

PhD Thesis

Assessing and improving prognosis in patients with high risk acute coronary syndrome

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Abstract

Endogenous fibrinolysis, if impaired, has been identified as a novel risk factor in patients with acute coronary syndrome (ACS). However, in-depth understanding of endogenous fibrinolysis, whether it varies day to day or reflective of a longer-term state, the relative importance of endogenous fibrinolysis in relation to different severity of coronary artery disease or if it can be modulated by pharmacotherapy remains unknown.

I assessed variability in endogenous fibrinolysis in a group of 17 healthy volunteers. I showed that there was no diurnal variation in thrombotic or thrombolytic status, nor variability from week to week within the cohort of healthy volunteers, nor between male and female volunteers. This showed that there was no evidence of short-term variation of endogenous fibrinolysis in healthy volunteers. Subsequently, I assessed thrombotic status in 80 patients with chest pain and suspected coronary artery disease, with the aim of relating fibrinolytic status to the extent of coronary disease. Patients had a CT coronary calcium score and fibrinolytic status assessed using point-of-care testing. Platelet reactivity and endogenous fibrinolysis were unrelated to the extent of coronary calcification, but endogenous fibrinolysis was more impaired in patients with obstructive coronary disease than in patients without obstructive disease (Lysis time (LT): 2524 [2425 – 2623] vs. 1865 [1636 – 2256]s, $p=0.0335$). This indicates that flow-limiting stenoses, possibly through creating a pro-thrombotic environment, are related to impaired endogenous fibrinolysis.

After this, I set out to investigate whether thrombotic status may differ amongst patients with different presentations of ACS. A total of 305 patients with ACS had blood tests to assess thrombotic and thrombolytic status: 143 ST-segment elevation myocardial infarction (STEMI), 125 Non-STEMI (NSTEMI) and 37 unstable angina (UA). Patients with NSTEMI

exhibited the longest endogenous fibrinolysis time, compared to patients with other ACS presentations (LT: NSTEMI 2008 [1615 – 2503] vs. STEMI 1764 [1411 – 2326] vs. UA 1803 [1483 – 2186] s, $p=0.0067$). However, the difference was no longer significant when a propensity matched cohort of patients was studied (LT: NSTEMI 1869 [1580.5 – 2202] vs. STEMI 1978.5 [1609.5 – 2645.5] s, $p=0.4832$). This indicates that co-morbidities or cardiovascular risk factors may impact on the effectiveness of endogenous fibrinolysis. In a small case series of patients with myocardial infarction with non-obstructive coronary arteries (MINOCA), an effective endogenous fibrinolytic system may potentially explain, in part, the absence of occlusive disease on angiography.

Lastly, I investigated the potential to modulate endogenous fibrinolysis using oral pharmacotherapy. In a randomised study, I assessed whether the addition of very low dose rivaroxaban could enhance fibrinolysis over and above that achieved with conventional dual antiplatelet therapy, in patients with acute coronary syndrome. The results showed that in patients with impaired endogenous fibrinolysis at presentation, this improved over time, irrespective of the antithrombotic medication prescribed. In particular, the addition of very low dose rivaroxaban did not improve endogenous fibrinolysis over and above that seen with dual antiplatelet therapy.

Larger cohort studies will be required to investigate if there are truly no diurnal variation amongst healthy individuals including a comparison to patients with different diseases such as diabetes mellitus, stable chronic kidney disease and coronary artery disease to verify firstly, if this lack of variation holds true in stable diseased states and secondly, if the level of impairment increases with stable diseased states.

Similarly, larger studies investigating endogenous fibrinolysis in patients with MINOCA may potentially provide further mechanistic insight into MINOCA with a coronary cause. With

regards to high-risk patients with out-of-hospital cardiac arrest, the potential role of endogenous fibrinolysis as an added risk predictor could be explored further in a well organised large cohort study.

Lastly, pharmacological modulation of endogenous fibrinolysis remains a potential novel pathway to prevent future cardiovascular events, but we may have to look beyond what the current pharmacological agents we have to offer.

Chapter 1. Assessing and improving prognosis in patients with CAD

Introduction

Cardiovascular disease, specifically ischaemic heart disease, is the leading cause of death in the developed world (G. A. Roth et al., 2018). Coronary artery disease (CAD) and the resultant reduction in myocardial blood flow can lead to symptoms of angina (Diamond, 1983). Such patients with chronic coronary syndrome (CCS) have a long-term condition which can be punctuated by acute events (Knuuti et al., 2020). Stable atherosclerotic plaques within the coronary arteries carry the risk of plaque erosion or rupture, which can subsequently result in thrombosis, leading to acute coronary syndrome (ACS) with the associated increased risk of morbidity and mortality.

The integral management of ischaemic heart disease, both CCS and ACS, involves modification of atherosclerotic risk factors and antithrombotic therapy (Ibanez et al., 2018; Knuuti et al., 2020; Roffi et al., 2016). However, despite this, approximately 10% of patients remain at risk of major adverse cardiovascular event (MACE) after an ACS, a risk mainly driven by thrombotic events (French et al., 2015). In this group of patients, further antithrombotic strategies have been proposed to mitigate this risk, which includes the addition of cilostazol (Suh et al., 2011), vorapaxar (Tricoci, Huang, Held, Moliterno, Armstrong, Van de Werf, et al., 2012), rivaroxaban (Mega, Braunwald, Wiviott, Bassand, Bhatt, Bode, et al., 2012) and dabigatran (Oldgren, Budaj, Granger, Khder, Roberts, Siegbahn, et al., 2011) to routine dual antiplatelet medications (DAPT). Unsurprisingly, the addition of further antithrombotic therapy carries a significant increase in the risk of bleeding, which could outweigh the benefit of reducing thrombotic events and therefore would not be suitable for all patients. Hence, to achieve maximum net benefit, risk stratification to identify patients at higher ischaemic risk for targeted, personalised therapy is pertinent.

Endogenous fibrinolysis, if impaired, has been identified as a novel risk factor in patients with ACS for future major adverse cardiovascular events (MACE) (Frag et al., 2019; Saraf et al., 2010; Sumaya et al., 2018). After adjusting for other risk predictors, impaired endogenous fibrinolysis remained an independent risk factor for predicting future MACE (Frag et al., 2019; Sumaya et al., 2018) and therefore can play an important role in identifying high risk individuals who may be appropriate for more potent antithrombotic therapy. However, the in-depth understanding of endogenous fibrinolysis, whether it varies from day-to-day or is reflective of a longer-term state, the relative importance of endogenous fibrinolysis in relation to the severity of CAD or if it can be modulated by pharmacotherapy is unknown. This would be essential as understanding endogenous fibrinolysis could allow better utilisation of it as a risk stratification tool or even as a potential target for pharmacological treatment as a modifiable risk factor.

Aims

1. To assess thrombotic status, in particular endogenous fibrinolysis and whether its measurement varies over time or by diurnal changes

2. To assess the relationship of thrombotic status and severity of CAD
 - 2.1 Comparing between patients with and without CAD

 - 2.2 Comparing with different degrees of severity CCS

 - 2.3 Comparing with different types of ACS

3. To assess if endogenous fibrinolysis can be modulated by contemporary oral pharmacological agents.

Hypotheses

1. Although the circadian rhythm has an impact on individual factors, it does not affect the overall fibrinolytic status in healthy individuals.
2. Endogenous fibrinolysis becomes increasingly impaired in relation to severity of coronary artery disease.
3. Endogenous fibrinolysis is more impaired in patients with STEMI when compared to patients with other types of ACS.
4. In patients with a recent ACS and impaired endogenous fibrinolysis, addition of very low dose rivaroxaban will improve endogenous fibrinolysis, over and above that achieved with DAPT.

Background

In this chapter, I will review the process of atherosclerosis and the pathomechanism behind its clinical manifestation of chronic and acute coronary syndromes, including the role of endogenous fibrinolysis. Subsequently, I will examine different presentations of ACS, risk profiling and assessment of future risk. I will then explore the role of endogenous fibrinolysis in risk prediction and finally pharmacological modulation of the pathway.

Atherosclerosis – progression and chronic coronary syndrome

Atherosclerosis is a multifactorial disease of the arterial wall initiated by lipid accumulation, in the form of low-density lipoprotein (LDL) within the arterial intima, where it is oxidised by free radicals, provoking an inflammatory process (Insull, 2009). There are several risk factors which intensify the process including hypertension, smoking and diabetes.

Atherosclerotic plaque formation begins in childhood and adolescence with early fatty streak development (Strong et al., 1999) from accumulation of LDL within the arterial intima.

Enzymatic modification and oxidation of LDL incites an inflammatory response which attracts monocytes to the intravascular, subendothelial smooth muscle cells. These monocytes then ingest oxidised LDL which leads to formation of foam cells. Accumulation of foam cells results in cell necrosis and progressively distorts the normal architecture of the intima to the point of complete disruption. During this time, a fibrous cap forms over the lipid core under the endothelium surface, forming a fibroatheroma. Within the necrotic core, following apoptosis of macrophages, microcalcification ensues (Shioi & Ikari, 2018). The coalescence of these microcalcifications leads to formation of calcified lesions within these plaques (Otsuka et al., 2014). The atheroma continues to develop, and when proteolytic enzyme activity predominates, the fibrous cap becomes thinner, leaving it susceptible to rupture. The rupture of this thin cap fibroatheroma (TCFA) exposes thrombogenic surface and leads to

thrombosis. Following rupture, the healing process begins with fibrous tissue formation and calcification (Demer, 1995). This cyclical process can occur multiple times throughout a lifetime and can result in the clinical presentation of ACS or can occur silently, without any clinical manifestations (Figure 1.1).

The resultant development and growth of the atherosclerotic plaque leads to narrowing of the arterial lumen. The narrow lumen promotes high shear and disturbed flow which promotes platelet aggregation (Casa et al., 2015) and increases risk of thrombosis. Reduction in lumen size also decreases oxygenated blood flow to the myocytes and can induce ischaemia which can present clinically as angina. The clinical presentations of CAD are now categorised into (1) CCS in patients who have 'stable' symptoms with confirmed or suspected CAD or (2) ACS in patients presenting with unstable symptoms. CCS is considered a chronic, progressive disease with acute events interspersed in between, which increases the risk of MACE.

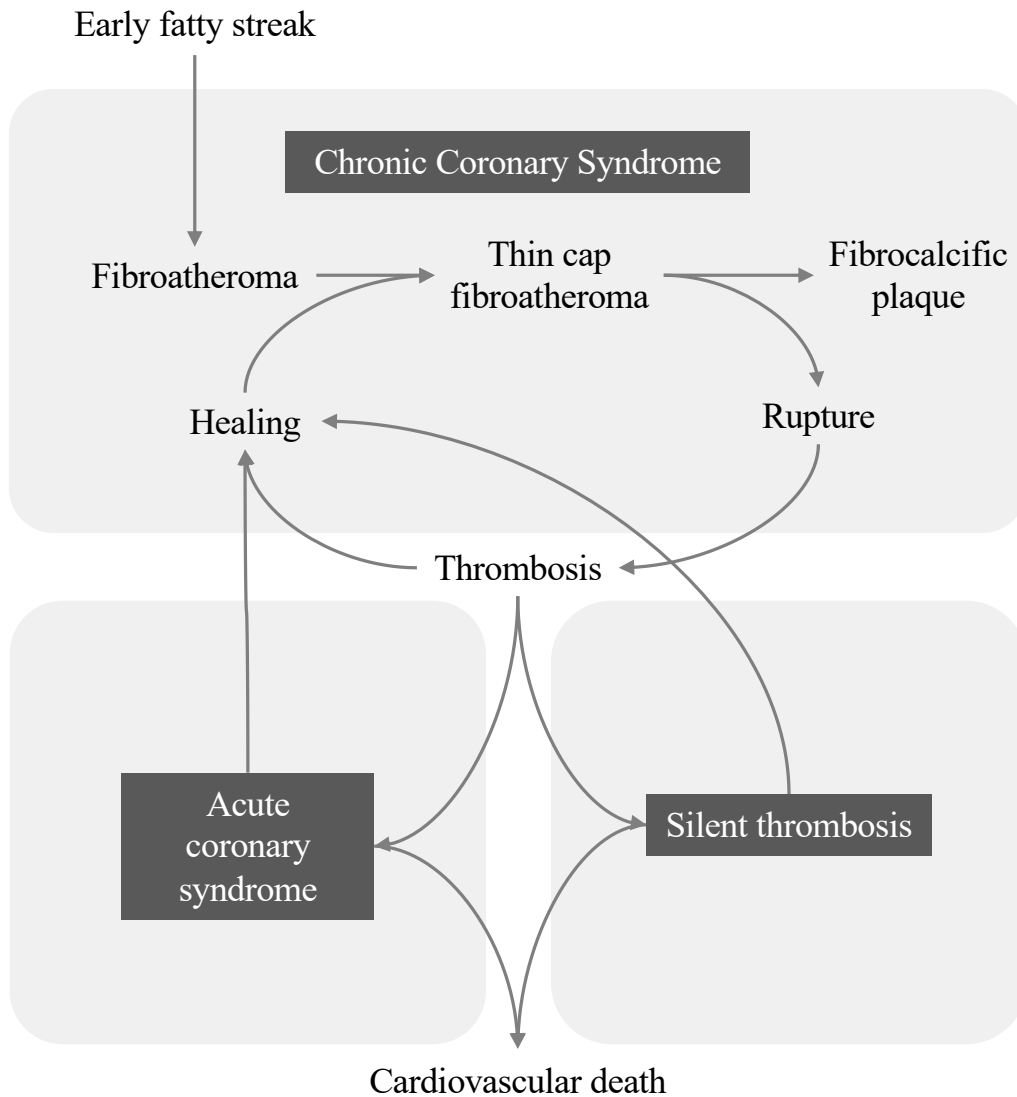


Figure 1.1 Process of atherosclerosis and its clinical manifestations as CCS and ACS.

Chronic coronary syndrome is an ongoing process which occurs throughout the lifetime of an individual due to atherosclerotic plaque progression. Following rupture of a thin cap fibroatheroma, as a sign of thrombosis, an individual could either present with symptoms of an acute coronary syndrome or the process could be silent. If an individual recover, healing occurs following the episode of thrombosis, further potentiating the atheroma. This cyclical process of rupture, thrombosis and healing continues until a stable fibrocalcific plaque is formed. The process of thrombosis, be it symptomatic or silent could prove fatal resulting in cardiovascular mortality.

Atherothrombosis – a mismatch between thrombosis and fibrinolysis

Formation of thrombus

Haemostasis is a complex sequence of biochemical responses to injury to allow formation of a blood clot and repair damaged endothelium. The maintenance of the equilibrium between coagulation and fibrinolysis is vital, as imbalance would lead to abnormal bleeding or thrombosis. Thrombosis is pathological clot formation within the blood vessels which in ACS, is typically caused by arterial thrombosis following the rupture or erosion of TCFA within the arterial wall (Bonaca et al., 2009; Fuster et al., 2005; Reininger et al., 2010). Following plaque disruption, the exposure of tissue factor and collagen creates a pro-thrombotic environment by initiating the coagulation cascade, platelet aggregation and thrombus formation (Reininger et al., 2010). vWF, platelet glycoprotein receptors, ADP, TxA₂ and thrombin all play important roles in platelet activation and recruitment of other platelets (Yun et al., 2016). Thrombin is also responsible for converting fibrinogen into fibrin, which stabilizes the platelet-platelet contacts leading to thrombus formation. This process is summarised in Figure 1.2.

Endogenous fibrinolysis

Endogenous fibrinolysis is the physiological countermeasure against thrombosis. It is divided into two key steps: (1) activation of plasminogen to serine proteinase plasmin by tissue (tPA) and urokinase (uPA) plasminogen activator; and (2) breakdown of fibrin into fibrin degradation products, thereby dissolving the thrombus (Longstaff & Kolev, 2015).

In a healthy individual, fibrinolysis is regulated by inhibition of plasminogen activator or antagonism of plasmin through α -2-antiplasmin (A2AP). This process is regulated by a complex system of enzymes including plasminogen activator inhibitor (PAI), thrombin-

activatable fibrinolysis inhibitor (TAFI), Factor XIII (FXIII), lipoprotein (a) (Lp(a)) and A2AP (Figure 1.2).

PAI acts as a fibrinolysis inhibitor and is the main enzyme responsible for inhibiting the actions of tPA and uPA, with PAI-1 being the most relevant as it is produced by platelets and endothelial cells. Upon stimulation by thrombin, PAI-1 is released from within platelets as a protective mechanism against premature lysis (Zhu et al., 1999). PAI-1 binds with tPA and uPA in a 1:1 ratio to form a stable compound which is then cleared by the liver (Epstein et al., 2000; Zhu et al., 1999).

TAFI is another fibrinolysis inhibitor which is converted into its active form, TAFIa, during coagulation after thrombin cleavage. It acts by reducing plasminogen binding to fibrin leading to increase in lysis time (Renucci et al., 2000; Rijken & Lijnen, 2009; Wang et al., 1998). Its generation is dependent on thrombin levels and greatly potentiated by thrombomodulin (Kokame et al., 1998; Renucci et al., 2000).

A2AP is a serine protease inhibitor which is produced in the liver. It inhibits fibrinolysis via three mechanisms: (1) forming a complex with plasmin; (2) inhibiting the adsorption of plasminogen onto fibrin; and (3) cross-linking with activated FXIII to make fibrin more resistant to plasmin (Carpenter & Mathew, 2008).

FXIII is a clotting factor which inhibits fibrinolysis. It is activated by thrombin in the presence of calcium. It plays a role in generating cross-links between fibrin strands within the fibrin mesh, making it stronger and more resistant to lysis. It also cross-links inhibitors of fibrinolysis, such as A2AP and TAFI, onto fibrin reducing its solubility and making it more resistant to the effects of plasmin (Mitchell et al., 2014). FXIII is present in large quantities within platelets, thereby making platelet rich clots more resistant to lysis than whole blood clots (Jang et al., 1989).

Lp(a) competitively inhibits plasmin generation as its molecular structure is similar to plasminogen, leading to an anti-fibrinolytic effect (Deb & Caplice, 2004). It also increases synthesis of PAI-1 by endothelial cells which further reduces plasmin levels (Deb & Caplice, 2004). This downregulation of plasmin results in the inhibition of fibrinolysis.

Loss of equilibrium

When haemostasis is disrupted, pathological conditions can occur. In cases where fibrinolysis is excessive, haemorrhagic complications can occur due to the inability of the body to form clots. On the other hand, an overwhelming thrombotic system can result in ischaemic organ damage. The vast majority of Type 1 myocardial infarctions (MI) are due to atherosclerotic plaque disruption (erosion or rupture) (Thygesen et al., 2018). The formation of thrombi and the resultant occlusion of blood flow distally may be due to inefficiency of the endogenous fibrinolytic pathway to counteract this rapid process to lyse the presenting thrombus, and this may culminate in an ACS. The ACS spectrum is divided into three main categories, determined by changes on the electrocardiogram (ECG) and changes in cardiac ischaemic biomarker (Figure 1.3). The rationale behind its classification is to guide immediate management strategy (Ibanez et al., 2018) as the ECG changes reflect the underlying pathophysiological differences (Nable & Brady, 2009).

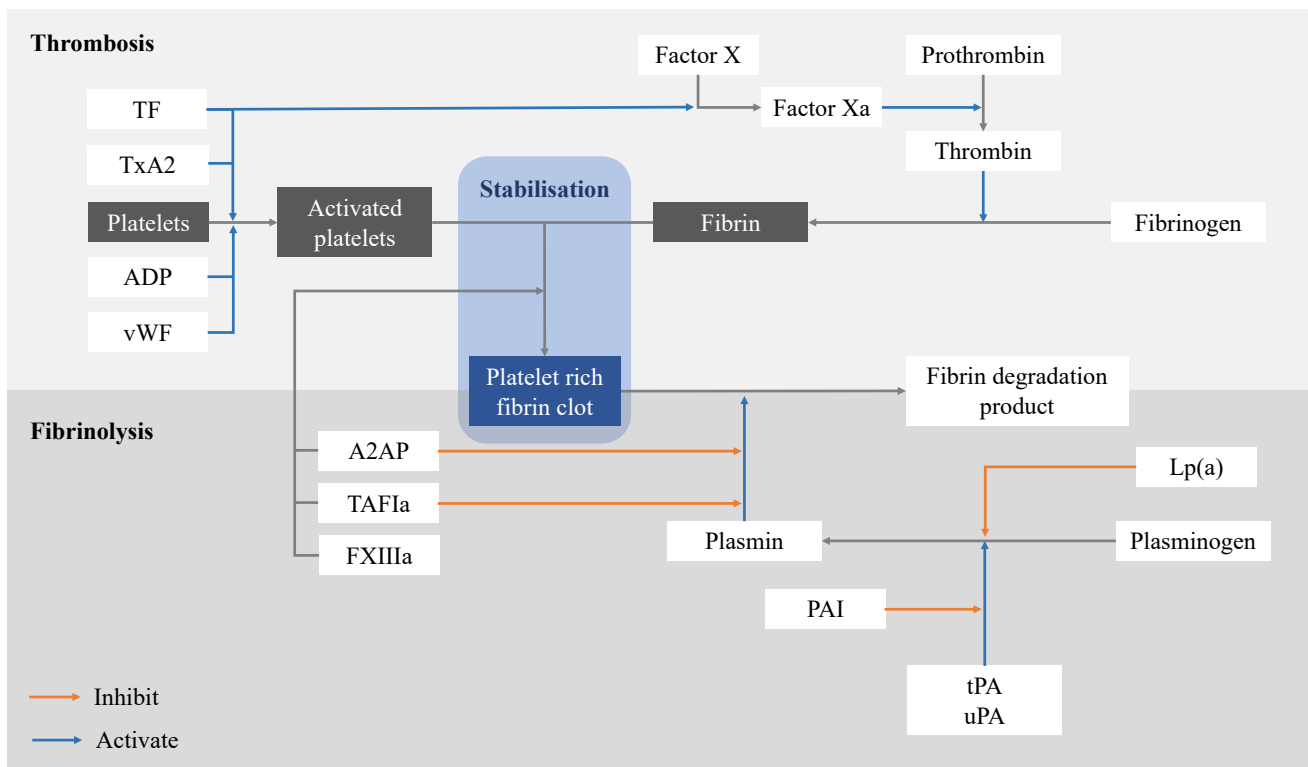


Figure 1.2 Process of thrombosis and fibrinolysis

The complex process of thrombosis and fibrinolysis requires the presence of multiple factors.

The formation and stabilisation of a platelet-rich fibrin clot requires (1) the activation of platelets with the help of tissue factor (TF), thromboxane A2 (TxA2), adenosine diphosphate (ADP) and von Willebrand factor (vWF) all of which are present during endothelial injury and presence of high shear stress and (2) the activation of fibrinogen to fibrin which is driven by thrombin formation, triggered by activated Factor X through the common pathway of the coagulation cascade.

On the other hand, fibrinolysis is mainly driven by plasmin. The activation of plasmin requires tissue (tPA) and urokinase (uPA) plasminogen activator. Inhibitors of fibrinolysis works on different segments of the pathway - plasminogen activator inhibitor (PAI) inhibits tPA and uPA, lipoprotein (a) (Lp(a)) reduces formation of plasmin from plasminogen, thrombin-activatable fibrinolysis inhibitor (TAFI) and α -2-antiplasmin (A2AP) reduces binding of plasmin to fibrin clot and in combination with Factor XIII (FXIII), they strengthen and stabilises the fibrin clot, making it more resistant to lysis.

Spectrum of ACS presentation and risk

ST-segment elevation myocardial infarction (STEMI)

In patients with ST-segment elevation on an electrocardiogram (Figure 1.4) combined with symptoms suggestive of MI, the underlying pathology in majority of cases is an acutely occluded coronary artery resulting in transmural infarction (Coppola et al., 2013). This requires immediate treatment with reperfusion, preferably with primary percutaneous coronary intervention (PCI) to restore blood flow and minimise myocardial necrosis (Ibanez et al., 2018). Mortality in STEMI is influenced by a multitude of factors and has greatly improved with the implementation of primary PCI and modern pharmacotherapy for secondary prevention (Gale et al., 2014; Puymirat et al., 2012). However, in-hospital mortality remains between 4 – 12% (Kristensen et al., 2014) and about 10% after 1 year (Pedersen et al., 2014).

Non-ST-segment elevation myocardial infarction (NSTEMI)

Patients without ST-segment elevation but with positive biomarkers i.e. a significant rise and/or fall of cardiac troponin are classified as NSTEMI or non-ST elevation ACS. Their ECG may be normal or include changes such as ST-depression or T-wave abnormalities. In contrast to STEMI, NSTEMI with thrombotic coronary aetiology has lower incidence of total coronary occlusion although most patients demonstrate severe narrowing (>70% luminal narrowing) of one or more epicardial arteries (Ambrose & Singh, 2015). Hence, the extent and risk of myocardial necrosis is lower. Revascularisation still plays an important role but is less emergent when compared to STEMI unless patients exhibit ongoing chest pain with clinical instability (Roffi et al., 2016). In-hospital mortality in NSTEMI is similar to STEMI but is numerically higher at 1 year (Montalescot et al., 2007).

Unstable angina (UA)

Patients with unstable angina demonstrate signs and symptoms suggestive of cardiac ischemia in the absence of changes in troponin, indicating myocardial ischemia without necrosis. Most such patients have at least one coronary artery with severe stenosis at angiography. These patients have lower risk of mortality (Reichlin et al., 2012, 2013) and derive less benefit from invasive management with PCI (Morrow et al., 2001) although aggressive pharmacotherapy as treatment to reduce future cardiovascular risk still applies.

Myocardial infarction with non-obstructive coronary arteries

10% of patients presenting with classical signs and symptoms of ACS do not have evidence of obstructive CAD to account for their presentation, namely those with myocardial infarction with non-obstructed coronary arteries (MINOCA) (Dokainish et al., 2005; Pasupathy et al., 2015; Safdar et al., 2018). The definition of MINOCA is predicated on the fulfilment of all three main diagnostic criteria, namely (1) Universal Definition of Acute Myocardial Infarction, (2) presence of non-obstructive coronary arteries on angiography (defined as no coronary artery stenosis $\geq 50\%$) in any potential infarct-related artery and (3) absence of another specific, clinically overt cause for the acute presentation.

