher mean OT in patients with bleeding diathesis compared to patients without previous history of bleeding (506+/-289s vs 447+/-156s), no statistical significance was found [t(149)=0.726, p=0.469].

Otherwise, no relationship was observed between the rest of clinical characteristics and baseline medication with OT. However, we did observe a relationship between both urea and RBC level with OT [Pearson correlation 0.171, p=0.036 for urea, Pearson correlation -0.222, p=0.007 for RBC].

Furthermore, no difference was observed in mean value of OT between patients with events and patients with no events [460+/-134s vs 447+/-164s, t(149)=0.335, p=0.738]. Also, as per ROC curve analysis, OT does not allow for discrimination of events (aoc=0.46, p=0.6) (Figure 1).

Figure 1. ROC curve (Lysis time by event).



ROC curve showing that LT is not a predictor of future adverse events

Statistically, none of the baseline clinical characteristics, medications or biochemistry was associated with events. When we tested angiographic, interventional and echocardiographic characteristics, only the number of diseased coronary vessels was found to predispose to future events. In particular, as the number of diseased coronary vessel increased from 1 to 3, the likelihood of future event also increased from 7.9% (1VD) to 27.8% (3VD) [$x^2(2)=5.97$, $p=0.05$].

Discussion

The most important finding from the statistical analysis, is the proven relationship between ET and the level of troponin in ACS. Patients with LT<3000s had a mean troponin of 4.6ng/ml, whereas patients with LT>3000s had a mean troponin of 13.6ng/ml, which is much higher. In patients with delayed ET during ACS, lasting coronary thrombus can cause more extensive myocardial damage due to prolonged myocardial ischaemia, which is associated with a higher incidence of complications and mortality. Furthermore, patients who survive, have a higher degree of heart failure, which at the moment is not a treatable condition, it is associated with high mortality rates and patients can only have palliation for their symptoms.
Despite the fact that we were not able to show statistical relationship between LT and future events, ROC curve analysis showed that there is a tendency towards this. Therefore, it is possible that a greater number of recruited patients, could have provided us with the result we aimed to show.

On the other hand, and similarly to previous studies of global thrombosis, no relationship was observed between OT and future events. However, OT was evidently influenced by both the level of RBC and urea. A previous study has shown that RBC can cause aggregation in the form of rouleaux, a process that is still not fully understood, but can also activate the process of thrombosis, which under normal circumstances leads to homeostasis (Wagner, Steffen, & Svetina, 2013). Regarding urea level, in a previous study of global thrombosis (Sharma et al., 2012), we observed prolongation of OT in patients with end-stage renal failure, in whom the levels of urea are very high, but our study has shown that this relationship still exists for lower levels of urea as well.

Finally, statistical significance was observed between previous CAD and number of diseased coronary vessels with future event occurrence, which is expected, not only because it has been previously proven (Arbab-Zadeh, Nakano, Virmani, & Fuster, 2012), but also because statistically, it is expected that with more diseased coronary vessels there is a higher risk for coronary events.
Proving once more the role of ET in CAD, reinforces our strong belief that further studies should be carried out, aiming at first to establish ET as a strong

predictor of future adverse events in patients with CAD and secondly to stimulate for further research for the development of pharmacotherapy enhancing ET, which will revolutionize the mode of treatment offered for primary and secondary prevention of CVD.

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CHAPTER 5

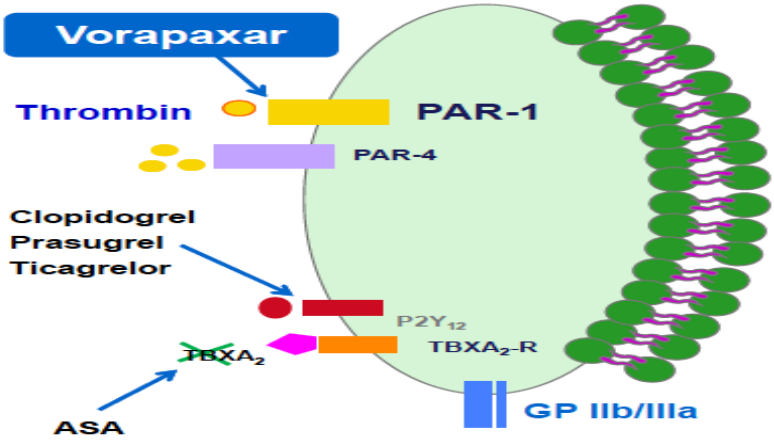
PAR-1 antagonist Vorapaxar and global thrombotic status in patients with coronary artery disease

Background

Current pharmacotherapy for patients with CAD aims to prevent platelet aggregation and subsequently thrombosis by deactivating TXA-2 and P2Y12 adenosine diphosphate (ADP) receptor mediated platelet activation pathways. Although new antiplatelet regimens have proven to be fairly effective in reducing the occurrence of coronary events, they fail to prevent platelet activation through thrombin receptors, the role of which is very important in the process of thrombosis, therefore occurrence of thrombotic events is still possible (Gao, Zhao, & Li, 2015). All platelet activation finally results in thrombin generation, therefore inhibition of thrombin would be highly desirable to reduce thrombosis risk (Figure 1). Theoretically, concomitant use of thrombin receptor antagonists with other types of antiplatelet agents could be a very useful and promising pharmacological therapy for the prevention of acute coronary events.

Vorapaxar is a novel, oral protease-activated receptor (PAR-1) antagonist that prevents platelet aggregation via thrombin inhibition. The role of this pharmacological agent was tested in the Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome (TRACER) and Trial to Assess the effects of vorapaxar in Preventing heart attack and stroke in patients with atherosclerosis-Thrombolysis In Myocardial Infarction 50 (TRA2P-TIMI 50) studies (Leonardi et al., 2013) (David a. Morrow et al., 2012), in which our cardiac unit, at East and North Hertfordshire NHS Trust, participated as a recruiting centre and as investigators. Both were randomised double blind multicentre studies, comparing vorapaxar with placebo in patients with cardiovascular disease.

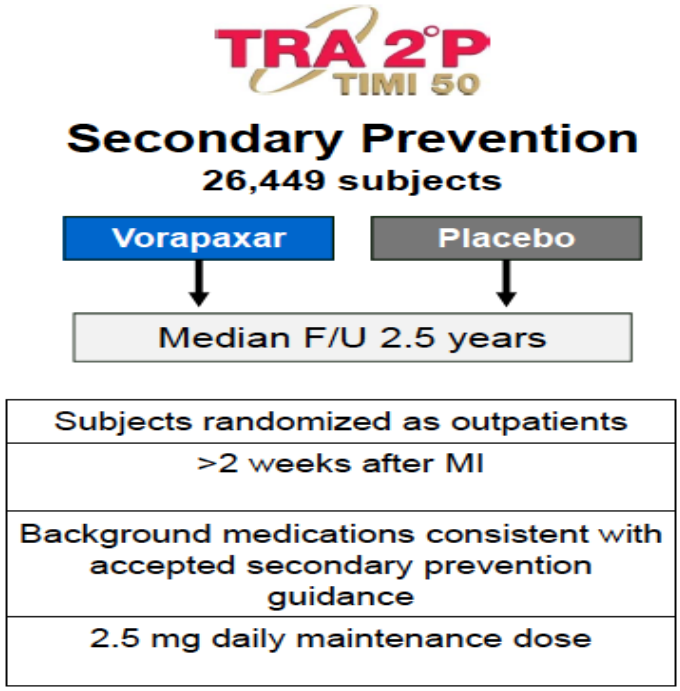
Figure 1. Antiplatelet activity of various pharmacological agents.



The aim of TRA2P-TIMI 50 study, was to assess the effectiveness of adding vorapaxar to standard therapy, in patients with a history of MI, for secondary prevention of cardiovascular events (Cavender et al., 2015).

In the TRA2P-TIMI 50 study, patients with established atherosclerosis in the form of MI or ischaemic stroke within the previous 2 weeks to 12 months or peripheral arterial disease with associated claudication and either ankle brachial index of <0.85 or previous revascularisation for limb ischaemia, were enrolled. Eligible patients were then randomly assigned to either receive a 2.5mg oral tablet of vorapaxar or a placebo tablet, along with standard pharmacotherapy for their condition (Thrombolysis et al., n.d.) (Figure 2).