This phenomenon has been overlooked historically (Kemp et al., 1986; Lichtlen et al., 1995). However, there is increasing evidence showing that this syndrome is not as benign as previously thought, with 1 year mortality of about 5% (Pasupathy et al., 2015). Although this is lower when compared with conventional ACS, it is by no means a benign condition.

Its underlying aetiology includes primarily (1) coronary causes such as plaque disruption or spontaneous coronary artery dissection, (2) non-coronary cardiac causes such as myocarditis or Takotsubo syndrome and (3) extra-cardiac causes such as type 2 myocardial infarction and

pulmonary embolism. The heterogeneity makes diagnosing, treating and assessing prognosis in this cohort of patients highly challenging.

In the context of endogenous fibrinolysis, this phenomenon is of particular interest due to the clinical manifestation of myocardial infarction but in the absence of significant epicardial coronary stenosis. Theoretically, such presentations could result from a strong prothrombotic environment with a highly effective fibrinolytic system.

Out-of-hospital cardiac arrest

Fatal arrhythmias, in the form of ventricular tachycardia (VT) or fibrillation (VF), leading to OHCA, occur in a minority of patients with acute ischemia and are often associated with genetic predisposition (Bezzina et al., 2010). The severe electrical storm causes disorganised contraction of the cardiac myocytes which induces a loss of mechanical cardiac output. This condition, if not treated promptly with electrical cardioversion and reversal of underlying aetiology, carries a high mortality (Gorenek et al., 2015; Ibanez et al., 2018) and therefore constitutes an extremely high risk cohort. Only in 28% of patients with OHCA is there return of spontaneous circulation and of these, only 10% survive to hospital discharge (Gräsner et al., 2016).

In patients with OHCA and ST-elevation or high index of suspicion of a coronary aetiology, emergency angiography with PCI is indicated (Ibanez et al., 2018). However, in other patients, angiography can be considered (as coronary events are common) but clinicians should take into consideration prognosis before making decisions. Predicting which patients do well is a difficult task as accurate prognostication requires consideration of a whole variety of factors.

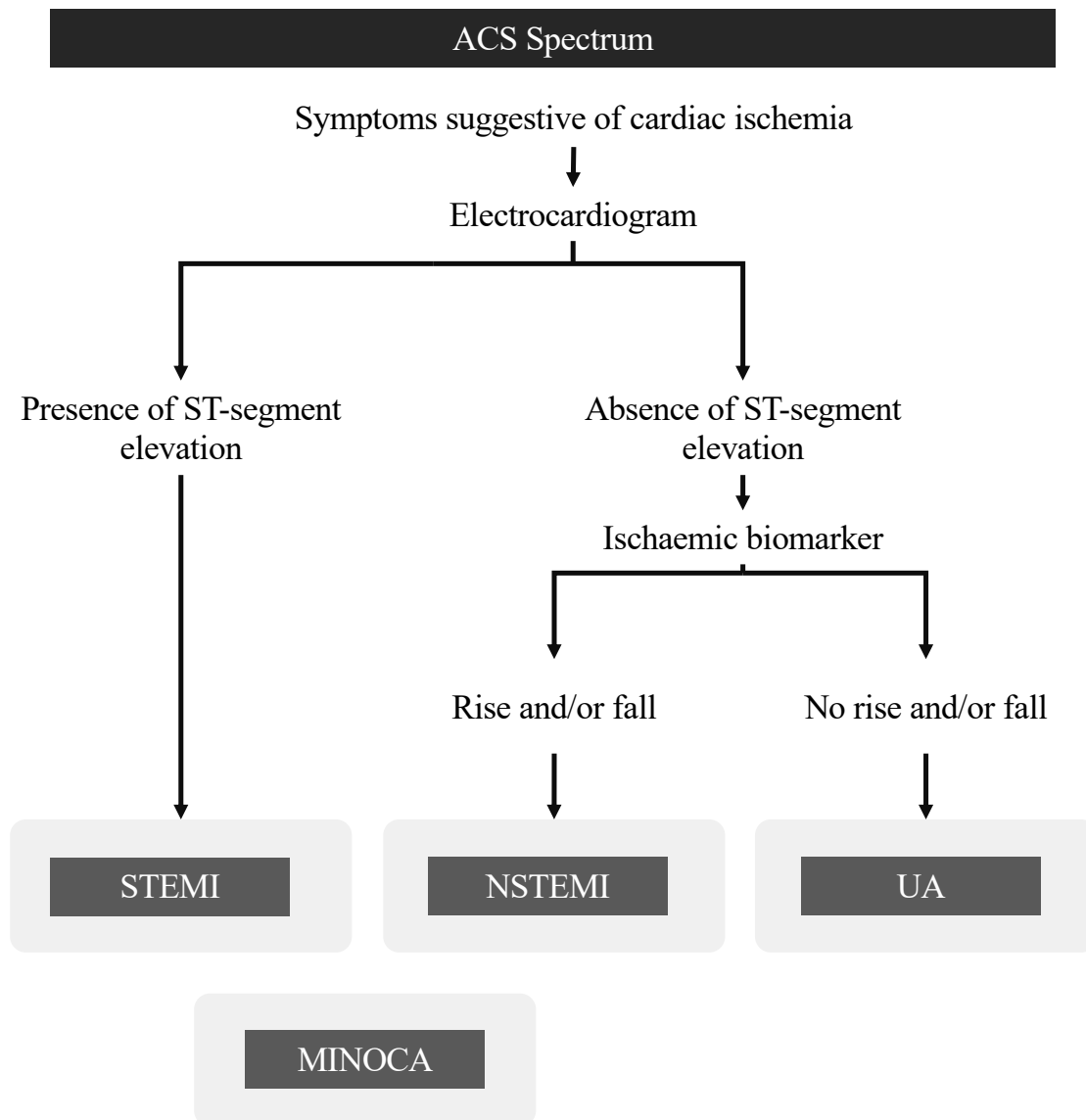


Figure 1.3 The ACS Spectrum

The umbrella term of acute coronary syndrome (ACS) comprises of 3 different diagnoses depending on (1) presence of ST-segment elevation and (2) rise and/or fall of cardiac ischaemic biomarkers. Within the cohort of ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI), a small group of patients will present with similar signs and symptoms without obstructive coronary arteries (MINOCA). Patients without biomarker changes but has signs and symptoms of cardiac ischaemia will be classed under unstable angina (UA).

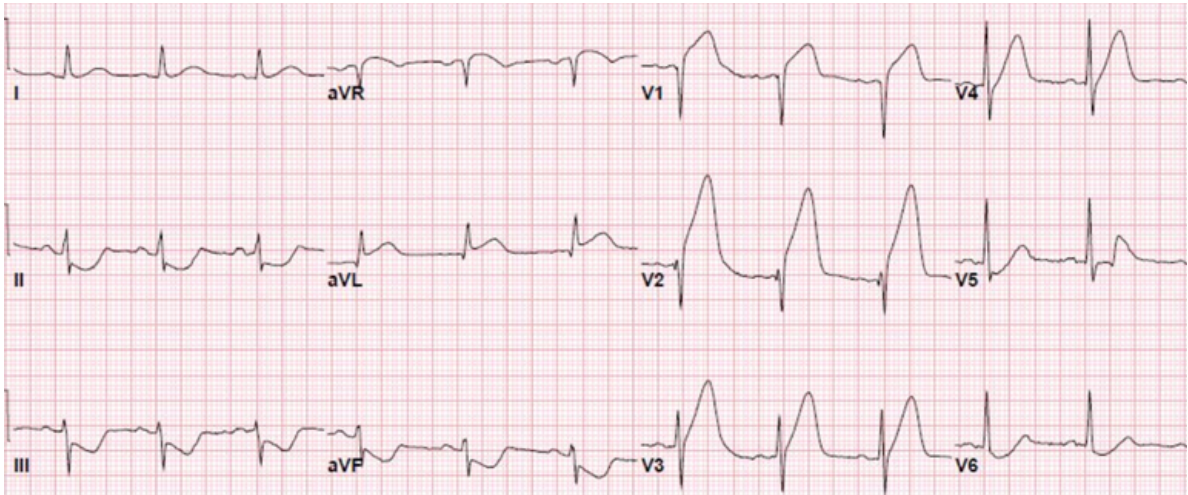


Figure 1.4 12-lead ECG showing anterior ST-segment elevation

Endogenous fibrinolysis as a risk predictor

The risk of future MACE in different presentations of ACS varies to some extent but the overall risks of mortality and morbidity remain relatively high, despite currently available treatments including revascularisation techniques and advanced pharmacotherapy. The mainstay treatments in ACS to prevent further thrombotic complications include antiplatelet therapy and in the acute setting, also anticoagulation. Following the results of the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) study showing the benefits of adding the P2Y₁₂ inhibitor clopidogrel to aspirin in patients with ACS (Yusuf et al., 2001), more potent P2Y₁₂ inhibitors ticagrelor (Wallentin et al., 2009) and prasugrel (Wiviott et al., 2007), showed further reduction in ischaemic events compared to clopidogrel. However, the mortality and morbidity in these ACS patients is not solely attributed to the underlying ischemic risks but also to the risk of major bleeding with potent antithrombotic therapy. Therefore, there has been a drive towards profiling patients to personalise therapy with the aim to optimise their risk profile with the correct therapy.

There is increasing evidence that impaired endogenous fibrinolysis is a strong predictor of residual cardiovascular risk in patients with ACS. Altered fibrin clot structure and increased resistance of the clot to lysis have been associated with myocardial infarction and stent thrombosis (Collet et al., 2006; Leander et al., 2012; Undas et al., 2008, 2010). In the last 2 years, two large prospective studies have confirmed that impaired fibrinolysis in patients with ACS is a novel, independent marker of increased cardiovascular risk (Farag et al., 2019; Sumaya et al., 2018). In a sub-study of >4000 patients in the PLATO trial, assessment of fibrin clot lysis using a validated turbidimetric assay revealed that impaired fibrin clot lysis was an independent predictor of adverse outcome in ACS (Sumaya et al., 2018). After adjusting for established cardiovascular risk factors, each 50% increase in lysis time was

associated with cardiovascular death/spontaneous MI (hazard ratio (HR) 1.17, 95% confidence interval (CI) 1.05-1.31; $P < 0.01$) and cardiovascular death alone (HR 1.36, 95% CI 1.17-1.59; $P < 0.001$). Earlier work employing a point-of-care assay of whole blood fibrinolysis showed that some 23% of patients with NSTEMI exhibit impaired endogenous fibrinolysis (lysis time, $LT \geq 3000$ sec) despite DAPT, and this was predictive of recurrent adverse cardiovascular events (HR: 2.52, 95% CI: 1.34 - 4.71, $p=0.004$) and cardiovascular death (HR: 4.2, 95% CI: 1.13 - 15.62 $p=0.033$) over the subsequent year with hazard increasing with increasing lysis time (Saraf et al., 2010). More recently, the RISK PPCI study, involving nearly 500 patients with STEMI showed that impaired endogenous fibrinolysis ($LT \geq 2500$ s) detected in 14% patients on admission was strongly related to recurrent major cardiovascular events (HR 9.1, 95% CI 4.28-15.03, $p=0.001$), driven by cardiovascular death and myocardial infarction (Farag et al., 2019).

These studies revealed endogenous fibrinolysis as a novel biomarker which can be utilised to detect patients who are at a much higher risk and could justify more potent pharmacotherapy.

Assessment of endogenous fibrinolysis

Given the apparent importance of endogenous fibrinolysis in determining the risk of future adverse events in patients with ACS, the assessment or measurement of this is very important in order to not only assess risk, but also to see if favourably improving fibrinolysis may reduce cardiovascular risk.

Factorial assays of fibrinolysis

The activity of fibrinolytic pathways can be assessed by various factorial assays and biomarkers such as tPA (Kinlay et al., 2009), PAI-1 (Akkus et al., 2009), LP(a) (Pineda et al., 2009) and d-dimer (Akgul et al., 2013).

There are limited studies of fibrinolytic assays assessing their use in predicting prognosis in ACS. Studies have shown that paradoxically, an increasing level of tPA correlated with an increased risk of MACE post-ACS (Kinlay et al., 2009) and that patients with ACS exhibited higher tPA levels when compared to patients with stable angina (Tousoulis et al., 2007). PAI-1 was found to independently predict in-hospital and one year mortality in patients with ACS (Akkus et al., 2009) and increased levels were associated with higher all-cause mortality and ACS (Battes et al., 2014). This was however not seen in some other studies exploring tPA and PAI-1 (Pineda et al., 2009; Sargento et al., 2005). With regards to Lp(a), previous studies have shown weak correlations (Pineda et al., 2009) whereas more recent studies have not shown any association of Lp(a) at time of ACS with mortality (C. Roth et al., 2020).

Similarly, contradictory results were seen in d-dimer, with the ESTEEM (Efficacy and Safety of Oral Direct Thrombin Inhibitor Ximelagatran in Patients with Recent Myocardial Infarction) trial sub-study showing no prognostic value in measuring d-dimer (Christersson et al., 2007) and Akgul et al. showing a positive association of d-dimer levels with in-hospital and 6 month mortality in patients with STEMI undergoing primary PCI (Akgul et al., 2013).

Although the measurement of these markers reflects the activity of the fibrinolytic pathway, their clinical use and predictive values remains limited (Gorog, 2010). This is likely due to the complex nature of the fibrinolytic pathway, with complex interactions between each factor, making it very difficult to build up a comprehensive overview based on individual components/enzymes and assess its overall function.

Global assays of fibrinolysis

Turbidimetric lysis assay

As the structure of the fibrin network regulates mechanical stability and its resistance to fibrinolysis, the assessment of clot structure can provide information about thromboembolic risk and susceptibility to lysis (Blombäck & Okada, 1982). Turbidimetric lysis analysis uses citrated plasma with addition of agonists - a calcium / thrombin buffer to induce thrombosis and tPA to induce fibrinolysis. Analysis is performed utilising principles of light transmittance aggregometry (LTA) in detecting absorption of light. Fibrin clot permeability is the measure of how tightly packed the fibrin clot is and has been utilised more in the prediction of venous thromboembolism (Undas, 2014; Undas et al., 2009; Zabczyk et al., 2016). Patients with conventional cardiovascular risk factors such as a family history of premature coronary artery disease (Mills et al., 2002) and type 2 diabetes (Alzahrani et al., 2012; Konieczynska et al., 2013) display more dense fibrin clots. In a matched case control study, patients with in-stent stenosis were found to have denser clots which were more resistant to lysis (Undas et al., 2010).

Fibrin clot lysis is the measure of time to achieve lysis of the clot. This has also shown promise in assessing fibrinolysis and its clinical impact, faring better in independently predicting outcomes in patients following ACS (Sumaya et al., 2018) and also specifically in patients with diabetes (Sumaya et al., 2020). However, this test is cumbersome and requires

dedicated and experienced laboratory staff trained to perform this reliably, making it unattractive as a clinically-useful test.

Thromboelastography

Thromboelastography or TEG[®] (Haemonetics, UK) and rotational thromboelastometry or ROTEM[®] (Pentapharm GmbH, Munich, Germany) are point of care, global test of coagulation status, simultaneously assessing clot development, stabilization, and dissolution based upon the same principle. It utilizes a pin suspended by a torsion wire into a cylinder to measure the physical properties of a clot. As blood clot formation occurs around the pin, fibrin strands form between the cylindrical cup and pin. The rotation of the cylindrical cup will be transmitted to the pin whose displacement is then picked up by the torsion wire. This is analysed and presented in graphical form by the instrument to allow analysis of different stages of coagulation and fibrinolysis (Thakur & Ahmed, 2012). It is able to measure clot formation time and rate, maximum amplitude (MA) or clot strength and clot lysis time (CLT). It was designed to be used with native blood but modification with different activators and inhibitors have been used (Chen & Teruya, 2009; Young et al., 2009; Zambruni et al., 2004) although the correlation between activated and non-activated samples has been poor (Thalheimer et al., 2008). Thromboelastography is well-established in the prediction of bleeding and the requirement for blood and blood products in the settings of trauma resuscitation and in surgery (Davenport & Khan, 2011; MacIvor et al., 2013; Shore-Lesserson et al., 1999; Whiting & Dinardo, 2014). Although its usefulness to detect hyperfibrinolysis, namely bleeding risk, is well described in the literature, its success is limited in the assessment of hypofibrinolysis or thrombosis risk (Okafor & Gorog, 2015; Pepperell et al., 2014). Another shortcoming, in terms of prediction of arterial thrombosis, is the employment of the low-flow, static-type situation which resembles more venous, rather

than arterial thrombosis. This is less reflective of the physiological response to high shear thrombosis, which typically occurs during ACS.

Global Thrombosis Test

The Global Thrombosis Test (GTT) (Thromboquest Ltd., London, UK) is a relatively new automated, point-of-care test that simultaneously assesses platelet reactivity, thrombosis, and fibrinolytic activity, from a native whole blood sample (Yamamoto et al., 2014). Blood passing through a plastic conical tube with narrow gaps is exposed to high shear stress that mimics flow within a narrowed vessel, activates platelets and induces thrombus formation. Thrombus formation gradually reduces and finally occludes flow. Reduction of flow, as detected by an optical sensor, is expressed as occlusion time (OT). Blood flow resumes following spontaneous fibrinolysis, and the time taken to do so is expressed as lysis time (LT). The GTT provides comprehensive evaluation thrombosis and lysis under high shear stress.

Clinical studies evaluating the GTT have shown a relationship between LT, a measure of endogenous fibrinolysis, and MACE in patients with ACS. The assessment of endogenous fibrinolysis has been shown to independently predict MACE in ACS (Saraf et al., 2010) and specifically in STEMI (Farag et al., 2019). In patients undergoing primary PCI, pre-primary PCI impaired endogenous fibrinolysis is associated with subsequent MACE, whilst effective (short) fibrinolysis was associated with spontaneous reperfusion, ST resolution and Thrombolysis In Myocardial Infarction (TIMI) 3 flow pre-primary PCI (Christopoulos et al., 2017). There have been clinical studies evaluating the use of the GTT in the context of cardiovascular risk factors like smoking and metabolic syndrome (Ikarugi et al., 2003; Suehiro et al., 2012, 2014) and there is a relationship between LT and cardiovascular risk factors.

The main advantage of the GTT is that it is an easy to use, point-of-care test that can assess platelet reactivity, thrombus stability and endogenous fibrinolysis, providing an overall assessment. When compared with other forms of testing for platelet function and fibrinolytic potential, the GTT has other advantages. The use of native, non-anticoagulated whole blood allows the measurement of the effects of thrombin generation in platelet aggregation without depletion of calcium (as opposed to citrated blood which is commonly required in other tests). Secondly, the presence of high shear as the key initiator of platelet activation is analogous to the physiological mechanism of platelet activation within a stenosed artery. Lastly, the assessment of spontaneous lysis through the measurement of LT is again comparable to the physiological recanalization of an occluded artery. However, the plastic tubes are not able to mimic actual endothelial surfaces in the face of stressors and hence unable to fully replicate the conditions but when compared to other conventional assessment of fibrinolysis, the GTT produces a more global assessment which is more reflective of the physiological conditions that occur within medium-sized arteries.

Modulation of endogenous fibrinolysis

Being able to identify high-risk patients is an important step towards improving outcomes in ACS but the next part of the equation is harnessing this risk by favourably modulating it. Unlike the enhanced platelet reactivity which can be targeted and reduced from admission to discharge, through the effect of antiplatelet therapy, fibrinolysis in ACS patients appears unaffected by DAPT (Farag et al., 2019; Sumaya et al., 2018). In a small study which investigated the effects of P2Y₁₂ inhibitors on endogenous fibrinolysis, clopidogrel and ticagrelor have been shown to produce an impact on platelet reactivity but no effect on fibrinolysis. On the other hand, cangrelor, an intravenous P2Y₁₂, displayed potent ability to both decrease platelet reactivity, increased thrombus instability and enhance fibrinolysis (Spinthakis, Farag, et al., 2019). Hence, a potential method for modulating endogenous fibrinolysis could involve the destabilisation of the platelet-rich fibrin clot. Apart from the conventional aspirin and P2Y₁₂ inhibitors, there are other oral pharmacological agents available which have been used in the context of ACS in addition to DAPT (Figure 1.5). I will discuss these agents in more detail, exploring their current use and the potential to modulate endogenous fibrinolysis.

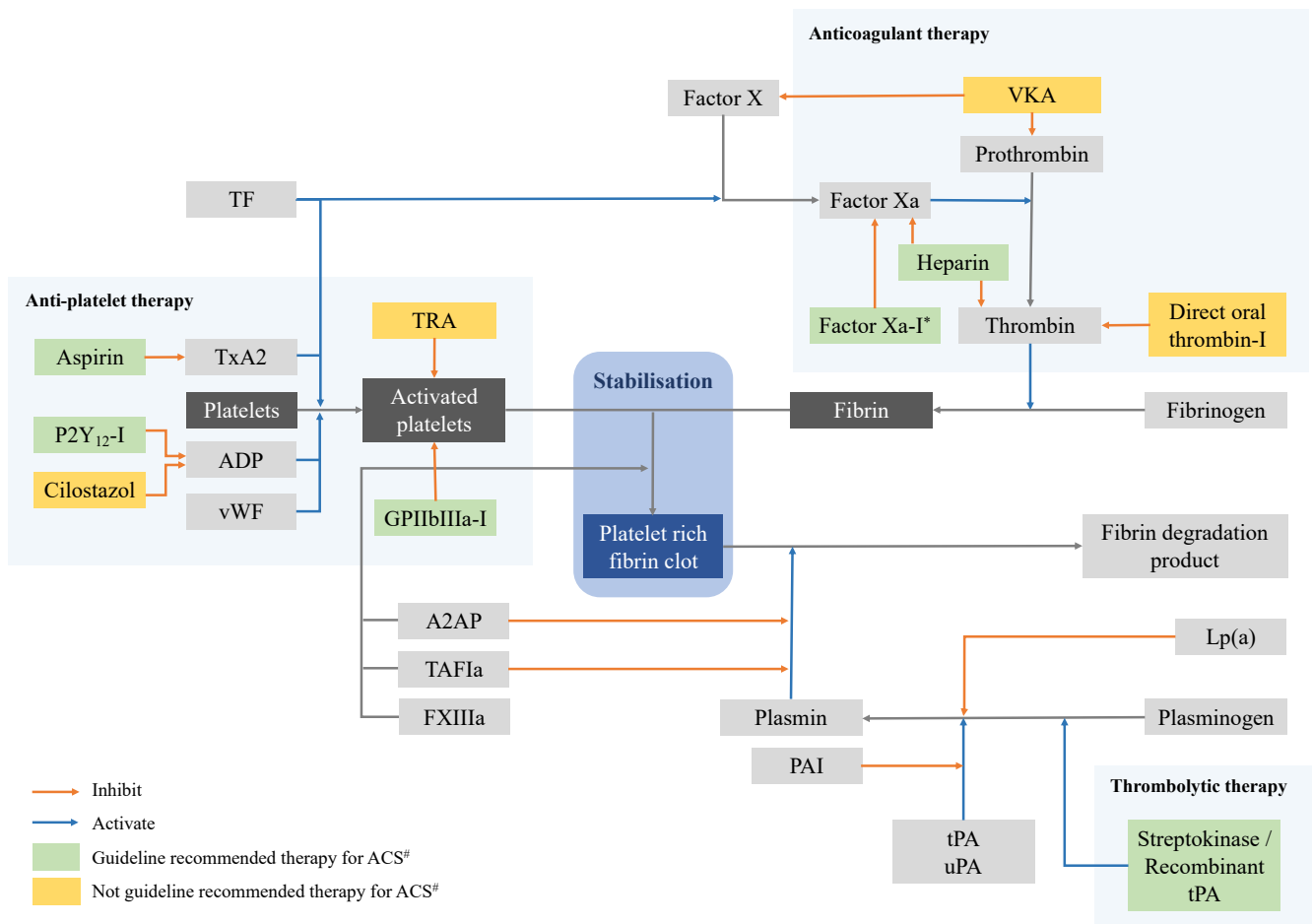


Figure 1.5 Mechanism of actions of antithrombotic therapies in ACS

There are 3 classes of antithrombotic therapy used for treatment of ACS.

Anti-platelet therapy includes [not orally administrated] GPIIb/IIIa-I – Glycoprotein IIb/IIIa inhibitors, [orally administrated] Aspirin, P2Y₁₂ inhibitors (Clopidogrel, Ticagrelor, Prasugrel), TRA - thrombin receptor antagonist (Vorapoxar) and Cilostazol.

Thrombolytic therapy includes [not orally administrated] Streptokinase, Recombinant tPA (Alteplase, Tenecteplase etc.)

Anticoagulant therapy includes [not orally administrated] Heparin, [orally administrated] VKA – Vitamin K antagonist (Warfarin), Direct oral thrombin inhibitors (Ximegalatran, Dabigatran) and Factor Xa inhibitors (Rivaroxaban*, Apixaban, Darexaban, Edoxaban).

*Only low-dose rivaroxaban (2.5mg b.i.d.) is recommended under the guidelines

#ACS refers to patients with ACS with no indication for oral anticoagulation

Anticoagulants

Direct oral Factor-Xa inhibitors

Direct oral factor Xa (FXa) inhibitors, have been used in ACS and rivaroxaban is licensed for use in ACS at the “vascular dose” of 2.5 mg *bis in die* (b.i.d) (Windecker et al., 2014). This group of drugs act directly upon activated Factor X in the coagulation cascade, inhibiting the conversion of prothrombin to thrombin. In the phase II ATLAS ACS-TIMI 46 (Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects With Acute Coronary Syndrome ACS 2 – Thrombolysis In Myocardial Infarction 46) study, the investigators assessed the safety and efficacy of various doses of rivaroxaban (5mg, 10mg, 15mg or 20mg once or twice-daily) in addition to DAPT with clopidogrel or single antiplatelet therapy with aspirin in 3,941 patients with a recent ACS (Mega et al., 2009). Bleeding complications were found to increase in a dose-dependent manner (in relation to rivaroxaban dose) in all triple therapy (TT) groups ($p < 0.0001$) with reduction of death, MI and stroke ($p = 0.027$). The subsequent phase III ATLAS ACS 2-TIMI 51 (Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects With Acute Coronary Syndrome ACS 2 – Thrombolysis In Myocardial Infarction 51) study, compared the addition of 2.5 mg b.i.d. and 5 mg b.i.d. rivaroxaban to DAPT with clopidogrel in 15,526 patients with ACS (Mega, Braunwald, Wiviott, Bassand, Bhatt, Bode, et al. 2012). Rivaroxaban at the 2.5 mg dose significantly reduced cardiovascular death (CVD) compared to placebo (2.7% vs. 4.1%; $p = 0.002$) but not at the 5 mg dose (4% vs. 4.1%; $p = 0.63$).