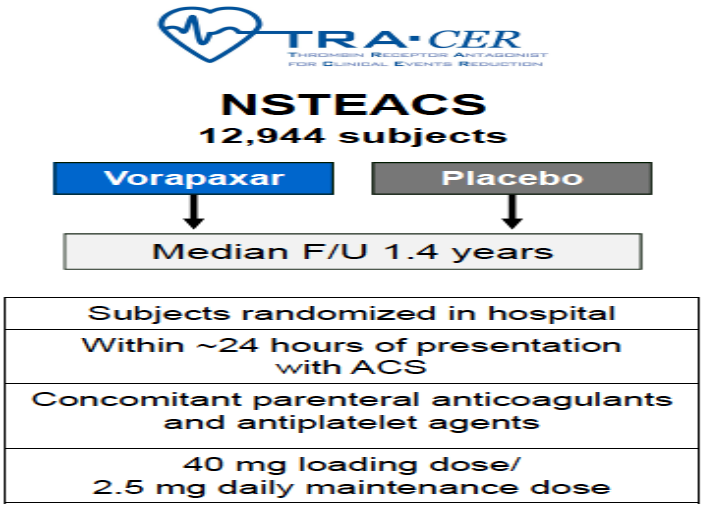
Figure 2. Summary of the TRA2P-TIMI 50 study



The TRACER study, aimed to investigate whether use of vorapaxar, in addition to standard therapy would be superior to placebo in reducing the occurrence of recurrent ischemic cardiovascular events, as well as to determine its safety profile in patients with ACS, without ST-segment elevation (Tricoci et al., 2012). In the TRACER study, patients were enrolled following diagnosis of NSTEMI and symptoms of coronary ischaemia within 24 hours of hospital admission (Figure 3). For diagnosis of NSTEMI, patients should have exhibited at least one of the following biochemical and electrocardiographic features: rise in cardiac troponin or creatine kinase MB level above the upper normal limit, new ST-segment depression of more than 0.1mV or transient ST-segment elevation for less than half an hour of more than 0.1mV in at least two contiguous ECG leads. Furthermore, at least one of the following criteria was necessary: patients to be not younger than 55 years of age, to have had previous MI, PCI or CABG, DM or PVD. Following randomisation, patients received either vorapaxar or placebo, along with standard pharmacotherapy in a blinded fashion, once daily until the end of follow-up, after an initial loading dose of

40mg vorapaxar vs placebo, at least 1 hour prior to the coronary revascularisation procedure [(Thrombolysis et al., n.d.)].

Figure 3. Summary of the TRACER study.



Aim

Our sub-study aimed to investigate the potential influence of vorapaxar compared to placebo, on the thrombotic and thrombolytic status of each participant.

Methods

We obtained permission in the form of informed consent from 57 patients (42 patients from the TRA2P-TIMI 50 and 15 from the TRACER study), in order to assess their thrombotic and thrombolytic status using the global thrombosis test (GTT), on and off treatment with the study medication (Thrombolysis et al., n.d.)

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Participants were initially tested after at least 2 months in the study, and once more at least 1 month following discontinuation of the treatment. Baseline demographics collected and follow-up sessions were performed according to the TRA2P-TIMI 50 and TRACER study protocols. Furthermore, all study protocol and GTT blood tests, were performed by investigators blinded to study drug allocation (Thrombolysis et al., n.d.).

Following termination of the studies, we were informed about initial drug allocation, which enabled us to proceed to statistical analysis of the data collected for our study's purposes (Thrombolysis et al., n.d.).

We compared the LT and OT between the intervention and control groups and assessed them using the non-parametric Mann-Whitney U test. We considered treatment effect to be significant if the p value was found to be less than 0.05.

We used the non-parametric Wilcoxon test in order to assess the significance of changes in OT and LT between periods on and off vorapaxar treatment. Mann-Whitney U test for continuous outcomes and two proportions Z-test for dichotomous outcomes were used to investigate differences between the clinical characteristics of the two groups, with $p<0.05$ considered as level of significance.

Statistical analysis was carried out using the R and Stata software (Thrombolysis et al., n.d.)

Results

Patients receiving vorapaxar exhibited significantly longer OT whilst on medication, than off medication. They also exhibited significantly shorter LT whilst on medication, than off medication. Patients receiving placebo showed no difference in OT or LT, on and off treatment. During treatment, OT was longer, but LT was similar in the vorapaxar compared to the placebo patients.

Following discontinuation of treatment, OT was similar in vorapaxar and placebo groups, but LT was longer in the vorapaxar group. LT was shorter in the placebo group whilst on treatment compared to LT on vorapaxar group off treatment. There was no difference in OT and LT between patients on TRA2P-TIMI 50 and TRACER studies on both vorapaxar and placebo groups.

Table 1. Results of thrombotic status using the GTT (Thrombolysis et al., n.d.).

	On treatment	Off treatment	P
Vorapaxar group OT	561s (422-654)	372s (338-454)	0.03
Placebo group OT	419s (343-514)	411s (346-535)	0.658
Vorapaxar group LT	1158s (746-1492)	1733s (1388-2230)	0.016
Placebo group LT	1236s (985-1594)	1400s (1092-1686)	0.524
OT	Placebo (on treatment) 419s (343-514)	vorapaxar (off treatment) 372s (338-454)	0.274
LT	Placebo (on treatment) 1236s (985-1594)	vorapaxar (off treatment) 1733s (1388-2230)	0.033

Data are shown as median (IQR)

OT: occlusion time, LT: lysis time, s: seconds

Figure 4. OT in seconds, whilst on and off treatment, in vorapaxar and placebo groups (Thrombolysis et al., n.d.).

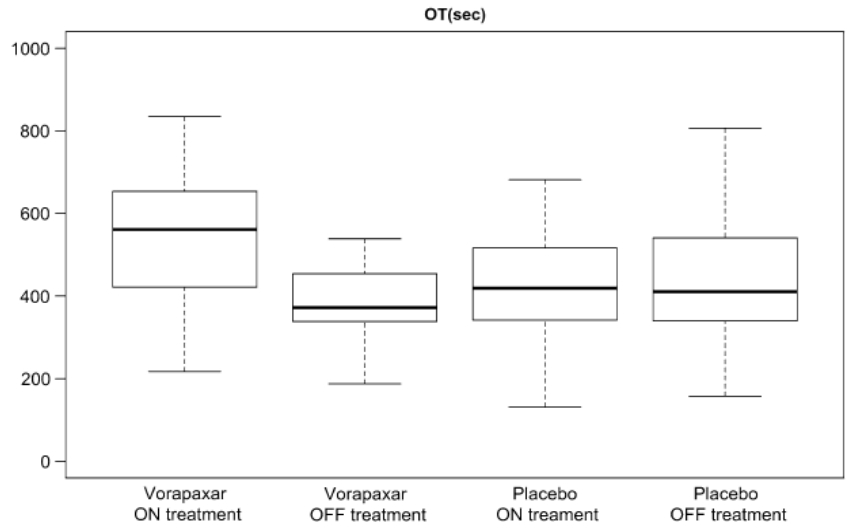

Figure 5. LT in seconds, whilst on and off treatment, in vorapaxar and placebo groups (Thrombolysis et al., n.d.).

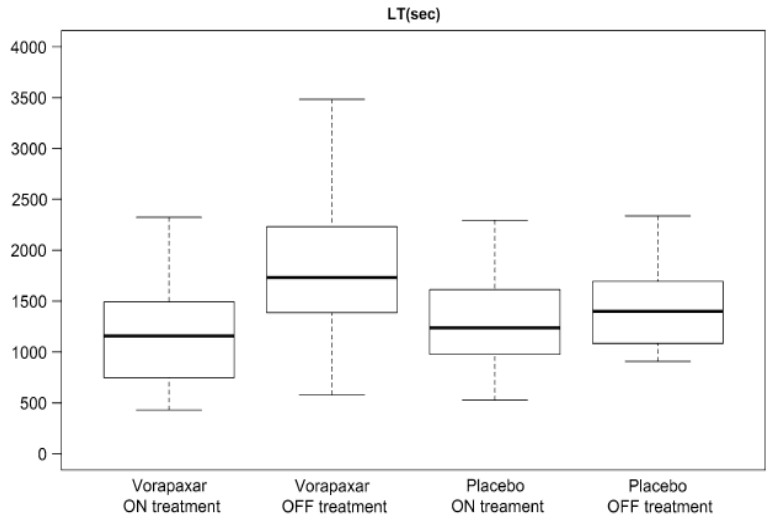

Table 2. Thrombotic status assessment using the GTT on and off treatment (Thrombolysis et al., n.d.).