Following this, 2015 European Society of Cardiology (ESC) Guideline for the management of NSTEMI-ACS stated that “rivaroxaban 2.5mg twice daily, while not recommended in those receiving ticagrelor or prasugrel, might be considered in combination with aspirin and clopidogrel if ticagrelor and prasugrel are not available for NSTEMI patients who have high ischaemic and low bleeding risks” (class IIb, level of evidence B) (Roffi et al., 2016) and the

2017 ESC Guidelines for the management of STEMI added that rivaroxaban 2.5 mg b.i.d. may be considered in selected patients who receive aspirin and clopidogrel after STEMI and who are at low bleeding risk (class IIb, level of evidence B) (Ibanez et al., 2018).

Apixaban is another direct oral FXa inhibitor which has been evaluated in ACS. In the phase II APPRAISE (Apixaban for Prevention of Acute Ischemic and Safety Events) study, the investigators assessed the addition of apixaban (placebo, 2.5mg b.i.d., 10mg once daily (o.d.), 10mg b.i.d. and 20mg o.d.) to aspirin or DAPT (J. H. Alexander, 2009) in 1715 patients. Apixaban significantly increased bleeding compared to placebo, in a dose dependent manner, with a trend to reduction in ischemic events. This led to the phase III APPRAISE-2 (Apixaban for Prevention of Acute Ischemic and Safety Events 2) which assessed the safety and efficacy of adding apixaban 5mg b.i.d. to DAPT in 7,392 patients with a recent ACS (J. H. Alexander et al., 2011). The trial was prematurely terminated due to the lack of impact on the primary efficacy endpoint of the composite of CVD, MI and ischaemic stroke (7.5% vs. 7.9%, 95% CI 0.80 - 1.11; p=0.51) but significantly increased TIMI major bleeding (1.3% vs. 0.5%, 95% CI 1.50 - 4.46; p=0.001) with apixaban.

In RUBY-1 (A Randomized, double-blind, placebo-controlled trial of the safety and tolerability of the novel oral factor Xa inhibitor darexaban (YM150) following acute coronary syndrome) study, 1279 patients with recent ACS were randomised to placebo or darexaban at different doses (5mg b.i.d., 10mg o.d., 15mg b.i.d., 30mg o.d., 30mg b.i.d., 60mg o.d.) (Steg et al., 2011). There was dose-dependent increase in bleeding with no impact on the primary combined efficacy endpoint of all-cause death, MI, stroke and recurrent ischemia. No further studies were reported after.

In the EDOX-APT (The Effects of EDOXaban on the cellular and protein phase of coagulation in patients with coronary artery disease on dual AntiPlatelet Therapy with aspirin

and clopidogrel) study which aimed to assess the pharmacodynamic effects of edoxaban on platelet aggregation and clot kinetics, the investigators assessed the addition of edoxaban (60mg o.d. or 30mg o.d.) to DAPT or aspirin on TEG and LTA parameters (Francesco Franchi et al., 2020). They recruited 75 patients with CAD and found that edoxaban prolongs thrombin generation in a dose dependent manner but did not impact clot kinetics. Another study conducted with edoxaban had found that it enhances tPA-induced clot lysis via inhibition of TAFIa (Bouma & Mosnier, 2006) and enhancement of plasmin generation (Morishima et al., 2018). This could potentially explain the mechanism behind apixaban's ability to enhance endogenous fibrinolysis in patients with non-valvular atrial fibrillation (NVAf) (Spinthakis, Gue, Farag, Srinivasan, et al., 2019). Therefore, the use of oral direct FXa inhibitors could potentially be a novel way to modulate endogenous fibrinolysis.

Direct oral thrombin inhibitor

Dabigatran is a direct oral thrombin inhibitor (DTI) which delays coagulation through direct inhibition of the enzyme thrombin (Factor IIa). The efficacy and safety of dabigatran in ACS was evaluated in the phase II RE-DEEM (Randomised Dabigatran Etextilate Dose Finding Study In Patients With Acute Coronary Syndromes Post Index Event With Additional Risk Factors For Cardiovascular Complications Also Receiving Aspirin And Clopidogrel) study (Oldgren, Budaj, Granger, Khder, Roberts, Siegbahn, et al., 2011). The study recruited 1861 patients with recent ACS, and assessed the addition of different doses of dabigatran (50mg, 75mg, 110mg and 150mg b.i.d) to DAPT with clopidogrel. Dabigatran dose-dependently increased major bleeding compared to placebo ($p < 0.001$) but did not reduce the composite endpoint of CVD, non-fatal MI and non-haemorrhagic stroke compared to placebo.

Theoretically, through action of inhibiting thrombin, DTI has the potential to delay clot formation by decreasing fibrin formation and destabilising clots with inhibition of TAFIa.

However, no mechanistic studies have been performed with DTI, used in the context of ACS, to investigate its role in enhancing fibrinolysis.

Vitamin K antagonist

Warfarin is the most commonly used vitamin K antagonist (VKA), which inhibits the production of vitamin K-dependent coagulation factors II, VII, IX, X. Being one of the first oral licensed anticoagulants, VKA has been investigated in various ACS studies. A meta-analysis looking at the effects of warfarin with aspirin identified in a significant decrease in MI (rate ratio RR 0.56), stroke (RR 0.46) and revascularisation (RR 0.80) but increase in major bleeding (RR 2.5) when compared to aspirin alone, with no difference in mortality (Rothberg et al., 2005). The addition of standard dose warfarin to DAPT with clopidogrel after ACS has only been used in the context of patients with indication for anticoagulation after PCI. A registry of 40,812 ACS patients treated with multiple different regimens revealed a three-fold increase in bleeding with TT comprising of warfarin, clopidogrel and aspirin compared to DAPT (Sørensen et al., 2009). Hence, the ESC guidelines on myocardial revascularisation have recommended individualised duration of TT with step-down to dual therapy and a preference to a novel oral anticoagulants (NOAC) instead of VKA (Neumann et al., 2019).

Antiplatelet therapy

Thrombin receptor antagonists

As conventional aspirin and oral P2Y₁₂ inhibitors have not been found to modulate endogenous fibrinolysis in previous studies, I will move on to discuss other agents working on platelet aggregation which may provide potential novel approaches to improve fibrinolysis. Thrombin, a key mediator of platelet activation, binds to protease-activated receptors (PAR) -1 and -4 on the platelet surface. PAR-1 inhibition prevents binding and

thereby inhibits platelet activation. Vorapaxar is currently the sole approved PAR inhibitor and its addition to aspirin or DAPT with clopidogrel, was assessed in 12,994 patients following an NSTEMI in the TRACER (Thrombin Receptor Antagonist for Clinical Event Reduction in ACS) trial which was prematurely terminated due to excess bleeding (Tricoci et al., 2012). Over a median follow-up of 30 months, moderate and severe Global Utilisation of Streptokinase and TPA for Occluded arteries (GUSTO) bleeding occurred more frequently with vorapaxar than placebo (7.2% vs. 5.2%; $p < 0.001$), most notably intracranial haemorrhage (ICH) (1.1% vs. 0.2%; $p < 0.001$) without significant reduction in composite endpoint of CVD, MI, stroke or recurrent ischaemia with hospitalisation or urgent coronary revascularisation (18.5% vs. 19.9%; $p = 0.07$). However, there was a reduction in the composite endpoint of CVD, MI and stroke (16.4 vs 14.7%, HR 0.89; $p = 0.02$).

In the TRA-2^oP (Thrombin Receptor Antagonist in secondary Prevention of atherothrombotic ischemic events) trial, addition of vorapaxar to aspirin or DAPT in secondary prevention led to a reduction of the composite endpoint of CVD, MI and stroke (9.3% vs 10.5%; $p < 0.001$) but increased the risk of bleeding, leading to premature termination of the study (Morrow et al., 2012). However, in the subgroup analysis of patients with MI, vorapaxar significantly reduced the composite endpoint of CVD, MI and stroke (HR 0.80; $p < 0.001$) with increased moderate to severe bleeding (HR 1.61; $p < 0.0001$) but not an increase in ICH ($p = 0.076$) (Scirica et al., 2012).

Although vorapaxar is not approved in patients with recent ACS, the 2015 ESC Guidelines on NSTEMI state that whilst vorapaxar is approved “for reducing ischaemic events in patients with a history of MI, the benefit of vorapaxar in addition to aspirin and clopidogrel is modest and must be carefully weighed against the increase in bleeding events, including intracranial haemorrhage” (Roffi et al., 2016).

In the OPTIMUS (Optimizing anti-platelet therapy in diabetes mellitus)-5 study (F Franchi, Rollini, Kairouz, et al., 2019), the authors found that addition of vorapaxar to DAPT with clopidogrel blocks platelet aggregation but does not affect markers of clot kinetics, including thrombin generation. However, interestingly, when vorapaxar is added to more potent P2Y₁₂ inhibitors (ticagrelor and prasugrel), it does show a transient and modest effect on thrombin generation (F Franchi, Rollini, Faz, et al., 2019). This may be a potential area for investigation when vorapaxar is licensed for use in ACS.

Cilostazol

Cilostazol is a selective phosphodiesterase III inhibitor which has antithrombotic effects through inhibition of adenosine uptake, eventually resulting in rising cyclic adenosine monophosphate (cAMP) levels (S. Goto, 2005; Schrör, 2002). This is a similar pathway to P2Y₁₂ inhibitors, which competitively inhibit ADP P2Y₁₂ receptors inducing downstream accumulation of cAMP. Elevation of cAMP levels leads to phosphorylation of proteins that inhibits activation of glycoprotein IIb/IIIa leading to reduced platelet activation and aggregation (Albert & Christopher, 2012). It is most commonly prescribed in patients with intermittent claudication to provide symptomatic relief (Bedenis et al., 2014), due to its vasodilatory effects. The use of cilostazol in ACS has been considered as part of TT in patients who have high on-treatment platelet reactivity (HTPR) despite DAPT (Lavie & Dinicolantonio, 2013). A meta-analysis of 11 randomised and observational studies comprising of 9553 studies showed that TT including DAPT with cilostazol reduced all-cause mortality compared to DAPT (odds ratio [OR] 0.72, 95% CI 0.61 - 0.85; p<0.001) without an effect on MI, stroke or bleeding (Fan et al., 2016). However, other meta-analyses involving only randomised controlled trials (RCT), with one including 8 studies involving 3590 patients (Sakurai et al., 2013) comparing TT with cilostazol to DAPT and another with 19 RCTs with 7464 patients (Chen et al., 2015) showed no difference with respect to the

occurrence of MACE. The lack of strong evidence of benefit has prevented cilostazol from featuring as an option for antithrombotic therapy by the ESC (Windecker et al., 2014) and the American Heart Association (AHA) (O’Gara et al., 2013). Its use has remained restricted to only patients with intermittent claudication.

With its mechanism of action on the same pathway to that of P2Y₁₂ inhibitors, it is unlikely that cilostazol would be able to modulate endogenous fibrinolysis any differently than the established oral P2Y₁₂ inhibitors.

Conclusion

Coronary atherosclerosis is a progressive disease which spans the lifetime of an individual. The continuum of CCS interspersed with episodes of ACS increases the risk of mortality and morbidity. Despite current optimal treatments, about 10% of patients with ACS still experience a major adverse cardiovascular event in the year following an ACS. Endogenous fibrinolysis has been identified as a potential biomarker to risk stratify these patients to allow more targeted and potent treatments. There is a need to identify pharmacological treatments which can modulate this pathway to improve outcomes in these patients. Additionally, although the role that endogenous fibrinolysis plays is clear in the prediction of future events in patients with ACS, its role in other presentations of ACS is unclear. Through my thesis, I hope to address these issues and provide some direction for future studies exploring this interesting pathway and its impact on cardiovascular mortality and morbidity.

Chapter 2. Materials and Methods

Regulatory approval and funding

For my main project in Chapter 6 and 8 - Can Very Low Dose Rivaroxaban (VLDR) in addition to dual antiplatelet therapy (DAPT) improve thrombotic status in acute coronary syndrome (VaLiDate-R), I drafted the study protocol and documents, which were critically appraised and reviewed by my principal supervisor. This was subsequently reviewed internally by the Research and Development department of East and North Hertfordshire NHS Trust and submitted for ethical review via the Integrated Research Application System (IRAS), to the National Research Ethics Service (NRES) London – Hampstead Research Ethics Committee [IRAS ID: 246526, REC reference: 18/LO/1608], Medicines and Healthcare products Regulatory Agency (MHRA) [MHRA reference: 31057/0006/001-0001] and the NHS Research and Development office of East and North Hertfordshire NHS Trust in August 2018. Once favourable opinion was obtained in October 2018, the study was listed on Clinicaltrials.gov [Identifier: NCT03775746] and European Union Drug Regulating Authorities Clinical Trials Database [EudraCT: 2018-003299-11].

Recruitment commenced in January 2019 following formal approval. The study was carried out in accordance to the European Union and international standards of Good Clinical Practice (GCP) and monitored according to the pre-determined monitoring plan which includes an assessment by an independent clinician, regular on-site monitoring by an independent Trial monitor, 6 monthly reviews through the Data Safety Monitoring Board (DSMB) and Trial Steering Committee (TSC).

The process was carried out similarly with studies in Chapter 3, 4 and 5 where I drafted the study protocol and documents which were critically appraised and reviewed by my principal supervisor. Following internal review, it was submitted through IRAS to NRES [INSigHT – IRAS ID: 230520, REC reference: 17/LO/1885; Calcific potential – IRAS ID: 235341, REC

reference: 17/LO/2067; OHCA risk scoring – IRAS ID: 246483, REC reference: 18/SC/0355] and the Research and Development office of East and North Hertfordshire NHS Trust. The studies were only commenced after formal approval from all parties involved.

Appendix 2 contains all the documents of approval from the different regulatory authorities for each study. All further amendments had to go through the same procedure, requiring regulatory approvals before changes were implemented in the protocol.

Study population

My main study examined patients who were admitted with ACS (including those with STEMI, NSTEMI and UA) who exhibit impaired endogenous fibrinolysis to assess the impact different pharmacotherapies have on the fibrinolytic status. Within this cohort, I also investigated a selected subgroup of patients with myocardial infarction with non-obstructive coronary arteries or MINOCA.

Apart from patients with ACS, I explored participants with different cardiac risk profiles – from healthy volunteers to assess the individual variations in fibrinolytic profile, patients with suspected coronary artery disease (with different levels of coronary artery disease) and the association to fibrinolytic status to patients presenting with out-of-hospital cardiac arrest to risk stratify and prognosticate based on different variables.

Participant identification and consent process

VaLiDate-R

Patients were identified from the cardiac catheterisation suite and inpatient wards including the acute cardiac unit and acute medical units at East and North Hertfordshire NHS Trust, where patients with a heart attack are admitted routinely. Written informed consent was obtained by members of the research team with GCP qualification through a two-stage consent process comprising of a screening consent followed by a main study consent if eligible. The screening consent allowed for the initial blood test for assessment of fibrinolytic status to determine eligibility for randomisation.

In the case of patients presenting acutely to the hospital with STEMI requiring immediate emergency treatment with angiography and stenting, a delayed consent approach was undertaken, with ethical approval, whereby an extra 5ml of blood was taken in addition to routine clinical bloods for assessment of fibrinolytic status. This approach was considered appropriate via the NRES London – Hampstead Research Ethics Committee and the NHS Research and Development department of East and North Hertfordshire NHS Trust.

Following clinical stabilisation, these patients were approached with provision of the patient information sheet to obtain written consent for screening to allow informed decision making. Patients who declined to participate were excluded from the study and the samples were discarded. Patients that did not meet eligibility criteria were recorded as a screen fail. For patients who met the criteria for randomisation, full written full informed consent was obtained within 24 to 48 hours.

INSigHT

Healthy participants were recruited through posters placed throughout the hospital inviting volunteers to join the study where contact details were provided for the research office.

Participants who then made contact with the research team were provided with an invitation letter and Participant Information Sheet before written informed consent by a GCP trained member of the research team.

Calcific potential

Patients were identified through cardiology outpatient clinics and recent computed tomography coronary angiogram (CTCA) reports and approached with an invitation letter and participant information sheet. Written informed consent by a GCP trained member of the research team was obtained before recruitment into the study.

OHCA Risk Scoring

Eligible patient data were identified through hospital admission records where case notes of patients admitted following an OHCA were retrieved. As the data was accessed by physicians employed within the trust and fully anonymised with no identifiable data recorded, informed consent was not required to access clinical information. However, permission was obtained to undertake this as part of a formal service evaluation and audit, with internal approval at East and North Hertfordshire NHS Trust.

Blood sampling for tests of thrombosis

Blood was drawn through an 18G butterfly needle without application of a tourniquet, in order to avoid platelet activation. In patients presenting with STEMI for primary PCI, blood sampling is performed through the arterial line prior to administration of heparin. The initial 5ml blood was used for baseline tests and the second sample used for tests of thrombotic status. A two-syringe technique was used in order to collect the samples required for GTT and TEG.

The Global Thrombosis Test

Thrombotic status was assessed using the Global Thrombosis Test (Thromboquest Limited, London, UK), a point-of-care instrument that is fully automated (Figure 2.1). The instrument consists of 4 ports where disposable tubes are inserted. Each disposable tube consists of two parts, with the top part comprising of two plastic surfaces leading to a conical shape ending with two ceramic balls, and the lower part comprising of a clear glass reservoir (Figure 2.2). The instrument works at a temperature of 37°C i.e. physiological body temperature.

When the selected channel is primed and the operator injects 4 ml of native, non-anticoagulated blood, into one of the ports loaded with a disposable tube, the measurement is initiated in a fully automated process which comprises of 2 phases – the assessment of platelet reactivity / thrombus stability and endogenous fibrinolysis. Inside the tube, blood flows under the influence of gravity in the conical tube through narrow apertures where it is subjected to high shear at 180 dynes/cm². This induces platelet reactivity, resulting in thrombus formation and arrest of blood flow downstream (Figure 2.2). The instrument uses a photosensor to detect the time interval between consecutive drops that fall into the reservoir (d, sec). Once $d \geq 15$ sec then the instrument records this digitally as the Occlusion Time

(OT, sec). OT reflects platelet reactivity, corresponding with the initial formation of the primary haemostatic plug which is platelet induced. A short OT reflects enhanced platelet reactivity, whilst on the other hand, a long OT reveals reduced platelet reactivity. The instrument would not record an occlusion time if it has not occurred by 900 sec. This indicates that an occlusive thrombus was not formed under high shear which can commonly occur under the effects of thrombolytic medications.

Following this, the GTT-2 will continue to measure thrombus growth and thrombus stability under atmospheric pressures through the photosensor's appreciation of rebleeds (D, number). This is because as the thrombus propagates and stabilises to the point of complete occlusion, blood will continue to flow downstream and trigger the photosensor. A higher number of rebleeds corresponds to a less stable thrombus. There is a pre-set thrombus stabilisation period which is set at a default of 300 sec. During this "blinking" time, the photosensors are inactive following complete occlusion. This marks the completion of the platelet reactivity / thrombus stability phase of the measurement. If occlusion time has not occurred by 900 sec the instrument does not record OT.

Assessment of endogenous fibrinolysis occurs during the second phase and is denoted by the measure – Lysis Time (LT, sec). LT is the time required to spontaneously dissolve the thrombus formed in the first phase through endogenous fibrinolysis and is calculated as the time from the last drop before thrombus formation to the first drop after complete occlusion i.e. following the stabilisation period. A rapid and effective endogenous fibrinolysis is shown through a short LT whilst impaired endogenous thrombolysis is reflected in prolonged LT. If lysis does not occur by 6000 sec, which is the pre-set cut-off time, the instrument does not record LT.

The results are displayed on the screen of the machine and simultaneously recorded in a memory card and can be further analysed with the GTT-draw software, projecting the results in a graphical format (Figure 2.3).



Figure 2.1 Global thrombosis test and disposable tube

(Adapted from www.globalthrombosis.com)

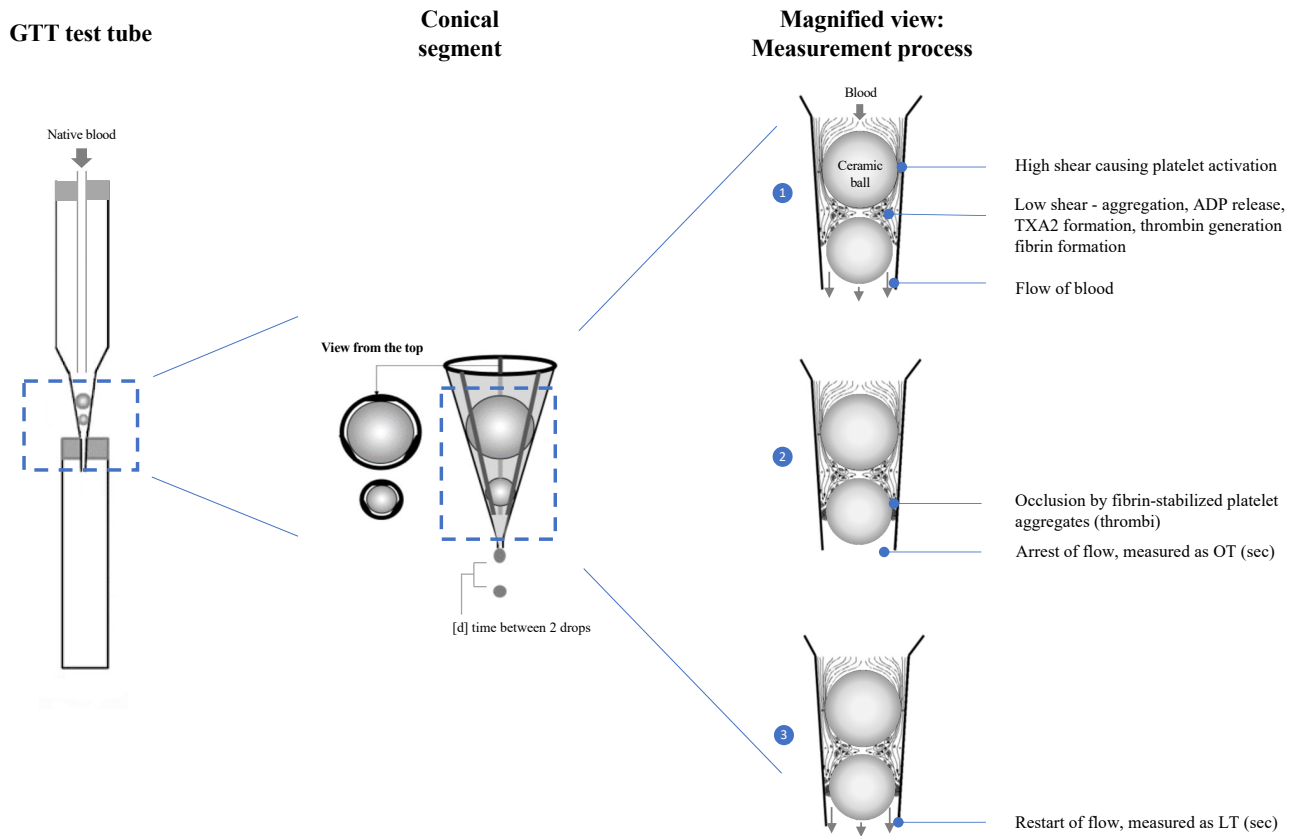


Figure 2.2 Basic principle of GGT

(Modified and adapted from www.globalthrombosis.com)

GGT test tube: top part comprising of two plastic surfaces leading to a conical shape ending with two ceramic balls, and the bottom part comprising of a clear glass reservoir

Conical segment: narrow apertures present between ceramic balls and walls of the tube which generates high shear, inducing platelet activation. Photosensor detects time interval between consecutive drops as d

Measurement process:

1. High shear promotes platelet activation and initiates the process of thrombus and fibrin formation. Blood flows through into the glass reservoir and is detected by the photosensor during this time.
2. Fibrin stabilised aggregate occludes the apertures, arresting flow into the reservoir. This is recorded in seconds as Occlusion Time or OT.
3. When the occluding thrombi breaks down through the process of endogenous fibrinolysis, blood flow is restarted and again detected by the photosensor. This is measured as Lysis Time or LT.

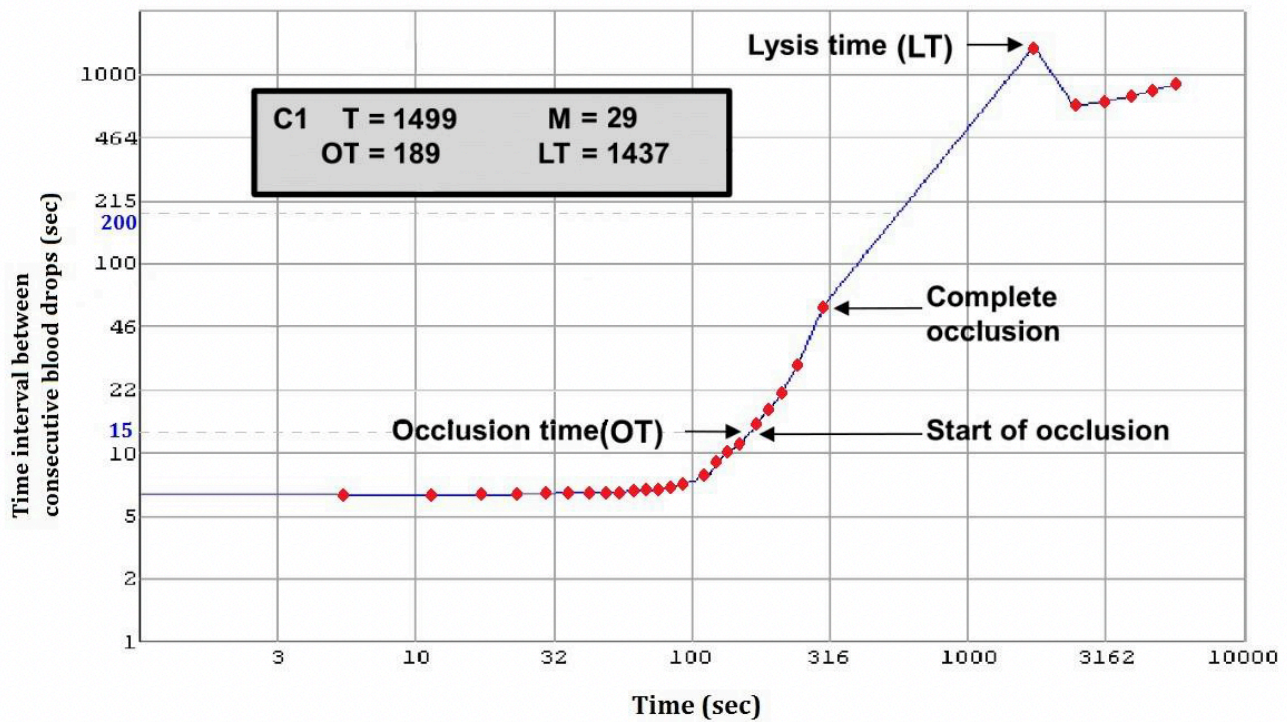


Figure 2.3 Graphical display of GTT analysis

(Adapted from www.globalthrombosis.com)

As the formation of thrombus begins, the interval between consecutive blood drops (d, sec) gradually increases. When d reaches ≥ 15 sec, the instrument records this as the occlusion time (OT, sec). The number of drops (D, number) following OT is measured, to assess thrombus stability until the complete occlusion. The instrument has a thrombus stabilisation period (300 seconds) during which the photosensors are inactive. The first drop detected after that period is recorded as the lysis time (LT, sec) indicating the spontaneous restoration of flow by endogenous fibrinolysis.