	Vorapaxar group	**Placebo group**	**P**
OT (on treatment)	561s (422-654)	419s (343-514)	0.09
LT (on treatment)	1158s (746-1492)	1236s (985-1594)	0.277
OT (off treatment)	372s (338-454)	411s (346-535)	0.079
LT (off treatment)	1733s (1388-2230)	1400s (1092-1686)	0.031

Data are shown as median (IQR)

OT: occlusion time, LT: lysis time, s: seconds

Table 3. Thrombotic status assessment using the GTT in the two sub-groups, for TRA2P-TIMI 50 and TRACER (Thrombolysis et al., n.d.).

	TRACER	TRA2P	P
V on treatment OT	611s (498-649)	541s (379-608)	0.201
P on treatment OT	526s (381-608)	414s (342-493)	0.288
V off treatment OT	338s (298-374)	375s (362-479)	0.224
P off treatment OT	435s (370-595)	394s (340-506)	0.331
V on treatment LT	814s (688-1113)	1282s (756-1788)	0.161
P on treatment LT	1212s (1087-1537)	1260s (959-1613)	0.734
V off treatment LT	1946s (1585-2278)	1726s (1311-2212)	0.330
P off treatment LT	1394s (1002-2337)	1405s (1127-1452)	0.700

Data are shown as median (IQR)

V: vorapaxar, P: placebo, OT: occlusion time, LT: lysis time, s: seconds

Discussion

This is the first time that an oral antiplatelet agent exhibits, apart from anti-thrombotic properties (prolongation of OT), also demonstrated by other agents such as aspirin and clopidogrel on previous studies of GTT (Sharma et al., 2012) (Saraf et al., 2010), enhancement of endogenous thrombolytic status (Thrombolysis et al., n.d.). It is not clear why vorapaxar has this effect on endogenous thrombolysis (ET), but a possible explanation would be the indirect reduction in thrombin generation, as a consequence of a stronger platelet inhibition mediated by the drug.

In both TRA2P-TIMI 50 and TRACER studies, there was significant reduction in cardiovascular death, MI and stroke by vorapaxar. In TRA2P-TIMI 50, significant reduction was also observed in unstable angina and coronary revascularisation rate (Leonardi et al., 2013), results that are probably related to the antithrombotic properties of vorapaxar. In addition to the antithrombotic properties of vorapaxar, enhancement of ET, also exhibited in the vorapaxar arm of our sub-study, may be a very important mechanism of action of this drug. This is particularly important since shorter LT has been previously documented to be associated with reduction in the risk of MACE, as previously demonstrated in studies using the GTT for assessment of patients' global thrombosis status (Sharma et al., 2012) (Saraf et al., 2010).

On the other hand, in both TRA2P-TIMI 50 and TRACER studies, there was a higher incidence of bleeding in patients receiving vorapaxar. In a previous study (Saraf et al., 2010), median LT in 100 healthy volunteers was 1052s, and in our sub-study 22% of patients on vorapaxar had LT below this value, which suggests that enhanced ET may promote bleeding in patients receiving vorapaxar. Therefore, clinicians should be careful when prescribing a combination of antiplatelet agents, including vorapaxar, always taking into consideration potential benefits versus risks. In theory the ideal candidate receiving vorapaxar, should be someone with prolonged LT and subsequently higher risk of cardiovascular events, as traditional antiplatelet agents do not enhance ET, although this theory would need to be tested in clinical trials.

Additionally, further studies should be carried out, in order to investigate what is a 'safe LT range' for avoiding bleeding, when treating patients with vorapaxar. For example, reducing LT from abnormally high levels to a normal range, close to the one found in normal volunteers mentioned earlier, rather than below this

level, could reduce the risk of bleeding, however, we should always take into account that at this level, LT may not prevent thrombotic events, making the decision for vorapaxar use, a two-edged sword. Theoretically, based on our knowledge from previous studies of global thrombosis, vorapaxar should be avoided in patients with prolonged OT (>300s) and very short LT (<1052s), as in theory it may not offer much benefit in improving thrombotic status, but might result in unprovoked bleeding.