Reproducibility

The intra-assay and inter-assay coefficients of variation (CV) for OT and LT were assessed in 10 stable patients on repeated sampling (48 hours apart) and also running samples in parallel. The intra-assay CV for OT was 6% and for LT 8%, and the inter-assay CV was 7% for OT and 9% for LT. For most of the studies except when investigating diurnal variation, patients were sampled at close to 4 hours post dose of their medications to standardise the effect, minimise error and measure activity at its peak concentration.

Thromboelastography

A second method of evaluating thrombotic status, viscoelastic potential of the formed thrombus and its ability to lyse was by thromboelastography (TEG 5000 Hemostasis Analyser System, Haemonetics, UK). This was used in parallel with the GTT in patients recruited for the study.

The TEG 5000 comprises of two channels that uses disposable plastic cups and pins which are “loaded” onto a temperature controlled (37°C) platform (Figure 2.4). Blood is placed into the cup which oscillates constantly at a preset speed through an arc of 4°45 producing a 2.4° rotation (clockwise and anti-clockwise) every 10 second intervals (Figure 2.5). The pin, which is suspended in the loaded cup, is connected to a torsion wire which measures the differences in rotation and presents it in the form of a graph and numerical values on the computer (Figure 2.6). The instrument was calibrated using a Level I and Level II quality control samples monthly according to the manufacturer’s instructions.

The TEG 5000 is a point-of-care platelet function test that assesses the global coagulation properties of the clot under low shear stress. In the initial phase of the test, the instrument assesses the platelet independent clot formation and fibrinogen contribution to clot integrity [Reaction time (R, min), Kinetics (K, min), Angle (A, °), Maximum Amplitude (MA, mm), Time to Maximum Amplitude (TMA, min)]. In the second phase, it evaluates the primary fibrinolytic potential of the clot [Lysis 30 (LY30, %), Lysis 60 (LY60, %), Clot Lysis Time (CLT, min)] (Figure 2.6). A shorter R, K and TMA represents a hypercoagulable state whereas a lower A and MA represents a weaker clot associated with hypocoagulable states. On the other hand, a higher LY30, LY60 and a shorter CLT reflects a more unstable clot and a more effective endogenous fibrinolytic system at a low shear environment (Table 2.1).

Immediately following withdrawal of blood, 1ml of blood was placed in plastic tube with no agonists. A pre-set volume pipette was used to aspirate 0.36 ml of native whole blood into one port and the cup was loaded into the pin in order to begin the test in the channel. The whole analysis normally takes up to 120 minutes. At the end of the test all the disposable paraphernalia were discarded safely into a sharps box.



Figure 2.4 TEG 5000 and disposable cups and pins

(Adapted from www.teg.haemonetics.com)

A: The instrument consists of 2 ports that is plugged and connected to power constantly in order to maintain a temperature of 37°C. The two disposable cups are loaded into the ports shown.

B: Disposable cup and pin

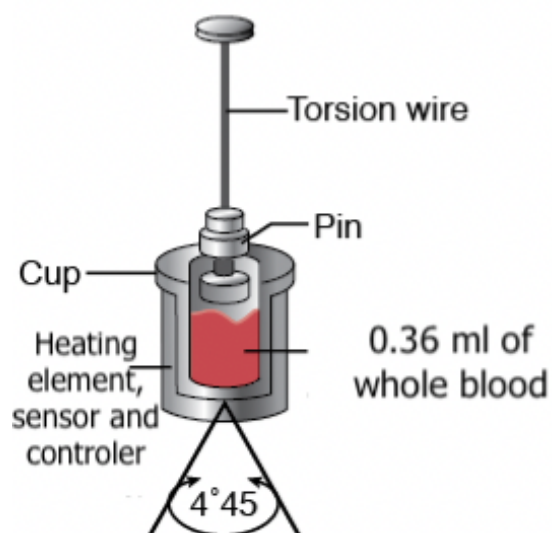


Figure 2.5 Principle of thromboelastography

(Adapted from www.teg.haemonetics.com)

A disposable pin connected to a torsion wire is suspended into the loaded disposable cup containing blood under a regulated temperature steady at 37°C. The cup oscillates constantly at a preset speed through an arc of 4°45' producing a 2.4° rotation (clockwise and anti-clockwise) every 10 second intervals which results in low shear stress conditions. The torsion wire picks up differences in rotation and translates the information into the computer.

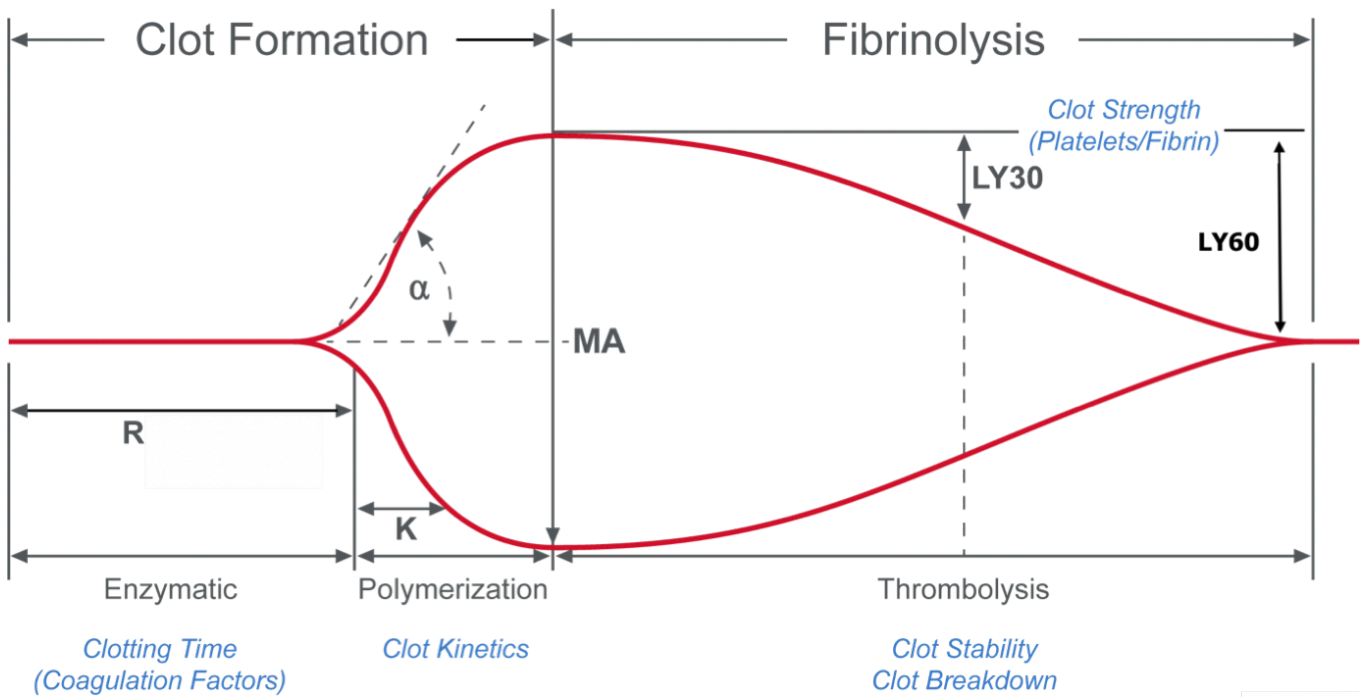


Figure 2.6 Graphical and numerical representation of results in TEG 5000

(Adapted from www.teg.haemonetics.com)

In the initial phase of the test, the instrument assesses the platelet independent clot formation and fibrinogen contribution to clot integrity [Reaction time (R, min), Kinetics (K, min), Angle (A, o), Maximum Amplitude (MA, mm), Time to Maximum Amplitude (TMA, min)]. In the second phase, it evaluates the primary fibrinolytic potential of the clot [Lysis 30 (LY30, %), Lysis 60 (LY60, %), Clot Lysis Time (CLT, min)]. The values (graphical and numerical) of these measurements are displayed and saved onto the software.

Table 2.1 TEG parameters

Assessment of clot formation	
Reaction Time (R), min	Measures the time from the start of a sample run until the first significant level of detectable clot formation Reduced in hypercoagulable state
Kinetics (K), min	Measures the time from R until fixed clot strength is reached Reduced in hypercoagulable state
Angle (A), degrees	Represents the rate of clot formation, reflecting fibrinogen activity Increased in hypercoagulable state
Maximum Amplitude (MA), mm	Represents whole clot strength, reflecting multiple aspects of clot formation (including platelet function, fibrin contribution to strength). Increased in hypercoagulable state
Time to Maximum Amplitude (TMA), min	Measures time to form maximum clot strength Reduced in hypercoagulable state
Assessment of fibrinolysis	
LY30, %	Represents percentage of clot lysed after 30 minutes of MA
LY60, %	Represents percentage of clot lysed after 60 minutes of MA
Clot Lysis Time (CLT), min	Measures time to 2mm amplitude reduction from MA

Reproducibility

The intra-assay and inter-assay CV for the TEG parameters were assessed in 10 participants on repeated sampling (48 hours apart) and also running samples in parallel for native whole blood assay. In detail, for the whole blood indices the inter-assay CV was 22% for R, 24% for K, 15% for A, 10% for MA, 56% for LY30, 45% for LY60, 33% for TMA and 23% for CLT. For the whole blood with kaolin activator the inter-assay CV was 19% for R, 21% for K, 11% for angle, 8% for MA, 43% for LY30, 61% for LY60, 24% for TMA and 20% for the CLT. The intra-assay CV for the whole blood indices was 27% for R, 21% for K, 15% for angle, 7% for MA, 47% for LY30, 67% for LY60, 12% for TMA and 15% for CLT. Similar to the GTT, patients were sampled at close to 4 hours post dose of their medications to standardise the effect, minimise error and measure activity at its peak concentration.

This was largely similar to a previously published paper showing whole blood inter-assay CV of 21.8% for R, 18.4% for K, 10.4% for A, 5.5% for MA and intra-assay CV of 16.4% for R, 24% for K, 14.3% for A, 7.8% for MA and 637.9% for LY30 (Ranjit et al., 2015).

Data collection

I examined the case notes and electronic records of all patients recruited into the studies and entered information into a database. There were 2 different databases used for the different studies. For studies in Chapters 3, 4 and 5, the databases were designed and built by me with the agreement of the principal supervisor using Microsoft Excel® following the “Good Clinical Practice” framework. For Chapters 6, 7 and 8, the database was designed by me and agreed upon by the sponsor before it was constructed and built by the team in University of Hertfordshire on Castor EDC®.

All confidential data was stored in anonymised format. Unique study ID numbers were assigned to every participant by the research team and henceforth identifiable information was completely anonymised. Appropriate Case Report Forms (CRFs) and electronic CRFs were prepared for the collection of the data as instructed by East and North Herts Research and Development department. All response variables were entered into the database which was kept in password protected NHS computers. Any written information was locked safely in the cardiology research offices at the East and North Herts Trust. Finalised data, in an anonymised electronic format, were transferred via an encrypted USB device in order to be analysed in a password protected computer at the University of Hertfordshire, as required.

A letter was subsequently sent to the general practitioner of the participating patients after their approval was sought. This letter along with all other documents were approved by the NRES and the local NHS Research and Development committee of East and North Hertfordshire NHS Trust (Appendix 2).

Patient follow up

Follow up was performed in accordance to the protocol as per each study detailed within each chapter. The follow-up appointments (face-to-face or telephonic) were undertaken by me or another adequately trained member of the research team who are able to carry out the follow up procedures which includes blood sampling and assessment of adverse events.

Statistical analysis

In all the studies described, the Shapiro-Wilk test is used to assess normality. Paired and unpaired t-tests were used for comparison of normally distributed variables and Wilcoxon signed-rank and matched-pair signed-rank test was used for non-normally distributed variables. Dichotomous variables were compared using Fisher's exact test. Correlations were analysed using Pearson's or Spearman's method. All tests were two-sided, and significance was taken as $p < 0.05$.

The analysis of variance (ANOVA) or Kruskal-Wallis test were used to assess differences between groups as appropriate. Non-parametric testing using the Friedman ANOVA test was employed with non-normally distributed variables. To investigate the relationship between variables and baseline characteristics, univariate and multivariate regression models were used. Where necessary post hoc analysis was performed in order to validate the model. Analyses were performed with Stata version 15.1 (StataCorp, College Station, TX, USA). Any study-specific analyses were mentioned in each chapter separately.

**Chapter 3. Assessing variation in
endogenous fibrinolysis within
healthy individuals**

Abstract

Diurnal variation in the presentation of acute thrombotic events such as cerebrovascular accident and acute myocardial infarction has previously been identified and attributed to the changes in autonomic nervous systems and thrombotic profiles. The suggestion that diurnal variation may exist stems from the historical observation that a large number of myocardial infarctions present in the early hours of the morning. Studies have previously shown the existence of diurnal variation of fibrinolytic factors, in particular tPA and PAI-1. However, whether such variation exists simply in these individual enzymes, or whether there is diurnal variation in overall thrombotic and thrombolytic status, is unclear.

I aimed to assess the variation in thrombotic and thrombolytic status in healthy volunteers. A total of 17 healthy volunteers had blood tests performed at two time points – between 0700 to 0900, and again between 1600 to 2000. Three further blood tests were performed on seven of the volunteers at weekly intervals to assess within individual variation over time.

There was no variation in thrombotic or thrombolytic status diurnally, or from week to week within the cohort of healthy volunteers, nor between male and female volunteers.

Thrombotic status appears not to display significant diurnal or weekly variation or difference by gender. However, due to the limitations of the study, particularly the small sample size, conclusions derived should be interpreted with caution. A larger cohort study in a well-controlled environment with more time-points for blood tests will be required to confirm the findings in this study.

Introduction

Endothelial dysfunction plays an important role in initiating arterial thrombosis. Endothelial dysfunction and the subsequent atherosclerotic plaque rupture, platelet aggregation, arterial thrombus formation and vessel occlusion lead to the clinical presentation of ACS. This stimulates thrombosis and initiates endogenous fibrinolysis.

Haemostasis represents the balance between thrombosis and fibrinolysis. This is a complex process comprising of many different factors, each playing a different role to achieve equilibrium. The process of thrombosis has been the focus of research, developing different ways to mitigate the risks of thrombosis including the use of antiplatelets and anticoagulants to reduce the risk of arterial thrombosis and its sequelae. Recently, the complementary process - endogenous fibrinolysis has gained traction as a potential biomarker to predict adverse outcomes in patients with ACS (Christopoulos et al., 2017; Farag et al., 2019; Sumaya et al., 2018).

Diurnal variation in thrombosis

The circadian rhythm is a natural, internal process that regulates the sleep-wake cycle over a 24-hour period. It controls various homeostatic and metabolic processes within the human body including temperature regulation and hormone secretion. The effect on the cardiovascular system is also evident and consequently affects the incidence of cardiovascular pathological events like MI (Muller et al., 1985).

Epidemiological studies have shown a morning peak occurrence of MI (between 6 am and noon) (Muller et al., 1985; Willich et al., 1989). This has been attributed to the changes in autonomic nervous systems and fibrinolytic profile over time (Manfredini et al., 1996) which in turn result in a transient increased risk during the morning hours. With regards to

thrombotic potential, studies have shown circadian variation in platelet aggregability (Guagnano et al., 2000; Haus et al., 1990), procoagulant factors and coagulation profile (Bertolucci et al., 2005; Haus et al., 1990; Soulban & Labrecque, 1989), fibrinolytic factors, in particular tPA and PAI-1 (Andreotti & Kluft, 1991; Angleton et al., 1989) and fibrinolytic activity (Rosing et al., 1970).

There appears to be a clear diurnal variation in thrombotic and fibrinolytic factors which persists in disease states (Andreotti & Kluft, 1991). This could provide the potential link to the diurnal variation in epidemiological findings barring the limitations of translating these individual factorial assays into clinical outcomes due to the complex interactions between these factors (Gorog, 2010). The presence of diurnal variation may potentially indicate a requirement to change timing of pharmacotherapy to target higher risk periods during the day to prevent thrombosis. The global assessment of the fibrinolytic pathway seems to better reflect overall fibrinolytic status than individual factors of the coagulation and fibrinolytic system, but circadian variation in global thrombotic status has not been assessed using contemporary methods of assessing global fibrinolytic activity.

Individual biological variation in endogenous fibrinolysis

Similarly, within the individual, biological variation in fibrinolytic status exists (de Maat et al., 1996) and can be affected by various factors including physical activity, lifestyle changes such as smoking and alcohol (Peace et al., 2009). The variation could have a bearing on trying to interpret longitudinal results and has not been previously investigated using current global tests of thrombosis.

Aim

I aimed to assess individual variation, including diurnal and weekly variation, in thrombotic status and endogenous fibrinolysis using point-of-care tests.

Methods

Study design and population

INSigHT (Assessing Individual variation in fibrinolytic Status in Healthy volunTeers) was a single centre prospective observational study involving healthy volunteers. The study was approved by the London Camden and King's Cross Research Ethics Committee and the relevant approvals are shown in Appendix 2 (Page 242 - 253).

Participants were recruited through posters within the hospital and through word of mouth. Following contact with the research team, participants were invited for an initial appointment to discuss the study and obtain written informed consent. Individuals who met the inclusion / exclusion criteria as shown in Table 3.1 were recruited.

Following full informed consent, participants provided details of their baseline demographics including age, gender, height and weight which were recorded. Blood pressure and heart rate were recorded on the first visit which was scheduled between 0700 to 0900. Blood was drawn during this first appointment for analysis of the baseline thrombotic status. Volunteers were then invited back for the second appointment between the hours of 1600 – 2000 for a repeat blood test. Participants who were willing were invited back once a week for weekly evaluation of their thrombotic profile (for a total of 3 further visits).

Assessment of thrombotic status

Thrombotic status was assessed using the point-of-care GTT (Thromboquest Ltd., London, UK) and TEG (Haemonetics Corporaion, USA) as detailed in Chapter 2.

Statistical analysis

Sample size was calculated using Stata version 15.1. Assuming $\alpha=0.05$ for two-sided tests and power of $1-\beta=0.80$ with a standard deviation of 100 identified from a previous study on normal volunteers (Saraf et al., 2010), a sample size of 20 was required to detect a change in $LT=70s$. However, due to the coronavirus disease 2019 (COVID-19) pandemic, recruitment was halted and a total of 17 participants were recruited for the analysis.

Baseline characteristics that are continuous variables are displayed as median values with interquartile ranges and binary variables are displayed as number and percentage. To assess for diurnal variation, given that both the occlusion time and lysis time are not normally distributed, paired comparison was performed using Wilcoxon matched-pairs signed-rank test. For the assessment of weekly variation, non-parametric testing using the Friedman ANOVA test was employed to investigate for significant differences between Week 1 to Week 4. Wilcoxon rank sum test was performed comparing the thrombotic profile between male and female volunteers to assess for gender differences in thrombotic status.

The analysis was repeated for parameters measured using TEG. Analyses were performed with Stata version 15.1 (StataCorp, College Station, TX, USA) and significance was taken at <0.05 .

Table 3.1 Inclusion and exclusion criteria for INSigHT

Inclusion	Exclusion
<ol style="list-style-type: none"> 1. Male and female participants aged 18 years or over. 2. The participant is willing and able to understand the Participant Information Sheet and provide informed consent. 3. The participant must agree to comply with the drawing of blood samples for the assessments. 4. The participant does not meet any of the exclusion criteria 	<ol style="list-style-type: none"> 1. Male and female participants aged < 18 years of age. 2. Participant actively using tobacco products (due to vasoactive and pro-aggregatory effects of nicotine). 3. The participant has any chronic medical conditions 4. The participant gives a history of substance abuse or demonstrates signs or clinical features of active substance abuse or psychiatric disease. 5. Alcohol consumption above recommended safe levels (i.e. more than 21 units per week for males, or more than 14 units per week for females) due to the potential effects of high alcohol levels on platelet reactivity. 6. Currently enrolled in an investigational device or drug trial. 7. Participant taking antiplatelet or anticoagulant medication 8. Participant taking medications (regularly or intermittently) that may, in the eyes of the investigators, affect fibrinolytic status (such as the oral contraceptive pill).

Results

17 volunteers were recruited into the study with seven of the volunteers participating in the weekly variation aspect of the study. The median age of the volunteers was 30 years (interquartile range (IQR) 29 – 33) with 9 males and 8 females. The median OT was 412.2 seconds and LT was 1781 seconds. The baseline characteristics of the volunteers and their thrombotic profiles measured using the GTT and TEG were summarised as shown in Table 3.2.

Comparison analysis was performed to identify differences in thrombotic status between male and female volunteers (Table 3.3). This showed no statistically significant differences between the thrombotic profiles of males and females except for TEG parameter of MA which was lower in the male cohort (57.1 [50.8 – 65.8] vs. 68.5 [62.4 – 74.6] mm, $p=0.0372$), indicating a less stable fibrin clot.

Diurnal and weekly variation

Paired comparison of the thrombotic profiles did not show any statistically significant diurnal variation in OT (morning OT 389.7[337.4 – 454.3] vs. evening OT 432.7[368.7 – 580.6] s, $p=0.2682$) and LT (morning LT 1939[1781 – 2097] vs. evening LT 1703.5[1588 – 1873] s, $p=0.1019$) within the cohort of healthy volunteers. Similarly, there was no difference in TEG measurements between morning and evening (Table 3.4). When assessing weekly variation over the period of 4 weeks, Friedman ANOVA test was conducted on the seven volunteers who returned for weekly blood tests. Results showed no significant difference between the OT ($Q(3)=0.7714$, $p=0.8563$) and LT ($Q(3)=3.8571$, $p=0.2773$) and indicating no evidence of significant weekly variation in the thrombotic profile in healthy volunteers. Likewise, thrombotic profile as measured with TEG did not show evidence of statistically significant variation (Table 3.5).

Table 3.2 Baseline characteristics and thrombotic profile of healthy volunteers

Demographics	
Age, years	30 (29 – 33)
Male	9 (52.9)
Female	8 (47.1)
Body mass index, kg/m ²	25 (22.9 – 27.5)
Observations	
Systolic blood pressure, mmHg	123 (120 – 130)
Diastolic blood pressure, mmHg	77 (74 – 81)
Heart rate, beats per minute	70 (67 – 75)
Global Thrombosis Test	
Baseline Occlusion time (OT), sec	412.2 (347.6 – 502.4)
Baseline Lysis time (LT), sec	1781 (1690 – 2031)
Thromboelastography	
Reaction time (R), sec	12.3 (6.95 – 15.6)
Kinetics time (K), sec	6 (3.3 – 7.8)
Angle (A), degrees	40.4 (29 – 45.7)
Maximum amplitude (MA), mm	63.6 (54 – 69.9)
Shear modulus strength (G), Kdynes/sec	8.7 (5.9 – 11.6)
Coagulation index (CI)	1.8 (0.4 – 3.1)
Lysis in 30 minutes (LY30), %	0 (0 – 0)

Lysis in 60 minutes (LY60), %	0.6 (0 – 1.5)
Time to maximum amplitude (TMA), sec	44.5 (33.4 – 50.6)
Clot lysis time (CLT), sec	60.8 (60.2 – 61.2)
Thrombodynamic potential index (TPI)	19.3 (7 – 44.9)

Table 3.3 Comparison thrombotic profiles at baseline in male and female volunteers

	Male (n=9)	Female (n=8)	p-value
Age, years	30 (29 – 31)	39.5 (27 – 56)	0.3320
Body mass index, kg/m ²	24 (22.3 – 25.5)	27 (23.6 – 28.7)	0.0920
Occlusion time (OT), sec	389.7 (337.4 – 454.3)	432.7 (368.65 – 580.55)	0.2682
Lysis time (LT), sec	1939 (1781 – 2097)	1703.5 (1588 – 1873)	0.1019
Reaction time (R), sec	12.3 (9.4 – 14.8)	12.9 (5.3 – 15.6)	0.9164
Kinetics time (K), sec	6.5 (3.8 – 8.1)	3.9 (1.1 – 6.7)	0.2239
Angle (A), degrees	30.3 (27.7 – 42.3)	45.1 (34.6 – 73.5)	0.0641
Maximum amplitude (MA), mm	57.1 (50.8 – 65.8)	68.5 (62.4 – 74.6)	0.0372*
Shear modulus strength (G), Kdynes/sec	6.7 (5.2 – 9.6)	10.9 (8.3 – 14.7)	0.0371
Coagulation index (CI)	1.1 (-0.8 – 2.7)	2.2 (1.4 – 4.9)	0.1824
Lysis in 30 minutes (LY30), %	0 (0 – 0.8)	0 (0 – 0)	0.7407
Lysis in 60 minutes (LY60), %	0.65 (0.1 – 1.7)	0 (0 – 1.1)	0.4733
Time to maximum amplitude (TMA), sec	41.7 (35.5 – 49.0)	45.5 (21.5 – 50.6)	0.9079
Clot lysis time (CLT), sec	61.1 (60.6 – 61.5)	60.5 (59.8 – 61.5)	0.1176
Thrombodynamic potential index (TPI)	11.9 (6.1 – 22.3)	25.6 (12.7 – 135.8)	0.0826

Table 3.4 Comparison of thrombotic profiles between morning and evening samples

	Morning (0700-0900)	Evening (1600-2000)	p-value
Occlusion time (OT), sec	412.2 (347.6 – 502.4)	456.2 (405.1 – 524.4)	0.102
Lysis time (LT), sec	1781 (1690 – 2031)	1726 (1517 – 1873)	0.227
Reaction time (R), sec	12.3 (6.95 – 15.6)	6.15 (4 – 15.1)	0.0858
Kinetics time (K), sec	6 (3.3 – 7.8)	3.7 (3.1 – 6.5)	0.5147
Angle (A), degrees	40.4 (29 – 45.7)	43.95 (30.4 – 54.9)	0.5147
Maximum amplitude (MA), mm	63.6 (54 – 69.9)	64.2 (58.2 – 68.8)	0.3743
Shear modulus strength (G), Kdynes/sec	8.7 (5.9 – 11.6)	8.95 (7 – 11)	0.3743
Coagulation index (CI)	1.8 (0.4 – 3.1)	2.85 (09 – 3.6)	0.7671
Lysis in 30 minutes (LY30), %	0 (0 – 0)	0 (0 – 1.7)	0.9032
Lysis in 60 minutes (LY60), %	0.6 (0 – 1.5)	1 (0 – 5.6)	0.8111
Time to maximum amplitude (TMA), sec	44.5 (33.4 – 50.6)	32.4 (25.5 – 49)	0.6784
Clot lysis time (CLT), sec	60.8 (60.2 – 61.2)	60.8 (59.6 – 61.3)	0.6784
Thrombodynamic potential index (TPI)	19.3 (7 – 44.9)	26.2 (13.5 – 39.6)	0.4413

Table 3.5 Friedman ANOVA test to assess for weekly variation of thrombotic profiles

Variable	Q (3)	p-value
Occlusion time (OT), sec	0.7714	0.8563
Lysis time (LT), sec	3.8571	0.2773
Reaction time (R), sec	3.4148	0.7454
Kinetics time (K), sec	0.9236	0.8197
Angle (A), degrees	3.6403	0.3030
Maximum amplitude (MA), mm	6.3943	0.0939
Shear modulus strength (G), Kdynes/sec	7.1935	0.0660
Coagulation index (CI)	6.6229	0.0849
Lysis in 30 minutes (LY30), %	2.8889	0.4091
Lysis in 60 minutes (LY60), %	2.2886	0.5147
Time to maximum amplitude (TMA), sec	0.2576	0.9678
Clot lysis time (CLT), sec	6.6229	0.0849
Thrombodynamic potential index (TPI)	0.7160	0.8694

Discussion

Diurnal Variation

When comparing between the morning and evening thrombotic status in the study, there was no statistically significant differences between the 2 time points (0700 – 0900 hours and 1600 – 2000 hours).

Within the literature, there are no published studies assessing the effect of diurnal variation on tests of global thrombotic status. However, there have been several studies exploring the impact of circadian rhythm on different coagulation and fibrinolysis factors and enzymes (Akiyama *et al.*, 1990; Andreotti *et al.*, 1988; Angleton *et al.*, 1989; Brezinski *et al.*, 1988; Budkowska *et al.*, 2019; Fujimura *et al.*, 1992; Haus *et al.*, 1990; Jafri *et al.*, 1992; Kapiotis *et al.*, 1997; Tofler *et al.*, 1987). Focusing on fibrinolytic factors, Angleton *et al.* investigated tPA activity and antigen levels with PAI-1 activity in 33 healthy men and 15 patients with coronary artery disease at 2 time points (0800 and 1900 hours). They showed a significantly lower PAI-1 activity and tPA antigen with a higher in tPA activity in the evening. There was also an inverse correlation identified between PAI-1 activity and tPA activity ($r=-0.57$, $p<0.0001$).

Akiyama *et al.* investigated 21 parameters of coagulation including tPA and PAI-1 in 16 healthy volunteers at 7 different time points (1600, 2000, 0000, 0800, 0900, 1200 and 1600 hours the next day). They found that levels of tPA antigen and PAI-1 antigen were lowest in the evening (1600 hours), reaching a peak at 0800 hours. The reverse was true for tPA activity, peaking at 1600 hours and lowest at 0900 hours. Andreotti *et al.* performed 3 hourly sampling in 6 healthy volunteers to identify fluctuations of PAI-1 activity and tPA activity. PAI-1 activity was lowest at 6pm, peaking at 3am whereas the opposite was true of tPA

activity. The activity between the 2 factors were complementary to each other, indicating a strong relationship between them.

One of the more recent papers is a study involving 66 healthy volunteers with bloods taken at 6 hourly-interval (0800, 1400, 2000, 0200 hours) to assess for variation in PAI-1 and tPA concentration. Budkowska *et al.* identified diurnal variation with highest level of PAI-1 at 0800 hours and lowest at 1400 hours again with a reversal with tPA concentration (Budkowska *et al.*, 2019). It is clear from these studies that the presence of circadian variation exists in the fibrinolytic factors (PAI-1 and tPA) of healthy volunteers. Potentially, the absence of diurnal variation in my cohort of volunteers could be explained by the timing of obtaining the blood samples. As the blood was drawn only at two time-points with a relatively large time window, the variation may not be apparent and therefore not seen in my study.

However, it is also possible that whilst there may be differences in levels in tPA and PAI-1 activity/antigen over the course of the day, it is possible that in fact overall thrombotic and thrombolytic status may not vary. Since this is the first study, to our knowledge, assessing global fibrinolytic status and diurnal variation, the data presented could be representative of the true nature of the overall thrombotic profile of the healthy volunteers. Although the circadian variation in individual fibrinolytic factors, especially tPA and PAI-1, is apparent in healthy volunteers, the overall thrombotic status, balanced out by other factors within the complex coagulation and fibrinolytic system during the state of health, may not alter.

Weekly variation

The study showed that thrombotic profile in healthy individual does not show significant variation in the short-term i.e. week to week. Short-term variability has not been reported in the literature. In studies comparing fibrinolytic factors tPA and PAI-1 in different age groups,

elderly individuals seems to have higher levels of tPA antigen and PAI-1 activity (Aillaud et al., 1986; Hashimoto et al., 1987; Rånby et al., 1986). In a large Swedish cohort study involving 272 subjects aged 30-60 years, the authors identified a weak positive correlation between age and tPA antigen in both males and females (Sundell et al., 1989). Although a similar correlation with PAI-1 activity was identified in, it was not seen in males. A further study identified that the correlation in PAI-1 only became apparent with the inclusion of younger participants (about 20 years old) (Rånby et al., 1990). Therefore, with a median age of 30 years in my study, the difference in fibrinolytic activity may not be apparent. Also, given the weak correlation between age and fibrinolytic activity in the literature, it is highly likely that there is little short-term variability as supported by the data.

Gender differences

In this small study assessing the fibrinolytic status in healthy volunteers using bedside clinical tests, there was no statistical significance between the thrombotic profile between male and female healthy volunteers except for MA (representing overall clot stability) which is higher in females. The age and body mass index (BMI) were comparable between the genders within the cohort of volunteers and the time of blood draw was consistent (between the hours of 0700 – 0900). In a large cohort study involving 1288 healthy volunteers (Eliasson et al., 1993), the levels of PAI-1 activity was largely similar except between the age group of 45 – 54 years old where women showed a significantly lower activity level than men. In terms of bedside testing, one study employing TEG showed that females were more hypercoagulable than men with a lower K (1.8 vs 2.5 min, $p < 0.0001$), higher angle (65.5 vs 57.6°, $p < 0.0001$), MA (62.8 vs 58.0mm, $p < 0.0001$) and coagulation index (-0.1 vs -1.9, $p < 0.0001$) (Scarpelini et al., 2009). Except for clot stability, which was higher in females, the results obtained from my cohort of volunteers was different to this earlier report. This difference could potentially be explained by the difference in technique where Scarpelini *et*

al. used citrated blood with kaolin used as an activator whereas in my study native blood with no additives or agonists were used. A study looking at the agonistic effect of platelet aggregation (using adenosine-5'-diphosphate, arachidonic acid, epinephrine and collagen) showed gender differences, with females showing a greater platelet activation irrespective of agonist used (Otahbachi et al., 2010). Although kaolin was not tested, it can impact on platelet aggregation (Hardisty & Hutton, 1965) which could potentially be exaggerated in females. The difference in response to external agonist, with females showing an exaggerated response could explain the significant differences identified by Scarpelini *et al.*, which was not seen in my study.

An interesting study (Kain et al., 2003) explored differences in PAI-1 activity according to gender in 546 stroke patients (292 males and 254 females) and 459 controls (187 males and 272 females). Kain *et al.* showed that in healthy controls, there were no significant difference in PAI-1 activity according to gender (9.9 U/mL vs 9.1 U/mL, $p=0.38$). However, when the measurement was performed within 48 hours of the diagnosis of an acute ischemic stroke, females showed significantly higher level of PAI-1 activity than men (14.6 U/mL vs 10.8 U/mL, $p<0.0001$). This study highlighted a potential interesting point, that as opposed to an intrinsic difference in fibrinolytic factors, it was the difference in gender-specific response to a diseased state that could account for differences in endogenous fibrinolysis. The more impaired thrombotic status in females when compared to males in disease states is also supported by a study in patients with non-insulin dependent diabetes mellitus, where females tended to have higher levels of PAI-1 activity than men (25.6 vs 17.0 U/mL, $p<0.0005$) (Mansfield et al., 1996) and another study assessing fibrin clot analysis which identified that females with type 2 diabetes have denser clot structure and longer lysis time when compared with males (Alzahrani et al., 2012).

These studies suggest that apart from differences in response to agonists, females may have a propensity towards a worse fibrinolytic profile during disease states when compared to males, but the differences may not be apparent in health.

Limitations

There are several important limitations in this study. Firstly, the study comprises of a very small sample size and therefore conclusions drawn from the study must be taken with caution. A larger sample size would provide more robust and reliable results. Secondly, the presence of only 2 specified time points to assess for diurnal variation limits reliability of conclusions drawn from the study. Ideally more frequent sampling to assess variation over a 24-hour period would provide a better assessment of the effect of circadian rhythm on the fibrinolytic profile. The lack of more time points for assessment of thrombotic status throughout the day could explain the absence of diurnal variation from my study cohort purely due to the wrong timing for sampling. The particular time windows were broad and could have missed a peak within this, due to spread of sampling times within the window. Thirdly, the definition of 'healthy' without comprehensive investigation could be misleading. Volunteers could potentially have asymptomatic disease which is not known and taken medications without informing us. The age of the cohort of volunteers within the study is significantly below from the mean presentation age of patients with coronary artery disease, therefore the comparison should be interpreted with caution. Lastly, lifestyle factors such as diet and exercise which can have an impact on thrombotic status were not explored in this study. Both the lack of baseline assessments such as blood test panel or other investigations to assess for underlying vascular disease and consideration of lifestyle factors such as diet and exercise could potentially confound the results obtained.

Conclusion

There appears to be no diurnal variation in thrombotic or fibrinolytic status, nor does it display significant variation from week to week. A larger cohort study in a well-controlled environment with more sampling time-points will be required to confirm the findings in this study.

**Chapter 4. Endogenous
fibrinolysis and severity of
coronary artery disease**

Abstract

Atherosclerosis is a multifactorial disease initiated by lipid accumulation within the arterial intima. The process occurs in stages and in a cyclical pattern with rupture, thrombosis and healing resulting in multiple layers of tissues in different stages within the plaques which can cause stenosis with progressive narrowing of the lumen or atherothrombosis from plaque rupture. There is evidence that increasing severity of atherosclerosis may be associated with increased pro-thrombotic tendency, with data showing increased platelet reactivity associated with increasing luminal narrowing and blood flow under high shear.

Currently, CTCA is the best non-invasive method of assessing plaque burden and severity of coronary atherosclerosis, since it can quantify the severity of any coronary stenosis.

However, it carries the risk of contrast administration, which is associated with a small risk of contrast allergy and contrast nephropathy, and excess X-ray radiation. CT coronary artery calcium scoring (CACS) carries less risk, since much smaller amount of radiation is used and no contrast is given, but it does not assess the severity of luminal stenosis. However, CACS has been shown to correlate well with CTCA with regards to likelihood of obstructive disease. To assess the relationship between the severity of coronary atherosclerosis (as defined by CT) and thrombotic status, in particular endogenous thrombolytic status, I conducted a study exploring the degree of calcification and stenosis within the coronary artery and its relationship to fibrinolytic status in patients with suspected coronary artery disease.

I recruited 80 patients with suspected coronary artery disease investigated with CT. Patients had a CT coronary calcium score and had their fibrinolytic status assessed using point-of-care testing. Results showed that platelet reactivity and endogenous fibrinolysis is unrelated to

extent of coronary calcification, but endogenous fibrinolysis is more impaired in patients with obstructive disease (1865 [1636 – 2256] vs. 2524 [2425 – 2623] s, p=0.0335).

In summary, the severity of maximal coronary stenosis, but not the extent of CACS, is related to the effectiveness of endogenous fibrinolysis at high shear *in vitro*, with patients with more severe stenoses exhibiting less efficient fibrinolysis.

Introduction

Role of endogenous fibrinolysis in development of atherosclerosis

Atherosclerosis is a multifactorial disease initiated by lipid accumulation, in the form of LDL within the arterial intima, where they are oxidised by free radicals, promoting an inflammatory process (Insull, 2009). This, in the presence of endothelial dysfunction, attracts monocytes, which transform to macrophages, into the intima to take up lipid and become foam cells. These foam cells undergo apoptosis and necrosis, further propagating the inflammatory process, resulting in accumulation of more foam cells, distorting the normal architecture of the intima until it is completely disrupted. A fibrous cap forms over the lipid-rich necrotic core, which is prone to rupture, thereby exposing the thrombogenic interior arterial wall. This leads to the process of atherothrombosis and, if occurs in the coronary artery, its resultant clinical presentation of myocardial infarction.

The processes of thrombosis and fibrinolysis play an important role, not only in the pathophysiology of acute coronary syndromes but also in the process of atherogenesis, especially in the progression of disease (Duguid, 1946; Meade et al., 1993; Salomaa et al., 1995). The normal function of healthy endothelium includes regulating vascular tone, exerting anticoagulant, antiplatelet and fibrinolytic effects (Davignon & Ganz, 2004). Endothelial dysfunction can lead to increased endothelial permeability, platelet aggregation and generation of cytokines causing progression of atherosclerosis and impaired fibrinolytic activity can contribute to it. The relationship between fibrinolytic factors and the process of atherosclerosis and clinical outcomes is contentious (Folsom et al., 2001; Juhan-Vague et al., 2002; Ridker et al., 1993, 1994; Salomaa et al., 1995; Wilhelmsen et al., 1984).

Detection of coronary atherosclerosis

In patients presenting with suspected CAD, invasive coronary angiography, utilising x-ray fluoroscopy and radio-opaque contrast to determine the severity of luminal obstruction is arguably still considered the gold standard for assessing the severity of coronary atherosclerosis. However, the relative balance of procedure-related potential complications and the overall low diagnostic yield of coronary angiography (Patel et al., 2010) suggest application of this test exposes a number of individuals to unnecessary risk, lending support to pursuing a safer, non-invasive initial investigative alternative.

The presence and extent of coronary artery calcification and the severity of disease on CTCA has been shown to be predictive of future MACE (Hou et al., 2012) with a corresponding increase in risk according to degree of coronary calcification as measured using the CACS.

This has contributed to the current National Institute of Health and Care Excellence (NICE) and ESC guidelines recommending the use of CTCA as a first line investigation for stable patients with suspected CAD (Knuuti et al., 2020; NICE, 2016), which can be used to diagnose and risk stratify patients with suspected CAD.

However, the use of CTCA carries its own risk with higher levels of radiation [2-5 mSv vs. 0.2-0.4 mSv] (Williams et al., 2019) and requires the use of intravenous contrast agents when compared to CACS. Given that CACS correlates well with CTCA and is reflective of a patient's overall coronary atherosclerotic plaque burden (Bajraktari et al., 2013; Williams et al., 2020) it would serve well as a surrogate marker of overall severity of CAD.

Flow and shear stress relating to thrombus and fibrinolysis

Patients with obstructive coronary artery disease exhibit abnormal blood flow across severely stenotic lesions, with high shear across the lesion and significantly disturbed flow immediately post (Sakariassen et al., 2015). High shear rate haemodynamic conditions

promote thrombus growth with rapid platelet aggregation (Casa et al., 2015), driven predominantly by vWF. This results in platelet rich thrombi (white thrombus) (Sakariassen et al., 2015) with presence of adverse clot features i.e. more compact fibrin fibres (Brass & Diamond, 2016; Campbell et al., 2010), making it more resistant to lysis (Spinthakis, Gue, Farag, Ren, et al., 2019). Impaired fibrinolytic factors have been more consistently shown to be correlated to severity of stenosis of coronary arteries with a few studies comparing individuals who have an assessment of coronary atherosclerosis, using invasive coronary angiography, to matched healthy volunteers (Koenig et al., 2001; Olofsson et al., 1989; Paramo et al., 1985; Silveira et al., 2000) or angiographically confirmed normal coronaries (Fernandes et al., 2015; Lima et al., 2007, 2012; Paramo et al., 1985; Schroeder et al., 2002).

In general, these studies appear to show some correlation between impaired fibrinolysis and severity of stenosis, and to some extent atherosclerotic burden (Table 4.1) but conclusions appear debatable. This could be partly explained by the mixed cohort of the population studied and/or the different types of biomarkers used in each study which have correlate variably with clinical outcomes.

Recently, the point-of-care testing of overall fibrinolytic status using the GTT (Thromboquest Ltd., London, UK), which assesses native blood *in-vitro* under high shear, has shown potential in predicting adverse outcomes in patients presenting with ST-segment myocardial infarction (Farag et al., 2019). It was the aim of my study here to determine if there is a correlation between severity of CAD and overall fibrinolytic status.

Table 4.1 Papers assessing different factors of fibrinolysis and CAD

Paper	Fibrinolytic factors tested	Assessment of CAD (n)	Association with CAD
(Schroeder et al., 2002)	TAFI antigen (ELISA)	Invasive coronary angiography for all subjects (n=496)	Significantly higher in patients with CAD compared to normal No correlation between number of plaques or number vessel disease
(Fernandes et al., 2015)	PAI-1 level (ELISA)	Invasive coronary angiography for all subjects (n=123)	Significantly higher with severe CAD compared with moderate/mild/normal Significantly higher with >70% vs <70% stenosis No significance between number of vessels diseased
	TAFI level (ELISA)		No significance with severe CAD compared with moderate/mild/normal Significantly higher with >70% vs <70% stenosis No significance between number of vessels diseased
(Lima et al., 2012) (Lima et al., 2007)	D-dimer	Invasive coronary angiography for all subjects (n=57)	No significance between different severity of stenosis
	Fibrinogen level		Significantly higher in patients with severe stenosis compared with mild/moderate or normal
	Plasminogen		No significance between different severity of stenosis
(Paramo et al., 1985)	PAI activity	Invasive coronary angiography for all subjects (n=118)	No significant difference between different severity of stenosis (severity defined by computer assisted scoring system evaluated using narrowing, length, number and site of stenoses)
		Invasive angiography (n=118) vs matched healthy (n = 57)	Significantly higher levels in patients with CAD

(Koenig et al., 2001)	D-dimer	Invasive coronary angiography (n=312) vs matched healthy (n=477)	Significantly higher levels when comparing CAD to normal
(Silveira et al., 2000)	TAFI level	Invasive coronary angiography (n=110) vs matched healthy (n=56)	Significantly higher in patients with CAD compared to normal.
(Oolofesson et al., 1989)	tPA antigen	Invasive coronary angiography (n=214) vs randomly selected elderly subjects (n=65)	Significantly higher in patients with CAD compared to control group
	PAI activity		Significantly higher in patients with CAD compared to control group
	Lp(a)		No significant difference identified
	Fibrinogen		No significant difference identified

CAD: Coronary artery disease; Lp(a): Lipoprotein (a); PAI: Plasminogen activator inhibitor; TAFI: Thrombin-activatable fibrinolysis inhibitor; tPA: tissue plasminogen activator; ELISA: Enzyme-linked immunosorbent assay

Aim

I aimed to assess the relationship between markers of thrombosis and fibrinolysis with the extent of coronary artery calcification and severity of coronary stenosis on CTCA.

Methods

Study design and population

This was an observational study which investigated 80 patients who had undergone a CTCA with CACS (for clinical indication due to presenting with chest pain) within 12 months of the recruitment date. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the National Research Ethics Service and the UK Health Research Authority as shown in Appendix 2 (Page 234 - 241). Patients were identified by through the cardiology referral pathway for cardiology clinics and recent CTCA reports. These patients were invited to participate in this observational study. All patients gave written informed consent prior to inclusion in the study.

The following exclusion criteria were applied: age < 18 years, presence of renal impairment (eGFR < 60ml/min), currently enrolled in another clinical trial of any investigational drug or device, unwilling or unable to provide informed consent, and those on oral anticoagulation or known malignancy.

Patients were recruited based on the results of their CACS into 4 groups. The first group consisted of 20 patients with CACS of 0. The recruitment and allocation of patients into the remaining 3 groups was dependent on the calcium score and the gender, age and ethnicity adjusted centile calculated using the Multi-Ethnic Study of Atherosclerosis (MESA) calculator (McClelland et al., 2006) with 20 patients in each of the second to fourth quartiles.

The degree of maximal stenosis was defined as the highest level of coronary luminal obstruction estimated on the CTCA. This was divided into 4 groups – no obstructive disease (0%), mild (1 – 49%), moderate (50 – 69%) and severe ($\geq 70\%$) luminal stenosis. The CTCA was reported by a consultant radiologist independent of the study.

Data collection and patient follow-up

Patients were recruited following their CTCA scan and brought in for their visit. Following informed consent, data concerning basic demographics, medical and social history were recorded on the visit. Results of the CTCA including calcification score and maximal coronary stenosis were also recorded in the CRF. No follow up visits were required.

Blood test

As part of the study protocol, approximately 30mls of blood were obtained. The first 5ml were used to assess global thrombotic status. The remaining blood samples were used for basic biochemical testing and biomarker profiling.

Assessment of thrombotic status

Fibrinolytic status was assessed using the point-of-care GTT (Thromboquest Ltd., London, UK), which assesses both platelet reactivity to high shear stress and endogenous fibrinolysis as explained in detail in Chapter 2. In brief, the test was performed on native, non-anticoagulated whole blood within 15 s of withdrawal. The instrument assesses time taken to form an occlusive thrombus under high shear – recorded in seconds as occlusion time or OT and the restart of flow due to endogenous fibrinolysis – recorded in seconds as lysis time or LT.

Statistical analysis

As there were no data available to perform a power calculation, this study was performed as a pilot study. Approximately 20 patients in each group was felt to provide adequate numbers for between group comparisons and pilot data (Julious, 2005). Data are presented as median (interquartile range) as they are non-normally distributed. Dichotomous variables were compared using Fisher's exact test. Paired comparison of continuous variables between groups were evaluated with Wilcoxon ranked sum test and Kruskal Wallis rank test.

Statistical significance was defined as $p < 0.05$. Analyses were performed with Stata version 15.1 (StatCorp, College Station, TX, USA).

Results

A total of eighty patients were recruited, specifically 20 patients from each CACS quartile (adjusted for age, gender and ethnicity) as previously described. Basic demographics, medical and social history are as shown in Table 4.2. There was a statistically significant difference in the baseline age and number of patients with hypertension between the quartiles. The use of aspirin and statins were significantly different between the quartiles, with significantly lower use of both in the first quartile.

Correlation of variables

Spearman's rank correlation analysis utilising all the variables showed a significant positive correlation of CACS with age ($r=0.4472$, $p<0.0001$) and aspirin use ($r=0.2868$, $p=0.0099$). A significant positive correlation between OT with use of statin ($r=0.2999$, $p=0.0069$). There was also a significant negative correlation between LT and OT ($r=-0.3356$, $p=0.023$).

Multivariate regression analysis

A multivariate regression was performed using OT as the dependent variable and other variables which potentially may be a confounder (age, gender, BMI, smoking status, use of aspirin, calcium channel blockers, statins, LT and CACS). The model showed that the use of statin ($\beta=59.68$, $p=0.02$) and LT ($\beta= -0.075$, $p=0.015$) was able to significantly predict OT.

When LT was used as the dependent variable, only OT was able to significantly predict LT ($\beta= -1.07$, $p=0.015$).

Relationship between coronary calcification and thrombotic status

Platelet reactivity (430.3 [339.1 – 477]s vs 458.4 [390.9 – 498.9]s vs 409.2 [351.5 – 487.6]s vs 413.1 [353.8 – 495.6]s, $p=0.76$) and endogenous fibrinolysis (1753.5 [1548 – 2161.5]s vs

1808.5 [1635 – 2290.5]s vs 2110.5 [1838 – 2312]s vs 1846 [1665.5 – 2089.5]s, $p=0.253$) were similar in the 4 quartiles of CACS. Furthermore, there was no difference in platelet reactivity ($p=0.829$) or endogenous fibrinolysis ($p=0.561$) when comparing patients within the lowest and the highest quartiles of CACS.

When comparing patients based on the absolute values of CACS in 2 groups: CACS 0 and >400 , there was no difference between platelet reactivity (430.3 [339.1 – 477]s vs 424.5 [385.9 – 537.9]s, $p=0.58$) or endogenous fibrinolysis (1753.5 [1548 – 2161.5] vs 2021 [1592 – 2425]s, $p=0.47$) between the two.

Difference in fibrinolytic status (maximal stenosis)

Patients were further divided into 4 groups according to maximal severity of coronary stenosis on CTCA (Table 4.3). Similarly, there was a statistically significant difference in the baseline age and number of patients with hypertension between the 4 groups.

Patients with the greatest severity of stenosis exhibited the least efficient endogenous fibrinolysis (1728 [1512-2102]s vs 2028 [1687-2288]s vs 1728 [1634-1927]s vs 2524 [2425-2623] , $p=0.04$) whilst platelet reactivity (438 [341-479]s vs 415 [357-484]s vs 444 [384-504]s vs 391 [357-425]s, $p=0.907$) appeared unrelated to severity of coronary stenosis. The relationship between endogenous fibrinolysis and degree of maximal stenosis was non-linear i.e. the level of impairment was not directly correlated to degree of maximal stenosis.