Another interesting point for discussion is the fact that none of the patients in our sub-study had prolonged LT, and this in combination with the fact that the number of participants was small, did not give us the opportunity to investigate the effect of the LT reduction on MACE. This is another reason why more studies are needed with greater number of participants.

Another interesting finding of our sub-study, was the extent of rebound prolongation in LT and reduction in OT, following discontinuation of vorapaxar. Further studies should also be carried out in order to investigate changes in OT and LT following discontinuation of Vorapaxar, in order for us to understand what measures to take when discontinuation of this medication becomes necessary. For instance, stepwise discontinuation of vorapaxar may prevent profound changes in global thrombosis and possible complications that could be associated with it.

We have identified a few limitations in our study. The first and main limitation is the small patient size, however, groups were well matched and randomly allocated. Secondly, the differing results observed in LT off treatment, in both placebo and vorapaxar groups, could be seen as a confounder to the study, however the fact that vorapaxar caused significant reduction in LT, strengthens the validity of our results. Thirdly the timing of the second blood sampling (off medication) made it just possible that since the half-life of vorapaxar is estimated at 187 hours, but at the time of the second blood sampling, one month following discontinuation of the medication, lasting anti-thrombotic and/or thrombolytic effects could still in theory be exerted. Finally, compliance was not confirmed with drug levels, so we had to rely on patients' account and

statements with respect to medication adherence.

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GENERAL CONCLUSIONS

In this study, we have once more proven that endogenous thrombolysis (ET) can predict future events in patients with cardiovascular disease. In particular, we showed that delayed ET is associated with cardiovascular death within one year from PPCI. Furthermore, we showed that LT≥3000s is the cut-off value that predicts MACE, which coincides with the results of previous studies of global thrombosis. Even though the numbers were small, all STEMI patients demonstrating full or partial resolution of ST-segment elevation on ECG prior to PPCI, had very short LT and subsequently enhanced ET. None of these patients had any complications during their hospitalization period or had any future coronary events, which is a very interesting finding and definitely something that should be re-investigated in larger scale studies.

Another important finding was the relationship between ET and troponin levels in NSTEMI patients. In patients with delayed ET, the mean level of troponin was significantly higher than in those with enhanced ET, meaning that lasting coronary thrombus in ACS, as a result of delayed lysis, is associated with more extensive myocardial damage and consequently with higher risk of complications and mortality. Unlike in other studies of global thrombosis, we did observe that increased thrombogenicity in the form of short OT, is associated with higher risk of non-fatal myocardial infarction and ischaemic stroke following PPCI.

Balance between thrombogenicity and ET is the determinant of the outcome following an acute coronary event, therefore any imbalance can lead to complications. Future therapy for both primary and secondary prevention of CVD, should aim to promote this balance, therefore development of novel pharmacotherapy targeting ET is essential. We have discussed the role of PAR-1 antagonist vorapaxar and its observed effects in lowering LT, thus enhancing ET, apart from its antiplatelet properties. However, usage of agents like vorapaxar would only be beneficial once establishment of ‘safe limits’ of OT and LT is achieved, for maintenance of the balance between thrombosis and thrombolysis and prevention of bleeding.

Current guidelines suggest fixed antiplatelet agent administration for primary and secondary prevention of CVD. However, thrombogenicity and ET vary among different individuals, therefore in the future, tailored pharmacotherapy could be provided, depending on the global thrombosis status of each patient, aiming to identify patients at high risk of thrombosis and bleeding. For this to be achieved, a platelet function test, such as the GTT, could be routinely used for patients receiving primary and secondary prevention treatment. However, in order for GTT to be established as a consistently accurate and safe screening test, larger studies confirming its value should be carried out. At present, the GTT cannot be a part of the management of CVD, as it has not been validated in large-scale trials. However, once its true value is proven in the future and subsequently its usage is approved for primary and secondary prevention of CVD, we strongly believe that has the potential to revolutionize future management of this condition for the benefit of millions of people worldwide.