When comparing endogenous fibrinolysis in clinically non-flow limiting ($<70\%$) versus clinically significant maximal stenosis i.e. maximal luminal reduction of $\geq 70\%$, the LT remained significantly longer in those with severe stenosis compared to those without (2524 [2425 – 2623] vs. 1865 [1636 – 2256] s, $p=0.0335$).

Table 4.2 Demographics, fibrinolytic status and CACS according to quartile

	1st Quartile n=20	2nd Quartile n=20	3rd Quartile n=20	4th Quartile n=20	p-value
Age, year	57 (50.5 – 62.5)	65 (61.5 – 75)	63.5 (56 – 70.5)	59 (52.5 – 67.5)	p=0.0028*
Male, n (%)	9 (45)	14 (70)	14 (70)	9 (45)	p=0.191
BMI, kg/m ²	25 (24 – 32.5)	27.4 (26 – 30.6)	26.3 (23.3 – 31)	28.8 (26.5 – 32.6)	p=0.191
Current smoker, n (%)	3 (15)	1 (5)	2 (10)	4 (20)	p=0.676
Hypertension, n (%)	4 (20)	12 (60)	6 (30)	11 (55)	p=0.028*
Diabetes, n (%)	1 (5)	2 (10)	1 (5)	4 (20)	p=0.507
eGFR, ml/min	80.5 (70.5 – 88)	75.5 (67.5 – 88.5)	81.5 (72 – 90)	84 (74.5 – 90)	p=0.3430
Medication, n(%)					
Aspirin	0 (0)	5 (25)	7 (35)	5 (25)	p=0.0483*
Statins	6 (30)	10 (50)	9 (45)	15 (75)	p=0.0403*
CCB	2 (10)	8 (40)	4 (20)	5 (25)	p=0.1638
Betablockers	2 (10)	5 (25)	2 (10)	3 (15)	p=0.508
PPI	3 (15)	4 (20)	2 (10)	5 (25)	p=0.6347
Metformin	1 (5)	2 (10)	0 (0)	5 (25)	p=0.0531
ACE-I / ARB	3 (15)	9 (45)	5 (25)	6 (30)	p=0.2106
OT, sec	430.3 (339.1 – 477)	458.4 (390.9 – 498.9)	409.2 (351.5 – 487.6)	413.1 (353.8 – 495.6)	p=0.76
LT, sec	1753.5 (1548 – 2161.5)	1808.5 (1635 – 2290.5)	2110.5 (1838 – 2312)	1846 (1665.5 – 2089.5)	p=0.253
CACS	0 (0 – 0)	17 (6 – 51.5)	70.3 (27 – 111.5)	192.6 (70.5 – 413.5)	p=0.0001*

BMI: Body mass index, eGFR: estimated glomerular filtration rate, CCB: Calcium channel blockers, PPI: Proton pump inhibitors, ACE-i: Angiotensin converting enzyme inhibitors, ARB: Angiotensin receptor blockers, OT: Occlusion time, LT: Lysis time, CACS: CT coronary artery calcium scoring

Table 4.3 Demographics, fibrinolytic status and CACS according to maximal stenosis

	No stenosis (0%) n=21	Mild stenosis (1 - 49%) n=44	Mod. stenosis (50 - 69%) n=13	Severe stenosis (≥70%) n=2	p-value
Age, year	57 (51 – 62)	64 (54 – 70.5)	64 (58 – 68)	63.5 (45 – 82)	p=0.018*
Male, n (%)	11 (52)	17 (39)	6 (46)	0 (0)	p=0.56
BMI, kg/m ²	25 (24 – 31.9)	28.2 (25.3 – 32)	26.1 (25.3 – 29)	24.6 (22.8 – 26.3)	p=0.4225
Current smoker, n (%)	3 (14)	5 (11.4)	2 (15)	0 (0)	p=0.856
Hypertension, n (%)	4 (19)	18 (41)	8 (62)	2 (100)	p=0.045*
Diabetes, n (%)	1 (4.8)	4 (9)	3 (23)	0 (0)	p=0.428
eGFR, ml/min	82 (72 – 90)	80.5 (70.5 – 89.5)	79 (74 – 90)	90 (90 – 90)	p=0.3788
Medication, n(%)					
Aspirin	1 (4.8)	9 (20.5)	5 (38.5)	2 (100)	p=0.0047*
Statins	7 (33)	23 (52.3)	9 (69.2)	1 (50)	p=0.2315
CCB	3 (14.3)	11 (25)	5 (38.5)	0 (0)	p=0.360
Betablockers	2 (9.5)	6 (13.6)	3 (23.1)	1 (50)	p=0.3756
PPI	3 (14.3)	7 (15.9)	4 (30.8)	0 (0)	p=0.5302
Metformin	1 (4.8)	4 (9.1)	3 (23.1)	0 (0)	p=0.3434
ACE-I / ARB	3 (14.3)	12 (27.3)	7 (53.8)	1 (50)	p=0.0878
OT, sec	438 (341-479)	415 (357-484)	444 (384-504)	391 (357-425)	p=0.907
LT, sec	1728 (1512-2102)	2028 (1687-2288)	1728 (1634-1927)	2524 (2425-2623)	p=0.040*
CACS	0 (0 – 0)	57.5 (17 – 132)	79.5 (30 – 227.2)	396.7 (374.3 – 419)	p=0.0001*

Pairwise comparison of LT (Dunn’s Test) showed significance only between severe stenosis and no stenosis (p=0.0483)

BMI: Body mass index, eGFR: estimated glomerular filtration rate, CCB: Calcium channel blockers, PPI: Proton pump inhibitors, ACE-i: Angiotensin converting enzyme inhibitors, ARB: Angiotensin receptor blockers, OT: Occlusion time, LT: Lysis time, CACS: CT coronary artery calcium scoring

Discussion

In this pilot study to assess the relationship between markers of thrombosis at high shear with degree of atherosclerosis as measured by CTCA, I showed an inverse association between maximal severity of coronary artery stenosis and effectiveness of endogenous fibrinolysis – patients with severe stenosis exhibit more impaired endogenous fibrinolysis compared with the rest. Although it does not appear to have a linear relationship, the presence of clinically significant i.e. ($\geq 70\%$) stenosis was associated with statistically significant impaired fibrinolysis when compared with patients without a significant epicardial artery stenosis. This is largely in keeping with previous studies exploring markers of fibrinolysis when comparing patients with angiographically proven stenosis and unobstructed arteries. There appeared to be no correlation between platelet reactivity or endogenous fibrinolysis and CACS (both in terms of raw score and quartiles adjusted to gender and ethnicity).

The presence of stenotic lesions within the coronary tree sufficient to cause disturbance of laminar blood flow, can lead to impaired fibrinolysis and in turn promote endothelial fibrin deposition (Glise et al., 2019) and may propagate the vicious cycle of atherosclerosis (Figure 4.1).

Activation of TAFI, in the presence of thrombin, results in reduced plasminogen binding to fibrin, leading to increase in lysis time. Schroder *et al.* found TAFI antigen to be significantly higher in patients with CAD when compared to patients with unobstructed coronary arteries and similar to my data, no correlation was observed between TAFI antigen and the number of plaques or vessels diseased i.e. the atherosclerotic burden (Schroeder et al., 2002). This indicates that more impaired fibrinolysis in patients with CAD is likely unrelated to disease burden but more closely aligned to flow dynamics (Fernandes et al., 2015).

Likewise, raised fibrinogen levels (Lima et al., 2012) have been found to be related to the severity of coronary stenosis but not related to plaque burden. The relationship of PAI-1 levels to luminal stenosis has been conflicting (Fernandes et al., 2015; Paramo et al., 1985). This difference in results regarding PAI-1 could be explained by the non-conventional way Paramo et al. defined severity – through a computer calculated score incorporating narrowing, length, number and site of different stenoses. The addition of plaque burden onto degree of stenosis may have confounded the outcome of the study.

Severity of stenosis and fibrinolysis

The presence of luminal stenosis, especially with severe stenosis, is commonly associated with disturbed laminar flow and higher shear stress (Sakariassen et al., 2015). In low shear conditions, platelets tend to bind to adsorbed fibrinogen or directly to collagen, which are present during endothelial injury, whereas at high shear, platelets binds preferentially to vWF (Savage et al., 1996) resulting in more rapid formation of platelet-rich thrombus. Thrombi formed under high shear stress produces more fibrin (Colace et al., 2012) which increases the clot strength and resistance towards lysis. Spinhakis *et al.* have shown a relationship between fibrin clot architecture on scanning electron microscopy and fibrinolysis measured by the GTT where the presence of denser fibrin meshwork is associated with more impaired fibrinolysis (Spinhakis, Gue, Farag, Ren, et al., 2019). This may explain the differences seen in endogenous fibrinolysis between patients with severe stenosis and normal coronary arteries.

Although the presence of luminal stenosis may be associated with local higher shear stress, this is not always the case. It is known that in some patients with visually defined severe coronary artery stenosis identified on coronary angiography, the use of fractional flow reserve (FFR) can show that there are no functionally significant stenoses. FFR employs the principle of pressure gradient and flow across stenosis during maximal arteriolar vasodilation

(Pijls et al., 1993) to provide an objective assessment of the physiological significance of a lesion, with an FFR < 0.80 being classed as significant (Tonino et al., 2010). Also, CTCA tends to overestimate hemodynamic severity (Meijboom et al., 2008), therefore, it is possible for patients to have visually stenotic lesions without corresponding high shear haemodynamics. This could potentially confound the findings described.

Plaque burden and fibrinolysis

The degree of plaque burden may not be correlated due to the method of defining plaque burden. The process of atherosclerosis occurs in stages and in a cyclical pattern with rupture, thrombosis and healing resulting in multiple layers of tissues in different stages within the plaques (Insull, 2009). Plaque burden defined by CACS, most commonly calculated by the Agatston method, uses a weighted sum of lesions i.e. multiplying area of calcium by a factor related to maximum plaque attenuation (Agatston et al., 1990). Recently, the Scottish Computed Tomography of the HEART or SCOT-HEART study (Williams et al., 2020) has provided insights into the predictive values of CTCA towards future risk of MI by showing that quantification of the type of plaque burden (i.e. calcified vs non-calcified vs low attenuation) can further improve the predictive value of CTCA. They showed patients with visually assessed obstructive CAD had higher total and subsets of plaque burden, with low-attenuation plaque burden carrying the strongest predictive potential for future MI. This shows the importance and difference in each type of plaque in causing MI. By only calculating CACS using the Agatston method, the plaque burden is only that of calcified plaques which disregards other non-calcified plaques which have the potential to progress and rupture leading to atherothrombosis and hence not a reflection of true plaque burden. Thus, it is not surprising that CACS alone was not related to thrombotic status.

Impaired Fibrinolysis

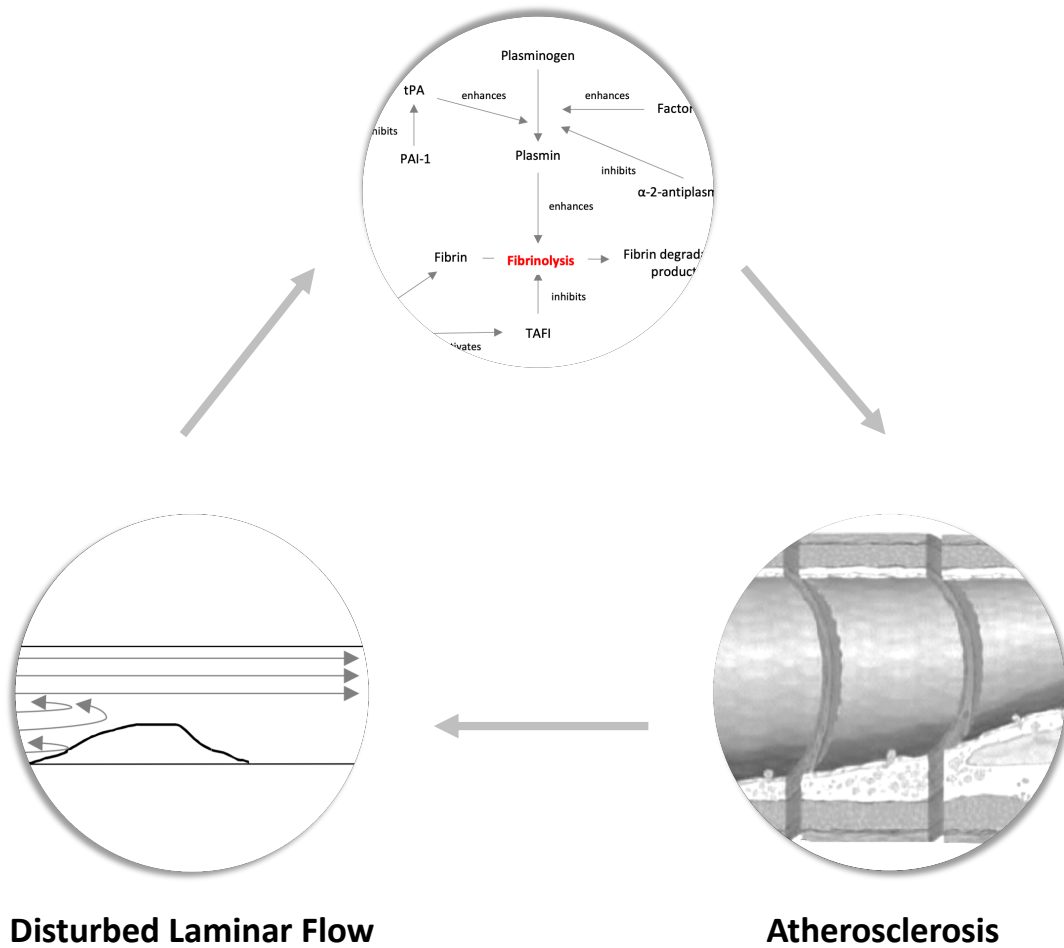


Figure 4.1 Cycle of propagation of atherosclerosis and role of impaired fibrinolysis

Limitations

The main limitation of the study is the small number of patients and as such the linearity of the relationship between severity and endogenous fibrinolysis may not be appreciated. This is even more so when assessing degree of maximal stenosis as the number of patients in each group is unequal with some groups much smaller than others. There are also difference in baseline characteristic in the way of significantly higher number of hypertensives within some groups. This can potentially confound results as patients with uncontrolled hypertension are known to display accelerated atherosclerosis (R. W. Alexander, 1995) and the control of hypertension within the cohort of patient studied was not defined.

Pharmacological treatments were not matched within the cohort with significantly higher number of patients on aspirin and statin therapy as primary prevention in the higher quartile. However, previous studies have shown that aspirin does not have a significant impact on high shear platelet reactivity and fibrinolysis (Spinthakis, Farag, et al., 2019) whilst statins have shown controversy with studies showing no impact on fibrinolytic factors (Balk et al., 2003; Kinlay et al., 2009; Tehrani et al., 2010) and a study showing increase in clot permeability (Undas et al., 2006) which may be due to a reduction in thrombotic potential (Tehrani et al., 2010; Undas et al., 2005). The impact of statins on global fibrinolytic status in high shear induced thrombosis is unclear.

The reliability and reproducibility of the GTT has been discussed in Chapter 2 with coefficients of variation between 6 – 9%. Given that all patients only had 1 sample taken for the study, there could be potential for error as intrinsic variability of thrombotic status has not been accounted for. Secondly, the time interval between the CTCA report and obtaining the blood samples also varies amongst patients which may potentially confound the findings.

As discussed earlier, although the presence of luminal stenosis may significantly impair coronary blood flow resulting in higher shear stress, this is not always the case as evident in

the Fractional Flow Reserve Versus Angiography in Multivessel Evaluation (FAME) study (Tonino et al., 2010). CTCA also tends to overestimate hemodynamic severity (Meijboom et al., 2008), therefore, it is possible for patients to have visually stenotic lesions without corresponding high shear haemodynamics. Hence, the interpretation of the analysis may not correspond to haemodynamic changes as presumed.

The results of the CTCA was reported as part of the clinical assessment of the patient and this may be reported by different radiologists for these patients without an independent central laboratory verification. There would be potential intra- and inter-observer variability in the interpretation of each CTCA which has not been taken into account for the analysis.

As the blood samples were obtained from a peripheral vein, the thrombotic status may not be reflective of the flow dynamics within the coronary artery.

Lastly, as this is a cross-sectional study, I could only assess the association between fibrinolysis and presence of CAD but unable to determine cause and effect.

Conclusion

The severity of maximal coronary stenosis, but not the extent of CAC, is related to the effectiveness of endogenous fibrinolysis at high shear *in vitro*, with patients with more severe stenoses exhibiting less efficient fibrinolysis. Further larger studies are required to investigate whether assessment of fibrinolysis in peripheral blood may be an indicator of the severity of coronary stenosis.

Chapter 5. Thrombotic status in patients with ACS

Abstract

The different clinical manifestations of ACS represent differences in pathophysiological processes amongst these patients. Although it is known that fibrinolysis plays an important role mediating the clinical presentation following an acute arterial thrombotic trigger, whether there is any difference in measurable fibrinolytic profile between patients presenting with different types of ACS remains unclear.

I aimed to investigate the difference in thrombotic and thrombolytic status of the different clinical presentations of patients with ACS.

A total of 305 patients with ACS had blood tests to assess thrombotic and thrombolytic status: 143 STEMI, 125 NSTEMI and 37 UA. When comparing the whole cohort, patients with NSTEMI displayed the most impaired endogenous fibrinolysis. (LT: NSTEMI 2008 [1615 – 2503] vs. STEMI 1764 [1411 – 2326] vs. UA 1803 [1483 – 2186] s, $p=0.0067$). However, the difference was no longer significant when a propensity matched cohort of patients was studied (LT: NSTEMI 1869 [1580.5 – 2202] vs. STEMI 1978.5 [1609.5 – 2645.5] s, $p=0.4832$).

Patients with NSTEMI displayed more impaired endogenous fibrinolysis when compared to patients presenting with STEMI, which is partly driven by the higher proportion of comorbidities within this cohort of patients. Another explanation may be differences in medication taken prior to sampling. A larger cohort longitudinal study examining fibrinolytic profile at the point of admission will be required to provide further insight into its differences in the different clinical manifestations of ACS.

Introduction

The ACS spectrum is divided into three main categories, determined by changes on the ECG and changes in cardiac biomarkers. The classification is based upon immediate management strategy (Ibanez et al., 2018) as the ECG changes reflect the underlying pathophysiological differences (Nable & Brady, 2009). In patients with ST-segment elevation on an electrocardiogram combined with symptoms suggestive of MI, the underlying pathology in majority of cases is an acutely occluded coronary artery resulting in transmural infarction (Coppola et al., 2013). This requires immediate treatment with reperfusion, preferably with primary PCI to restore blood flow and minimise myocardial necrosis (Ibanez et al., 2018).

Patients without ST-segment elevation but with positive biomarkers of myocardial necrosis, namely a significant rise and/or fall are classified under NSTEMI. Their ECG may be normal or include changes such as ST-depression or T-wave abnormalities. In contrast to patients with STEMI, patients presenting with NSTEMI have a much lower incidence of total coronary occlusion although there is nevertheless a high rate of severe coronary stenosis in the culprit vessel (>70% luminal narrowing) (Ambrose & Singh, 2015). Revascularisation still plays an important role in these patients but is less emergent when compared to STEMI, unless patients exhibit ongoing chest pain with clinical instability (Roffi et al., 2016).

Lastly, patients with UA present with signs and symptoms suggestive of cardiac ischemia in the absence of biomarker abnormality, indicating myocardial ischemia without necrosis. These patients have lower risk of mortality (Reichlin et al., 2012, 2013) and derive less benefit from invasive management with PCI (Morrow et al., 2001).

The majority of hospital ACS presentations are attributable to NSTEMI, with a smaller proportion of STEMI patients (Zeymer et al., 2013). UA represents the smallest proportion of patients being admitted.

Mortality in STEMI is influenced by a multitude of factors and has greatly improved with the implementation of primary PCI and modern pharmacotherapy for secondary prevention (Gale et al., 2014; Puymirat et al., 2012). However, in-hospital mortality remains between 4 – 12% (Kristensen et al., 2014) and about 10% after 1 year (Pedersen et al., 2014). In-hospital mortality in NSTEMI is similar to STEMI but is numerically higher at 1 year (Montalescot et al., 2007) whereas UA patients have the lowest risk of mortality (Reichlin et al., 2012, 2013).

Impaired endogenous fibrinolysis has been identified as a risk predictor of future adverse cardiovascular events in patients presenting with ACS. Shantsila *et al.* had compared fibrinolytic factors in which has shown significantly higher in PAI-1 activity and lower TAFI levels in STEMI when compared to NSTEMI patients (Shantsila et al., 2012). Otherwise, the difference in thrombotic profiles from the different presentations of ACS is limited within the literature.

Aim

I aimed to compare the thrombotic and thrombolytic status between patients with different clinical presentations of ACS.

Methods

Study design and population

All patients presenting to the Lister Hospital, East and North Hertfordshire NHS Trust, with ACS and satisfying the inclusion and exclusion criteria (Table 5.1) were recruited as part of the screening process prior to proposed consideration of enrolment in the VaLiDate-R study which will be detailed in Chapter 8. The study was approved by the London Hampstead Research Ethics Committee and the relevant approvals are shown in Appendix 2 (Page 260 – 273).

Patients admitted with an ACS were approached by a member of the research team. Following informed consent, a screening consent form was signed, to allow gathering of clinical data and to obtain a blood sample to assess thrombotic status in eligible patients. Data including basic demographics, medical history and type of presentation were collected as part of the process. In cases where patients were admitted as an emergency with STEMI for primary PCI, a delayed consent approach was undertaken, with ethical permission, whereby screening blood tests for assessment of thrombotic status were taken at the same time as standard of care blood samples on arrival, and patients were consented for the study after the primary PCI procedure following stabilisation, when they had time to carefully consider the study. This was to ensure the consent process was fully informed (patients presenting with STEMI are unwell, in pain, may be haemodynamically unstable, or they may have been given opiates for analgesia, thus impairing their ability to properly process information) and that the research did not negatively impact on the clinical care (explaining the study and obtaining full consent might cause delay to the emergency primary PCI procedure) whilst not compromising the integrity of the sampling process (drugs such as heparin given during the procedure may affect the thrombotic status of patients). In other situations, screening consent

and blood test for thrombotic status were performed as close to admission as possible. The cohort of patients were divided based on their type of presentation (STEMI, NSTEMI or UA) in order to understand the differences in thrombotic status. STEMI was defined as an acute MI with evidence of ST-segment elevation in 2 or more contiguous ECG leads and evidence of myocardial injury according to the ESC definition (Ibanez et al., 2018). NSTEMI was defined as an acute MI without the evidence of ST-segment elevation with evidence of dynamic change in biomarker of myocardial injury whilst UA is similar to NSTEMI but without a change in troponin (Roffi et al., 2016).

Assessment of thrombotic status

Thrombotic status was assessed using the point-of-care GTT (Thromboquest Ltd., London, UK) as detailed in Chapter 2. The instrument assesses firstly the time taken to form an occlusive thrombus under high shear (occlusion time, OT) and in the second phase, the restart of flow due to endogenous fibrinolysis (lysis time, LT).

Statistical analysis

As this was an exploratory pilot study utilising the screening cohort of patients, the sample size was not predefined.

Baseline characteristics will be presented with continuous variables are displayed as median values with interquartile ranges and binary variables are displayed as number and percentage. Fishers exact test was used to compare binary variables whilst Kruskal-Wallis test was used to compare between continuous variables between the three different presentations of ACS.

A matched cohort derived using propensity matched scoring method on Stata was used to further compare the thrombotic status of STEMI and NSTEMI patients. Analyses were

performed with Stata version 15.1 (StataCorp, College Station, TX, USA) and significance was taken at <0.05 .

Table 5.1 Inclusion and exclusion criteria for VaLiDate-R study

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

Results

The recruitment for the study started in January 2019 following approval from all the relevant organisations stated in Chapter 2. Over the course of 15 months of recruitment, a total of 305 patients were eligible and underwent the screening process.

The breakdown of the clinical presentations of the ACS patients are as follows – 143 patients presented with STEMI, 125 patients presented with NSTEMI and 37 patients presented with UA. The overall baseline characteristics, medical history and thrombotic status are shown in Table 5.2. Patients with NSTEMI when compared to STEMI and UA patients were more likely to have hypertension (52 [41.6%] vs. 36 [25.2%] vs. 17 [46.0%], $p=0.005$), diabetes (41 [32.8%] vs. 19 [13.3%] vs. 7 [18.9%], $p=0.001$) and dyslipidaemia (45 [36%] vs. 27 [18.9%] vs. 12 [32.4%], $p=0.005$). Both the OT (494.1 [399.7 – 592.9] vs. 425.1 [333.9 – 515] vs. 459.9 [361.9 – 597.4] s, $p=0.0002$) and LT (2008 [1615 – 2503] vs. 1764 [1411 – 2326] vs. 1803 [1483 – 2186] s, $p=0.0067$) were significantly higher in the NSTEMI cohort when compared to the STEMI and UA cohorts, respectively. When comparing NSTEMI to UA, both OT ($p=0.3444$) and LT ($p=0.0695$) were not significantly different.

Regression analysis

Multivariate regression analysis was performed using OT as the dependent variable and showed that type of ACS was predictive of OT ($p=0.009$). However, when LT was used as the dependent variable, the type of ACS was not predictive ($p=0.366$) of LT.

Subgroup analysis – STEMI and NSTEMI

Further analyses were performed comparing patients presenting with STEMI and NSTEMI, since the UA cohort was very small in comparison (Table 5.3). Patients presenting with NSTEMI were more likely to have diabetes (41 [32.8%] vs. 19 [13.3%], $p<0.0001$) and

dyslipidaemia (45 [36%] vs. 27 [18.9%], $p=0.002$). Both OT ($p<0.0001$) and LT ($p=0.0023$) were significantly higher in the NSTEMI cohort.

Propensity matched cohort

A paired cohort of 24 STEMI and 24 NSTEMI patients were identified using propensity matching for age, hypertension, diabetes and dyslipidaemia. As shown in Table 5.4, all baseline characteristics were matched between the 2 presentations. OT remained significantly elevated in the NSTEMI cohort (566.5 [380.7 – 607.5] vs. 427 [368.25 – 499.25] s, $p=0.0221$) whereas LT was similar between the two cohorts (1869 [1580.5 – 2202] vs. 1978.5 [1609.5 – 2645.5] s, $p=0.4832$)

Table 5.2 Baseline characteristics and fibrinolytic profile of patients screened

	STEMI n=143	NSTEMI n=125	Unstable Angina n=37	p-value
Age, median (IQR)	61 (52 – 72)	63 (56 – 71)	61 (55 – 71)	0.4685
Male, n (%)	111 (77.6)	94 (75.2)	22 (59.5)	0.085
Race, n (%)				
Caucasian	128 (89.5)	112 (89.6)	34 (91.9)	1.00
Asian	10 (7)	11 (8.8)	3 (8.1)	0.831
Black	5 (3.5)	0 (0)	0 (0)	0.088
Mixed	0 (0)	2 (1.6)	0 (0)	0.395
Current smoker	42 (29.4)	45 (36)	9 (24.3)	0.316
Hypertension	36 (25.2)	52 (41.6)	17 (46.0)	0.005*
Diabetes	19 (13.3)	41 (32.8)	7 (18.9)	0.001*
Dyslipidaemia	27 (18.9)	45 (36)	12 (32.4)	0.005*
OT in sec, median (IQR)	425.1 (333.9 – 515)	494.1 (399.7 – 592.9)	459.9 (361.9 – 597.4)	0.0002*
LT in sec, median (IQR)	1764 (1411 – 2326)	2008 (1615 – 2503)	1803 (1483 – 2186)	0.0067*

Table 5.3 Baseline characteristics and fibrinolytic profile of subgroup

	STEMI n=143	NSTEMI n=125	p-value
Age, median (IQR)	61 (52 – 72)	63 (56 – 71)	0.2328
Male, n (%)	111 (77.6)	94 (75.2)	0.667
Race, n (%)			
Caucasian	128 (89.5)	112 (89.6)	1.00
Asian	10 (7)	11 (8.8)	0.652
Black	5 (3.5)	0 (0)	0.063
Mixed	0 (0)	2 (1.6)	0.217
Current smoker	42 (29.4)	45 (36)	0.153
Hypertension	36 (25.2)	52 (41.6)	0.296
Diabetes	19 (13.3)	41 (32.8)	<0.0001*
Dyslipidaemia	27 (18.9)	45 (36)	0.002*
OT in sec, median (IQR)	425.1 (333.9 – 515)	494.1 (399.7 – 592.9)	<0.0001*
LT in sec, median (IQR)	1764 (1411 – 2326)	2008 (1615 – 2503)	0.0023*

Table 5.4 Propensity-matched cohort comparing STEMI and NSTEMI patients

	STEMI n=24	NSTEMI n=24	p-value
Age, median (IQR)	65 (57 – 80)	62 (53.5 – 74.5)	0.3693
Male, n (%)	5 (20.8)	8 (33.3)	0.259
Race, n (%)			1.000
Caucasian	21 (87.5)	22 (91.7)	
Asian	3 (12.5)	2 (8.3)	
Current smoker	6 (25)	5 (20.8)	1.000
Hypertension	6 (25)	5 (20.8)	1.000
Diabetes	5 (20.8)	6 (25)	1.000
Dyslipidaemia	7 (29.2)	7 (29.2)	1.000
OT in sec, median (IQR)	427 (368.25 – 499.25)	566.5 (380.7 – 607.5)	0.0221*
LT in sec, median (IQR)	1978.5 (1609.5 – 2645.5)	1869 (1580.5 – 2202)	0.4832

Discussion

The breakdown of the different types of ACS presentations within the study is reflective of the case mix that presents to local heart attack centres, with the majority of patients fulfilling the criteria for STEMI or NSTEMI and a small proportion of UA patients. The low number of UA patients was unsurprising, given that the majority of patients are managed as outpatients. The marginally higher STEMI:NSTEMI ratio highlights the commonly observed ‘more complex’ cohort of patients presenting with NSTEMI that may not fit the inclusion and exclusion criteria of the study for screening, including those with atrial fibrillation or with other indication for oral anticoagulation. This is also evidenced by the older age group and higher proportions of patients with co-morbidities in the NSTEMI cohort.

Comparison of platelet reactivity

The thrombotic status of the ACS presentations was significantly different with STEMI patients showing the lowest OT, followed by UA and NSTEMI patients displaying the highest OT (425.1 [333.9 – 515] vs. 459.5 [361.9 – 597.4] vs. 494.1 [399.7 – 592.9] s, $p=0.0002$). The higher platelet reactivity could reflect the timing of blood draw in STEMI patients compared to NSTEMI and UA. Given the small numbers in UA, I excluded and performed a separate analysis comparing STEMI and NSTEMI as a whole cohort and using a propensity matched cohort. In both groups, NSTEMI patients had a significantly higher OT irrespective of adjusting of co-morbidities. This likely means that differences in OT are not dependent on the patient’s clinical characteristics. In my study, patients with STEMI generally had their blood tests taken immediately as part of the admission into the cardiac catheterisation lab and loading doses of antiplatelet medications may not have taken effect, which may explain the resultant higher platelet reactivity. In support of this, when comparing NSTEMI and UA patients, where bloods are normally taken several hours after antiplatelet

loading, the OT was not significantly different. This shows that the OT measured within the study is likely examining the effects of antiplatelet medications, rather than simply comparing different presentations of ACS.

Comparison of endogenous fibrinolysis

Lysis time was shortest in STEMI patients and shorter than in with UA and NSTEMI patients (1764 [1411 – 2326] vs. 1803 [1483 – 2186] vs. 2008 [1615 – 2503] s, $p=0.0067$). This is surprising as the pathomechanism behind STEMI involves the complete occlusion of a coronary artery to produce changes on the ECG. Therefore, one could infer that endogenous fibrinolysis would be much more impaired in the STEMI cohort as compared to NSTEMI or UA. However, in my study, the opposite was true when examining the cohort as a whole and when comparing STEMI with NSTEMI (after excluding the small group of UA). However, in the propensity matched cohort, with matched comorbidities and comparable demographics, LT was not significantly different. The higher proportion of patients with comorbidity could potentially confound the findings and may explain the higher LT in patients with NSTEMI.

Furthermore, lysis time is clearly not the only determinant of vessel patency and the strength of the prothrombotic trigger may exceed the effectiveness of a similar fibrinolytic response in those with occluded vessels.

The only report in the literature comparing fibrinolytic profiles in different cohorts of ACS patients is a study by Shantsila *et al.* which recruited 50 patients with STEMI and 47 patients with NSTEMI who underwent PCI. Blood was taken within 24 hours of ACS, day 3 in STEMI or after PCI in NSTEMI, day 7 and day 30 post ACS. Fibrinolytic factors (tPA and PAI-1 antigen, PAI-1 activity and TAFI concentration) were quantified using enzyme-linked immunosorbent assay (ELISA). STEMI patients displayed significantly higher levels of PAI-1 activity (3.79 [2.16 – 6.82] vs. 1.92 [0.38 – 3.26] ng/ml, $p<0.05$) but lower concentration of

TAFI (126 ± 28.3 vs. $159 \pm 24.1\%$, $p < 0.05$) whereas tPA and PAI-1 antigen were not significantly different (Shantsila et al., 2012). The peak levels in different fibrinolytic factors also differed between the two ACS presentations, highlighting the importance of the timing of blood tests on the measurement of fibrinolysis. The complex interactions between fibrinolytic factors and specific time of blood draw could potentially explain the lack of difference between the 2 ACS presentations.

Limitations

One major limitation is the differences between the groups with regards to the timing of blood draw and the differential use of antiplatelet medication or anticoagulant such as fondaparinux in patients with different ACS presentations. Although every effort was taken to ensure that blood draw occurs at least 24 hours after administration of the last dose of fondaparinux or other thromboprophylactic anticoagulant, this may have resulted in a delay in obtaining blood samples and introduced heterogeneity of sample timing into each of the cohort of patients. Similarly, as patients were screened at different times of the day depending on the time of presentation, circadian variation could be a confounder. The underrepresentation of UA cohort limits the strength of the study in comparing UA with the other 2 presentations. As this is an observational, cross sectional study performed at a single time-point, there could be potential unknown confounders which are not accounted for and I am unable to determine cause and effect between the variables examined.

Lastly, the small sample size of the matched cohort of patients could only be hypothesis generating and conclusions derived from it should be interpreted with caution.

Conclusion

Patients with NSTEMI display more impaired endogenous fibrinolysis when compared to patients presenting with STEMI, which is partly driven by the higher proportion of comorbidities within this cohort of patients. The longer occlusion time in NSTEMI compared to STEMI patients may reflect lower platelet activation, but likely reflects the effect of antiplatelet medications also. A larger cohort longitudinal study examining fibrinolytic profile at the point of admission will be required to provide further insight into its differences in the different clinical manifestations of ACS.

**Chapter 6. Thrombotic status in
patients with myocardial
infarction with non-obstructive
coronary arteries presenting with
STEMI**

6.1 Incidence of mortality of MINOCA in STEMI

Abstract

Historical data indicate 10% of acute coronary syndrome patients have absence of CAD but contemporary incidence in STEMI is not clear. Effective endogenous fibrinolysis could potentially explain, in part, the absence of obstructive CAD despite classical signs and symptoms of ACS. In current literature, there is a lack of studies looking specifically at MINOCA STEMI and establishing the true incidence is vital to allow future trials of MINOCA STEMI to be adequately powered.

A retrospective database search was conducted of all patients presenting to the cardiac catheterisation laboratory with STEMI with a view to emergency primary PCI. 2521 patients with full electronic dataset were identified. Of these 196 (7.8%) patients had absence of obstructive CAD on angiography, of whom 167 (6.7%) had no stenosis (<30%) and 29 (1.1%) had mild coronary atheromatosis (stenosis >30% but <50%). A total of 110 (4.4%) patients met diagnostic criteria for MINOCA with a 30-day all-cause mortality of 3.6% and 1-year all-cause mortality of 4.5%

The results showed that one in 20 patients presenting with STEMI have MINOCA and 40% of these are due to plaque disruption. Further studies to assess endogenous fibrinolysis within this cohort may reveal insights into the underlying pathomechanism behind this syndrome.

Introduction

Cardiovascular disease is the leading cause of death globally, with 85% of cardiovascular deaths attributed to ACS and stroke (WHO, 2017). Development of coronary atherosclerosis due to lipid deposition followed by vulnerable plaque disruption and thrombosis is responsible for the majority of ACS presentations (Finn et al., 2010; Fuster et al., 2005). The resulting persistent occlusion of the coronary artery classically presents with symptoms of chest pain and ECG evidence of ST-segment elevation requiring urgent coronary angiography and revascularisation (Ibanez et al., 2018). This recommendation is based upon historical registry data (DeWood et al., 1980) which suggests that approximately 90% of patients with myocardial infarction have angiographic evidence of obstructive CAD.

Myocardial infarction with non-obstructed coronary arteries

In contrast, the remaining 10% of patients presenting with classical signs and symptoms of ACS do not have evidence of obstructive CAD to account for their presentation, namely those with MINOCA (Dokainish et al., 2005; Pasupathy et al., 2015; Safdar et al., 2018). This phenomenon has been historically overlooked and largely understudied in relation to prognosis and treatment as it was considered a benign condition with good prognosis (Kemp et al., 1986; Lichtlen et al., 1995). However, there is increasing evidence showing that this syndrome is not as benign as previously thought (Bugiardini & Bairey Merz, 2005; Pasupathy et al., 2015) and this led to the first authoritative paper by ESC Working Group on Cardiovascular Pharmacotherapy describing and defining the condition in detail (Agewall et al., 2016). Following on, the AHA released its scientific statement on MINOCA (Tamis-Holland et al., 2019), incorporating the new Fourth Universal Definition of Myocardial Infarction (Thygesen et al., 2018) into its description.

The definition of MINOCA is predicated on the fulfilment of all three main diagnostic criteria, namely (1) Universal Definition of Acute Myocardial Infarction, (2) presence of non-obstructive coronary arteries on angiography (defined as no coronary artery stenosis $\geq 50\%$) in any potential infarct-related artery and (3) absence of another specific, clinically overt cause for the acute presentation.

Aetiology of MINOCA

The heterogeneity of its underlying aetiology creates a diagnostic challenge to clinicians, requiring a systematic approach to evaluate and investigate these patients through various diagnostic algorithms (Agewall et al., 2016; Scalone et al., 2019; Tamis-Holland et al., 2019). The aetiology includes primarily (1) coronary causes such as plaque disruption or spontaneous coronary artery dissection, (2) non-coronary cardiac causes such as myocarditis or Takotsubo syndrome and (3) extra-cardiac causes such as type 2 myocardial infarction and pulmonary embolism. While atherosclerotic plaque disruption is the leading cause of type 1 myocardial infarction (Thygesen et al., 2018) and is responsible for the majority of STEMI presentations, it is also recognised, with the assistance of advanced intracoronary imaging, as not an uncommon cause of MINOCA (Opolski et al., 2018; Ouldzein et al., 2012; Reynolds et al., 2011). Therefore, it is possible that patients with mild luminal irregularities presenting with STEMI have underlying atherosclerosis with plaque disruption, thrombosis and transient epicardial occlusion with distal embolization.

Endogenous fibrinolysis and MINOCA

Impaired endogenous fibrinolysis has been identified as a potential mechanism to explain poor outcome in patients with obstructive CAD (Farag et al., 2019; Sumaya et al., 2018) despite contemporary revascularisation and pharmacotherapeutic treatments. On the opposite side of the same coin, an effective and efficient endogenous fibrinolytic system (Farag et al.,

2019) may potentially explain, in part, the reason why individuals with MINOCA do not exhibit angiographic evidence of significant plaque or visible thrombus when they present to a HAC, in other words these patients may have a transient occlusive coronary thrombus but with effective endogenous fibrinolysis, this thrombus is dissolved or embolised by the time the patient arrives in the HAC. Therefore, the study of a cohort of patients with MINOCA may provide further insights into the variability of endogenous fibrinolysis within different patient groups and presentations.

In patients presenting with STEMI, the recommendation for urgent revascularisation with target door-to-balloon times to reduce treatment delay and mortality (Ibanez et al., 2018) puts clinicians in a unique position to assess a patient's fibrinolytic status clinically before percutaneous and pharmacological intervention.

Representation of MINOCA in STEMI

STEMI occurs in the presence of transmural ischaemia due to transient or persistent complete occlusion of the infarct-related coronary artery whereas in patients presenting with NSTEMI, the infarct is subendocardial. This pathophysiological difference also seems to be present within the MINOCA cohort, where within the literature, MINOCA tends to present more commonly as NSTEMI rather than with STEMI. This has resulted in an under-representation of STEMI MINOCA patients in the literature. Most studies within the literature examined undifferentiated ACS cohorts (Pasupathy et al., 2015) with only a handful of studies providing separate data (Gehrie et al., 2009; Planer et al., 2014; Widimsky et al., 2006). Therefore, establishing the true incidence of MINOCA amongst patients presenting with STEMI is vital to allow future trials assessing pathomechanisms of and treatments for MINOCA STEMI to be adequately powered.

Aim

The aim of the study was to establish the contemporary incidence of MINOCA amongst patients with STEMI, delineate the underlying cause/diagnosis and assess 30-day and 1-year clinical outcomes.

Methods

Study design and population

I undertook a retrospective, observational study assessing the health records of all consecutive patients with STEMI admitted with a view to primary PCI, to East and North Hertfordshire NHS Trust and Norfolk and Norwich University Hospital, United Kingdom. These HACs serve a population of 1.5 million, supported by East of England Ambulance Service NHS Trust. According to standard protocol, all patients who meet the criteria for STEMI are brought directly to the HAC for emergency primary PCI. Criteria for primary PCI protocol activation are symptoms compatible with an acute myocardial infarction within 12 hours with any of the following ECG criteria: ST-segment elevation ≥ 1 mm in contiguous limb leads, > 2 mm in contiguous chest leads, bundle branch block believed to be new in the context of acute cardiac-sounding chest pain, or patients resuscitated from cardiac arrest with ECG criteria as above. All patients who met these criteria were included. Patients with MINOCA according to the ESC position diagnostic criteria (Agewall et al., 2016) were identified by review of all cases in the HAC activation database.

Initial MINOCA screening diagnosis required the presence of all of the following criteria:

- (1) meets Universal Definition of Acute Myocardial Infarction criteria (Thygesen et al., 2018),
- (2) no obstructive CAD at angiography, defined as no stenosis (diameter reduction) $\geq 50\%$ in any potential infarct-related artery and
- (3) no other clinically overt cause for the specific presentation.

Patients were identified and stratified using a predefined flowchart (Figure 6.1) to ensure a standardised approach.

Data collection

Electronic patient records, blood results, angiographic data and echocardiographic data were used to determine diagnosis, based on ESC recommendations for diagnostic work-up. Follow up visits and mortality information were obtained from electronic health records. The underlying diagnosis was subdivided into coronary, non-coronary cardiac and non-coronary extra-cardiac causes. Anonymised data were recorded electronically in a password protected document.

Statistical analysis

Data were analysed using Stata version 15.1 (StatCorp, College Station, TX, USA).

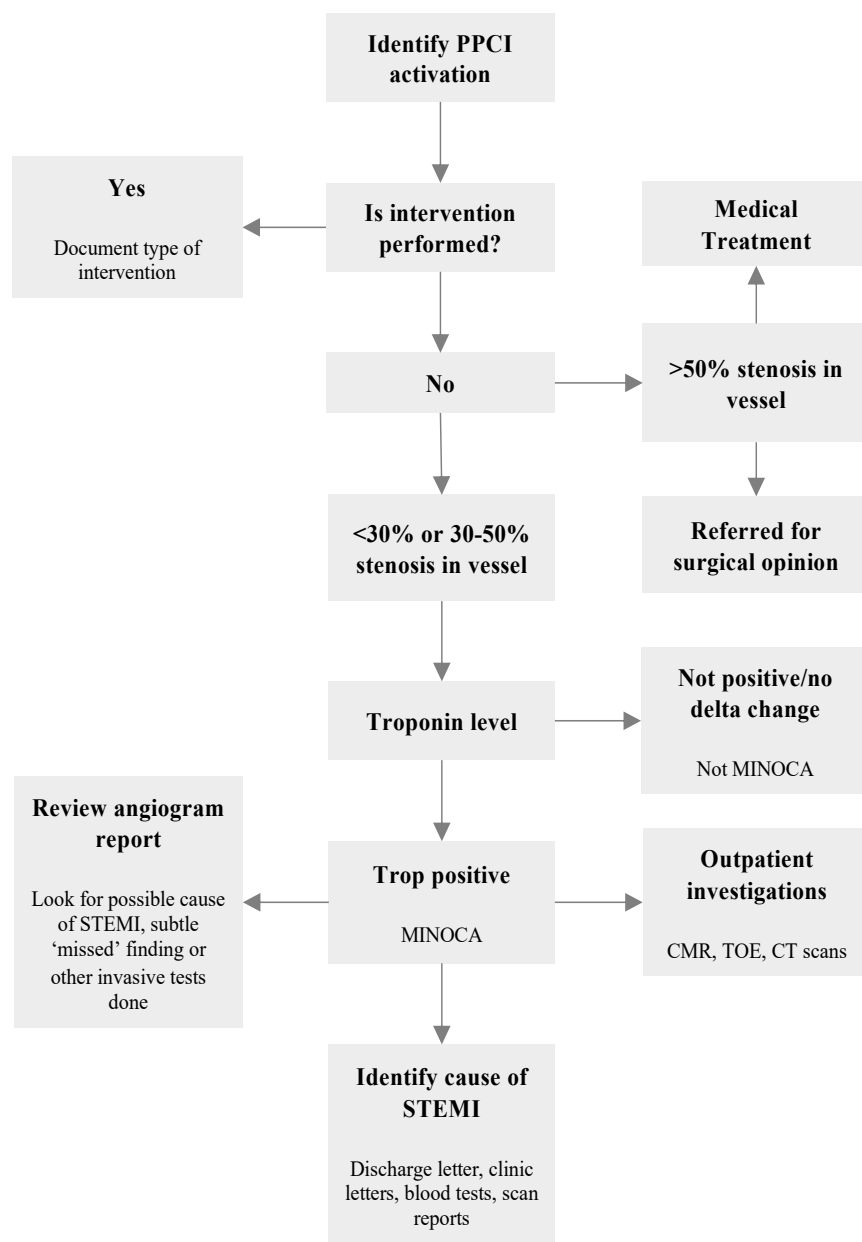


Figure 6.1 Flowchart to standardise patient identification

Results

From May 2015 to January 2018, a total of 2521 consecutive unselected patients with ST-elevation fulfilling criteria for primary PCI, with full electronic dataset (Figure 6.2) were identified within the 2 trusts. Of these, 2158 (85.6%) patients underwent primary PCI for obstructive CAD with a further 167 (6.6%) patients with evidence of obstructive CAD treated medically or with surgical revascularisation. 196 (7.8%) patients had angiographically non-significant CAD in any potential infarct-related artery.

A total of 110 patients (4.4% of all STEMIs) met diagnostic criteria for MINOCA. Of these, 59 (54%) were male, with mean age 63.5 ± 13.9 years. The underlying aetiology of MINOCA was determined to be of coronary cause in 28%, non-coronary cardiac cause in 61% and non-coronary extra-cardiac cause in 11% of patients.

Coronary causes included plaque disruption (39%), coronary spasm (10%), spontaneous coronary artery dissection (19%), coronary embolism (23%) and aortic dissection (10%).

Non-coronary cardiac causes included myocarditis (36%), Takotsubo syndrome (30%) and type 2 myocardial infarction (34%), with the latter comprising patients with cardiomyopathy, anaemia, valvular disease and arrhythmia. Non-coronary extra-cardiac causes included pulmonary embolism (50%), cerebrovascular event (8%), and other causes included sepsis, gallstone pancreatitis and extracardiac tumour compressing the heart.

In the remaining 86 patients, there was absence of angiographically-significant CAD but these did not fit the criteria for MINOCA i.e. there was no significant troponin rise and the final diagnoses were predominantly pericarditis, myocarditis and normal-variant ECG.

The 30-day all-cause mortality was 3.6% and 1-year all-cause mortality was 4.5% within the cohort of STEMI patients with MINOCA.

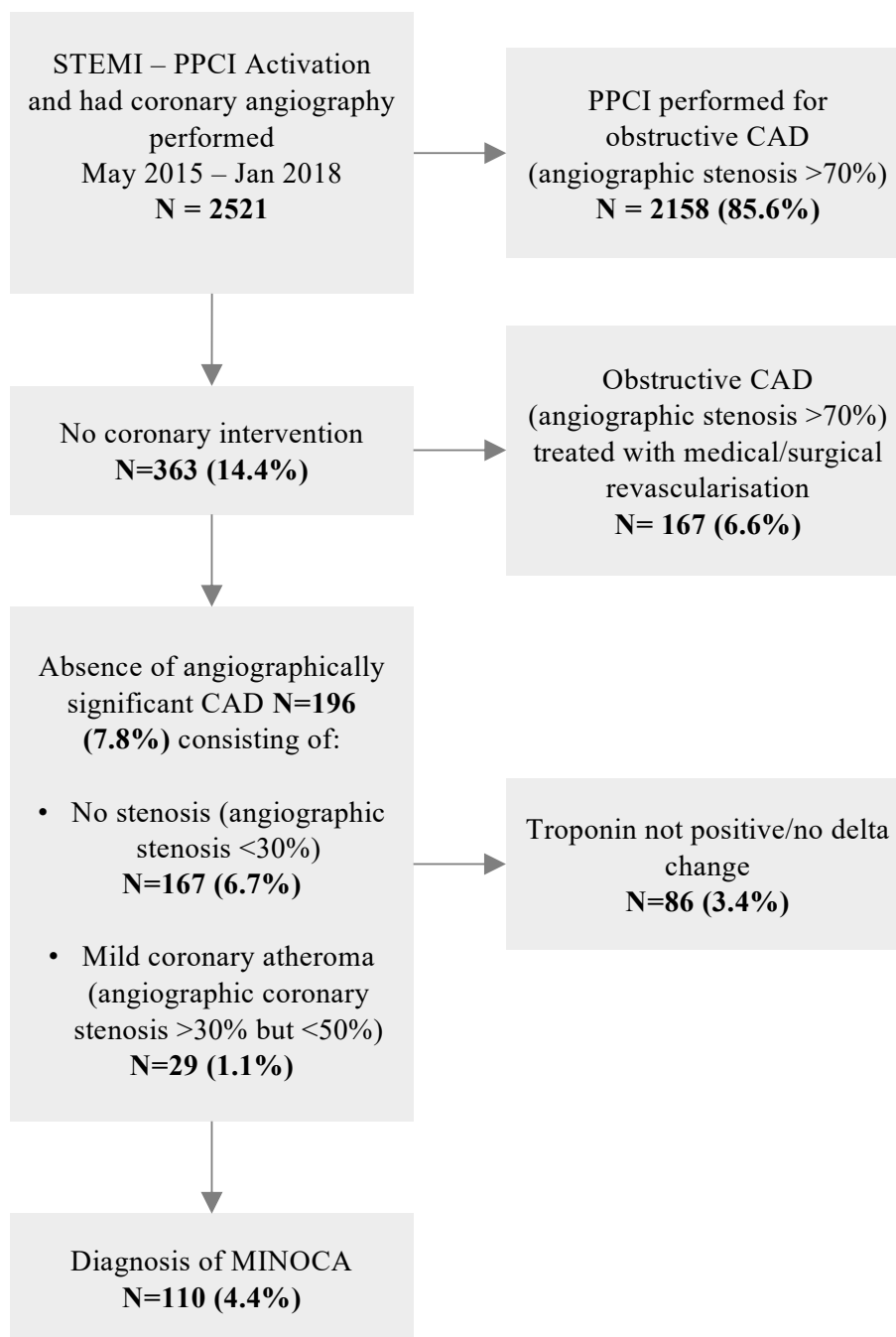


Figure 6.2 Breakdown of STEMI – primary PCI activation and the final diagnoses

Discussion

The main findings of the study are that the incidence of MINOCA in a contemporary cohort of patients presenting with STEMI is 4.4%, and that 30-day and 1-year mortality rate are 3.6% and 4.5%, respectively.

The incidence of MINOCA, using the diagnostic criteria proposed by the ESC position paper (Agewall et al., 2016) and specifically within the STEMI and primary PCI setting has not been reported previously. The only data available was from a retrospective analysis of the PRAGUE studies from pre-2002, where the incidence of angiographically normal coronary arteries in 1004 emergency angiograms performed for STEMI was reported as 2.6% (Widimsky et al., 2006). The incidence was lower within their cohort as even mild atherosclerosis of <50% was considered abnormal whereas the definition in the ESC paper classed <50% as non-obstructive. When looking at the MINOCA only cohort, the incidence of STEMI was reported to be between 17 – 30% (Lindahl et al., 2017; Pasupathy et al., 2015), with the majority presenting as NSTEMI. This results in an under-representation of STEMI when results are presented as an undifferentiated cohort.

The 1-year mortality data from our study are similar to that reported in an undifferentiated cohort in a prior systematic review (4.5% vs 4.7%) (Pasupathy et al., 2015). Mortality in our cohort is however slightly lower when compared with mortality reported for contemporary STEMI patients with CAD (30-day 3.6% vs 4.3%; 1-year 4.5% vs 7.3%) (Doost Hosseiny et al., 2016).

Future relevance

Recognition of the incidence and mortality of MINOCA STEMI is important for the planning of future treatment outcome studies for this cohort, particularly in the acute phase as treatments may differ markedly in STEMI patients with CAD and MINOCA patients. This

cohort of patients not only provide a diagnostic challenge for clinicians, they may offer possible insights into an efficient endogenous fibrinolytic system, particularly when the underlying aetiology is plaque disruption. In a recent paper assessing endogenous fibrinolysis in patients presenting with STEMI (Farag et al., 2019), a subgroup of patients with spontaneous reperfusion, manifesting as complete or partial resolution of ST-elevation pre-primary PCI, exhibited more efficient fibrinolysis as shown by a shorter LT [1050s vs 1501s, $p<0.001$] and reduced platelet reactivity, as evidenced by longer OT [448s vs. 338s, $p<0.001$] than patients without spontaneous reperfusion. Patients with MINOCA due to plaque disruption could have super-effective endogenous fibrinolysis and may benefit from different pharmacotherapy regimes.

Limitations

The main limitations of our data are the relatively small patient sample size and the retrospective nature of the analysis. Furthermore, coronary spasm provocation testing and intravascular imaging were not routinely performed, and therefore the underlying aetiology may have been inaccurately characterised in some patients. Similarly, as the functional significance of the coronary artery lesion was not assessed with the use of fractional flow reserve or instantaneous wave-free ratio, the visual assessment of the degree of coronary stenosis may have either under- or over-estimated the functional significance of the stenosis. Lastly, the location of the infarct related artery could be inaccurate due to the difficulty in localising the area of infarct in some cases of STEMI.

Conclusion

Approximately 1 in 20 patients presenting with STEMI for primary PCI have MINOCA, and these patients have a mortality rate comparable to that of patients with obstructive CAD.

6.2 Thrombotic Status in MINOCA STEMI vs MI-CAD STEMI

Abstract

Impaired endogenous fibrinolysis has been identified as a novel biomarker to predict future cardiovascular risk in patients presenting with STEMI. Patients with MINOCA presents with unobstructed coronaries on initial angiography but carries similar risk compared to patients with myocardial infarction with coronary artery disease (MI-CAD). A difference in endogenous fibrinolysis could potentially underlie the pathophysiology behind the disparity in angiographic findings between these 2 groups of patients.

I extracted data from the VaLiDate-R study to identify patients who were recruited for screening and subsequently had the diagnosis of MINOCA. The database was further explored to identify matching patients, in terms of demographics and medical history, and their data extracted to compare fibrinolytic profiles.

2 patients with MINOCA STEMI and 2 corresponding matched patients with MI-CAD STEMI were identified through the search. Endogenous fibrinolysis was more effective in patients with MINOCA when compared to closely matched patients with MI-CAD.

Effective endogenous fibrinolysis could possibly explain, in part, the phenomenon of MINOCA. It could offset the impact of increased platelet reactivity in young patients presenting with MI. Future larger cohort study to test the hypothesis is mandated to understand its role.

Introduction

Impaired endogenous fibrinolysis has been identified as a novel biomarker to predict future cardiovascular risk in patients presenting with STEMI (Farag et al., 2019; Sumaya et al., 2018). Within this cohort of patients, there are a subgroup of whom spontaneous reperfusion, manifesting as complete or partial resolution of ST-elevation pre-primary PCI, exhibited more efficient fibrinolysis than patients without spontaneous reperfusion (Farag et al., 2019).

In contrast to patients presenting with MI-CAD, MINOCA is a syndrome recently coined by the ESC to denote a group of patients presenting with classical acute coronary syndrome signs and symptoms without the presence of obstructive coronary artery disease. It has been recognised, contrary to historical belief, to carry comparable adverse prognosis to patients with obstructive disease (Pasupathy et al., 2015) which has prompted more focus on the investigation and management of this syndrome (Agewall et al., 2016; Tamis-Holland et al., 2019).

The main differentiating factor between the 2 diagnoses is the absence of obstructive disease during the time of angiography, which can vary depending on type of presentation i.e. NSTEMI vs. STEMI. In patients presenting with STEMI within 12 hours of symptom onset, international guidelines recommend urgent angiography with PCI within 90 minutes of the diagnosis to maximise benefits of early reperfusion strategy (Ibanez et al., 2018; O’Gara et al., 2013). Patients exhibiting signs and symptoms of STEMI but no evidence of obstructive disease during angiography could potentially be explained, in part, by the presence of efficient endogenous fibrinolysis.

Aim

I aimed to examine, in a case series of patients presenting with MINOCA STEMI and matched patients with MI-CAD, whether differences in fibrinolytic status could account for their presentation.

Methods

Study principle and population

The VaLiDate-R study (ClinicalTrials.gov Identifier: NCT03775746, EudraCT: 2018-003299-11) is an investigator-initiated, randomised, open-label, single centre trial comparing the effects of ticagrelor, clopidogrel and clopidogrel combined with VLDR on fibrinolytic status in patients with ACS. Patients admitted to hospital with ACS (STEMI, NSTEMI and unstable angina) fitting the inclusion and exclusion criteria underwent a screening process to determine their fibrinolytic status before consenting to randomisation if they displayed impaired endogenous fibrinolysis.

As part of the study, patients with suspected STEMI underwent a screening blood test, following screening consent, to identify their fibrinolytic status prior to angiography. Patients who do not fit criteria for dual antiplatelet therapy would then be withdrawn from the main study, but their results will be recorded for analysis.

From the database containing all the patients screened for the study, I looked into the angiographic findings and final diagnoses of the patients to identify patients with MINOCA. Following that, complementary patients with matched medical history and MI-CAD were identified and extracted for comparison analysis.

Definition of MINOCA

Criteria used to define MINOCA within the cohort is as per ESC working group position paper (Agewall et al., 2016): (1) meets Universal Definition of Acute Myocardial Infarction criteria (Thygesen et al., 2018), (2) no obstructive CAD at angiography, defined as no stenosis (diameter reduction) $\geq 50\%$ in any potential infarct-related artery and (3) no other clinically overt cause for the specific presentation.

Data collection

Basic demographic details including age, gender and medical history are recorded as part of the screening process. Admission blood tests and fibrinolytic status are documented within the electronic CRF. Results of investigations and final diagnosis are retrieved from the medical notes and recorded.

Blood test

As part of the study protocol, 5ml were used to assess global thrombotic status on arrival to the cardiac catheterisation suite as part of their admission for urgent angiography and PCI.

Assessment of thrombotic status

Fibrinolytic status was assessed using the point-of-care GTT (Thromboquest Ltd., London, UK), which assesses both platelet reactivity to high shear stress and endogenous fibrinolysis as explained in detail in Chapter 2. In brief, the test was performed on native, non-anticoagulated whole blood within 15 s of withdrawal. The instrument assesses time taken to form an occlusive thrombus under high shear – recorded in seconds as occlusion time or OT and the restart of flow due to endogenous fibrinolysis – recorded in seconds as lysis time or LT.

Results

A total of 305 patients were screened as described in Chapter 5. Within this cohort, we identified 143 patients who presented with STEMI. Out of the patients with STEMI, 2 patients with MINOCA were included in this case series. 2 patients, closely matched for age, gender and medical history, who presented with MI-CAD were selected as comparators.

Patient A is a 42-year old gentleman admitted with symptoms of chest pain radiating to his left arm, diaphoresis and nausea with no previous cardiovascular disease. His admission ECG showed 1 mm ST-segment elevation of the inferior leads (II, III and aVF) and was promptly brought to the cardiac catheterisation lab for angiography with the view to PCI. The procedure was performed subsequently which revealed unobstructed coronaries with minor atheromatous disease in the left circumflex and therefore no PCI was required. Subsequent blood tests confirmed a significant troponin change from 9.9ng/L to 1084ng/L, meeting the criteria for MINOCA. Echocardiography showed good left ventricular (LV) function with no obvious regional wall motion abnormality (RWMA). On further review of the coronary angiography, it was supposed that the aetiology behind his presentation was an underlying plaque event. His fibrinolytic profile showed an OT of 454.9 secs and LT of 1315 secs.

Patient B is a 45-year old gentleman admitted similarly with symptoms of chest pain at rest with an admission ECG showing inferior ST-segment elevation. He was brought urgently to the cardiac catheterisation lab which revealed moderate disease in his left coronary system and significant (90%) disease in his RCA requiring PCI. He was brought to the ward for routine post-PCI care and subsequently discharged after 48 hours with no immediate complications. His echocardiogram reveals normal LV function and hypokinetic inferoposterior wall, consistent with the infarcted coronary artery territory. His fibrinolytic profile showed an OT of 664.5 secs and LT of 1597 secs revealing lower platelet reactivity

but more impaired fibrinolysis when compared to his matched comparator with MINOCA (Patient A).

Patient C is an 82-year old lady admitted with symptoms of chest pain, radiating to both arms associated with sweating. Her past medical history included that of hypertension and dyslipidaemia, and she was taking aspirin, statin and an angiotensin receptor blocker. Her admission ECG showed inferior ST-segment elevation which resulted in her immediate transfer into the cardiac catheterisation suite for an emergency angiogram. Angiography revealed mild disease in her coronary system with no culprit lesions identified. Following admission into the ward, paired troponin showed significant delta rise (159.5 ng/L to 1527 ng/L) with no obvious RWMA but mild aortic valve disease identified on echocardiography. These findings fit the diagnostic criteria of MINOCA which prompted a further review of her coronary angiography and the final diagnosis was assumed to be due to plaque disruption. She was discharged after 48 hours with no immediate complications. Her admission fibrinolytic profile showed her OT to be 631.1 secs and an LT of 2124 secs.

Patient D is a 78-year old lady with past medical history of hypertension and dyslipidaemia was admitted with symptoms of chest pain and an ECG finding of ST-elevation of the anterior leads. Her admission drugs included aspirin, betablockers, statin and an angiotensin converting enzyme inhibitor. Urgent angiography showed severe distal left main stem disease (LMS) extending into both left anterior descending (LAD) and left circumflex (LCx) artery. PCI was performed to the LMS, extending into the LAD and LCx. Her echocardiogram has revealed good LV function with hypokinesia of the apical segment, mild aortic and mitral disease. She was subsequently discharged with no complications. Her fibrinolytic profile showed an OT of 574.1 secs and LT of 2629 secs revealing higher platelet reactivity and more impaired fibrinolysis when compared to the matched patient with MINOCA (Patient C).

Table 6.1 shows the summary comparison of baseline characteristics, admission drugs and blood tests results, fibrinolytic profile, echocardiographic and angiographic findings of the 4 patients.

Table 6.1 Demographics, characteristics, investigation and final diagnosis

	Patient A	Patient B	Patient C	Patient D
Age	42	45	82	78
Gender	M	M	F	F
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian
Medical history				
Hypertension	No	No	Yes	Yes
Diabetes	No	No	No	No
Dyslipidaemia	No	No	Yes	Yes
Current smoker	No	No	No	No
Admission drugs				
Aspirin	No	No	Yes	Yes
P2Y12 inhibitors	No	No	No	No
Anticoagulants	No	No	No	No
Statin	No	No	Yes	Yes
ACE-I / ARB	No	No	Yes	Yes
Betablockers	No	No	No	Yes
Calcium channel blockers	No	No	No	No
Nitrates	No	No	No	No
Admission blood tests				
Haemoglobin, g/L	149	145	129	103
White Cell Count, 10 ⁹ /L	9.5	10.5	10.3	8
Neutrophils, 10 ⁹ /L	6.94	8.93	7.62	6.48
Platelets, 10 ⁹ /L	291	215	173	205
Creatinine, micromol/L	74	67	82	44

eGFR, ml/min	>90	>90	58	>90
Fibrinogen, g/L	2.0	2.2	3.2	3.3
Prothrombin time, secs	11.2	10.6	10.8	11.6
INR	1.0	1.0	1.0	1.1
aPTT, secs	19.8	20.7	25.0	24.5
Troponin, ng/L (admission)	9.9	97	159.5	79.8
Troponin, ng/L (peak)	1084	-	1527	176.6
Fibrinolytic profile	444	225	1741	1762
OT, sec	454.9	664.5	631.1	574.1
LT, sec	1315	1597	2124	2629
Echocardiogram				
LV function	65%	63%	65%	64%
Presence of RWMA	No	Hypokinetic inferoposterior	No	Hypokinetic apex
RV function	Normal	Normal	Normal	Normal
Valve disease	Nil	Mild MR	Mild AS/AR	Mild AS/MR
Angiographic findings				
Left main stem	Unobstructed	Unobstructed	Unobstructed	90%
Left anterior descending	<30%	60%	<30%	<30%
Left circumflex	<30%	60%	<30%	<30%
Right coronary artery	Unobstructed	90%	<30%	40%
Intracoronary imaging	Nil	Nil	Nil	Nil
Intervention	Nil	PCI x 3 to RCA	Nil	PCI x2 to LMS/LAD/Cx
Diagnosis				
Discharge diagnosis	Inferior STEMI	Inferior STEMI	Inferior STEMI	Anterior STEMI
Underlying aetiology	Likely plaque event		Likely plaque event	

AS – aortic stenosis, Cx – left circumflex, LAD – left anterior descending, LMS – left main stem, MR – mitral regurgitation, PCI – percutaneous coronary intervention, RCA – right coronary artery, STEMI – ST-segment elevation myocardial infarction

Discussion

This small case series reflect some interesting findings when comparing the fibrinolytic status in patients with MINOCA to MI-CAD.

When comparing Patient A and Patient B who are relatively healthy with no past medical history and essentially similar blood profile, Patient B has evidence of obstructive coronary artery and required coronary intervention as treatment for STEMI. He had echocardiographic features of RWMA whereas in contrast, Patient A who had MINOCA, there was no evidence of RWMA on echocardiogram, likely indicative of a shorter ischemic time and therefore lesser myocardial damage (Rácz et al., 2015).

The presence of delta rise of troponin in the case of Patient A establishes that significant myocardial ischemia had taken place to explain the ST-segment elevation seen on his ECG and the symptoms of chest pain. Subsequent review of his coronary angiogram provided clues that his presentation was likely due to a plaque event as there was evidence of minor atheroma in the infarct related artery with the absence of other explanation of his symptoms such as Takotsubo cardiomyopathy or spontaneous coronary artery vasospasm. Although it is suggested that intracoronary imaging and vasospastic testing could be performed in such cases (Agewall et al., 2016; Tamis-Holland et al., 2019) to further delineate the underlying cause, this was unfortunately not performed for him.

Similarly, in Patients C and D, their medical background appears comparable. Patient D had confirmed plaque event with STEMI requiring PCI and corresponding echocardiographic changes whereas Patient C had MINOCA, confirmed with the delta rise in troponin and no other obvious attributable cause. Again, even though review of the coronary angiogram pointed towards likely plaque disruption, it is unfortunate that further investigations to confirm the underlying aetiology were not completed.

Platelet reactivity

Platelet reactivity appears more similar and reduced (C and D – 631.1 vs. 574.1 sec) in the elder pair as compared to the younger pair (A and B – 454.9 vs. 664.5 sec). This could potentially be due to the use of aspirin as primary prevention therapy in the older pair which decreases platelet reactivity (Gurbel et al., 2007) when compared to patients not on aspirin.

Endogenous fibrinolysis

Both pairs had a shown a similar pattern regarding their fibrinolytic profile – patients with MINOCA had more effective endogenous fibrinolysis i.e. shorter lysis time when compared to patients with MI-CAD (A and B – 1315 vs. 1597 sec; C and D – 2124 vs. 2629 sec). This small case series provides some signs, albeit limited due to the extremely small sample size, to explain the potential mechanism proposed previously – effective endogenous fibrinolysis resolves the coronary obstruction from the thrombotic event prior to angiography (Kovacs et al., 2006), giving rise to the MINOCA phenomenon.

Another interesting finding was when comparing between the 2 age groups, the older patients exhibited less favourable endogenous fibrinolysis (mean LT 1456 sec vs. 2377 sec). This has previously been seen in the literature with age-related deterioration of fibrinolytic profile (Abbate et al., 1993; Cesari et al., 2010; Eliasson et al., 1993; Gleerup & Winther, 1995; Ochi et al., 2016) and could potentially explain the comparatively younger median age in MINOCA (Pasupathy et al., 2015; Safdar et al., 2018). The differences between the 2 age groups also highlights the potential role of endogenous fibrinolysis in its contribution to age-related increase in clinical events such as ACS (Cesari et al., 2010).

The difference in platelet reactivity and endogenous fibrinolysis when comparing between similar age groups highlights that in younger patients, effective endogenous fibrinolysis

could potentially offset the negative impact of higher platelet reactivity (Table 6.2), resulting in a marginally less adverse clinical course.

Table 6.2 Comparative fibrinolytic profile between MINOCA and MI-CAD

Patient	Platelet Reactivity (OT)	Endogenous Fibrinolysis (LT)	Clinical Presentation
A	Less favourable	Favourable	MINOCA
B	Favourable	Less favourable	MI-CAD
C	Favourable	Favourable	MINOCA
D	Less favourable	Less favourable	MI-CAD

Table showing the comparative fibrinolytic profile between the 2 age groups and the clinical presentation. It highlights that endogenous fibrinolysis potentially plays a larger role in the resulting clinical presentation in younger patients.*

**Comparison is made between the 2 patients of the same age group.*

Limitations

The main limitation within this case series is the small numbers involved, with only 4 patients. This is due to the small number of patients who were screened and recruited to the VaLiDate-R study and fits criteria for STEMI and MINOCA. The paired patients are not perfectly matched as it was impossible to identify perfectly mirrored patients, but the closest match was chosen from all the patients available in the study. This could have introduced unknown confounders and bias into the dataset which were unaccounted for. Therefore, conclusions drawn from this small sample size are very limited and may only apply to a small subset of patients.

Secondly, investigations for the patients with MINOCA are not completed i.e. advanced intracoronary imaging, cardiac magnetic resonance imaging and vasospastic testing. Without a definitive test to confirm the underlying aetiology, which may be completely different or potentially attributed to multiple causes, the analysis of fibrinolytic status could potentially be invalid. This was due to clinical limitations within the study centre as there was no clinical protocol during the time of the study to mandate investigative pathways in patients with MINOCA. Hence, the final aetiology is based upon the highest likelihood upon available evidence – coronary angiogram with minor atheroma and normal echocardiogram.

Lastly, the cause and effect relationship between endogenous fibrinolysis and the presentation with MINOCA cannot be confirmed as this is a cross-sectional study. The impaired endogenous fibrinolysis could potentially be a reflection of the presence of a greater degree of stenosis as discussed in Chapter 4. Therefore, this case series can only highlight the potential interesting mechanism behind MINOCA and endogenous fibrinolysis. Larger cohort studies would be required to confirm the relationship between this phenomenon.

Conclusion

This small case series highlights endogenous fibrinolysis as a potential mechanism behind patients presenting with MINOCA with a coronary cause. It also accentuates the role that impaired endogenous fibrinolysis can play in younger patients with lower cardiovascular risk profiles. As this is a very small series case study, the findings are mainly hypothesis-generating. Further studies and analyses with a larger cohort of patients with MINOCA and MI-CAD are mandated to assess this relationship.

Chapter 7. Modulating endogenous fibrinolysis in ACS

Abstract

Whilst treatment with DAPT addresses the enhanced platelet reactivity in ACS, ongoing activation of the coagulation cascade and impaired endogenous fibrinolysis appear to be unaffected by current DAPT. There is increasing evidence that impaired endogenous fibrinolysis is a strong predictor of residual cardiovascular risk in patients with ACS. It was my aim to assess whether low dose anticoagulation with rivaroxaban may enhance endogenous fibrinolysis in patients with ACS, over and above standard DAPT.

I performed an investigator-initiated, randomised, open-label, single centre trial in patients with ACS, to investigate relative effects of ticagrelor, clopidogrel and clopidogrel combined with VLDR on fibrinolytic status in patients with ACS with impaired endogenous fibrinolysis. The study was called the VaLiDate-R (Can Very Low Dose Rivaroxaban [VLDR] in addition to dual antiplatelet therapy [DAPT] improve thrombotic status in acute coronary syndrome [ACS]) trial.

Out of a total of 305 patients with ACS, some 98 patients were found to have impaired endogenous fibrinolysis as defined by a lysis time of > 2000 s. Patients were randomised to one of 3 antithrombotic treatments: aspirin plus clopidogrel, aspirin plus ticagrelor, and aspirin, clopidogrel and VLDR. Comparison of the fibrinolytic profiles showed that all 3 regimes sustained improvement in LT at 8-week follow-up and the magnitude of change correlated to the initial baseline LT ($r = 0.6467$, $p < 0.0001$). When compared with clopidogrel or ticagrelor, the addition of rivaroxaban did not confer a larger magnitude of improvement in LT. However, rivaroxaban did produce a more significant prolongation of OT than that with P2Y₁₂-inhibitors alone.

The results of the VaLiDate-R study showed that in patients with impaired endogenous fibrinolysis at the time of ACS, there is an improvement in fibrinolytic status by 4 weeks post-

ACS, irrespective of the antithrombotic regimen prescribed post-ACS. The magnitude of improvement of endogenous fibrinolysis appeared similar amongst the regimens compared and was related to the baseline lysis time, with no additional benefit of VLDR. The addition of VLDR did significantly prolong OT compared to patients just taking P2Y₁₂-inhibitors. A larger cohort study to correlate these findings with clinical outcomes is required.

In conclusion, the addition of VLDR does improve thrombotic status by reducing platelet reactivity, but not by enhancing endogenous fibrinolysis.

Introduction

International guidelines for antithrombotic treatment for ACS patients, following the acute phase, predominantly entails administration of DAPT, which comprise of aspirin together with a P2Y₁₂ inhibitor (Ibanez et al., 2018; Roffi et al., 2016). This recommendation was put in place following the results of the CURE study (Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events) (Yusuf et al., 2001) which identified benefits of adding the P2Y₁₂ inhibitor clopidogrel to aspirin in patients with ACS.

Clopidogrel is a prodrug requiring two step activation to its active metabolite which irreversibly inhibit P2Y₁₂ receptors resulting in platelet inhibition. It was the first P2Y₁₂ inhibitor which was widely used. However, it was soon discovered that clopidogrel has important limitations – variability in platelet inhibition between patients due to “non-responders” (Serebruany et al., 2005) and consequently recurrent atherothrombotic events (Matetzky et al., 2004).

The quest for better alternatives to clopidogrel began with the TRITON-TIMI 38 (Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction) trial which showed that prasugrel, a more potent prodrug, was superior to clopidogrel in the ACS cohort undergoing PCI, with significant reduction in the risk of cardiovascular death, myocardial infarction, stroke and stent thrombosis, albeit at the expense of a significant increase in major bleeding (Wiviott et al., 2007). Subsequently, the PLATO (Platelet Inhibition and Patient Outcomes) study (Wallentin et al., 2009) showed similar reduction in ischemic events with ticagrelor when compared to clopidogrel but again, without an increase in the rate of overall major bleeding but with an increase in the rate of non-procedure-related bleeding.

Despite advances in antiplatelet pharmacotherapy, up to 10% of patients with ACS continued to suffer adverse ischemic events (French et al., 2015). Beyond DAPT, trials have explored the concept of using triple (rather than dual) antithrombotic therapy to further reduce ischaemic events following ACS (Spinhakis et al., 2018) with the addition of cilostazol (Suh et al., 2011), vorapaxar (Tricoci et al., 2012), dabigatran (Oldgren et al., 2011) and rivaroxaban (Mega et al., 2012) to conventional DAPT. These studies has shown that whilst additional antithrombotic therapy can further reduce recurrent ischaemic events, it significantly increases the risk of bleeding and therefore is not suitable for every patient (Spinhakis et al., 2018).

Hence, the identification of patients at higher risk of future adverse ischemic events would be extremely desirable, as they could be targeted with more potent antithrombotic medications, allowing the use of less potent therapies in low-risk groups to reduce bleeding risk and consequently improving net clinical benefit.

Endogenous fibrinolysis as a risk predictor

Following the onset of a thrombotic stimulus such as rupture or erosion of a thin-cap fibroatheroma, the likelihood of ACS and potential coronary occlusion is determined by the overall balance between factors which propagate thrombosis, mainly through enhanced platelet aggregation and activation of the coagulation cascade, and the effectiveness of the inherent defence mechanism of endogenous thrombolysis/fibrinolysis.

Whilst treatment with DAPT addresses the enhanced platelet reactivity in ACS, ongoing activation of the coagulation cascade and impaired endogenous fibrinolysis appears to be unaffected by current DAPT. There is increasing evidence that impaired endogenous fibrinolysis is a strong predictor of residual cardiovascular risk in patients with ACS. Altered fibrin clot structure and increased resistance of the clot to lysis have been associate with

myocardial infarction and stent thrombosis (Collet et al., 2006; Leander et al., 2012; Undas et al., 2008, 2010). In the last 2 years, two large prospective studies have confirmed that impaired fibrinolysis in patients with ACS is a novel independent marker of increased cardiovascular risk (Farag et al., 2019; Sumaya et al., 2018). In a sub-study of >4000 patients in the PLATO trial, assessment of fibrin clot lysis using a validated turbidimetric assay revealed that impaired fibrin clot lysis was an independent predictor of adverse outcome in ACS (Sumaya et al., 2018). After adjusting for established cardiovascular risk factors, each 50% increase in lysis time was associated with cardiovascular death/spontaneous MI (HR 1.17, 95% CI 1.05-1.31; $P < 0.01$) and cardiovascular death alone (HR 1.36, 95% CI 1.17-1.59; $P < 0.001$). Earlier work employing a point-of-care assay of whole blood fibrinolysis showed that some 23% of patients with NSTEMI exhibit impaired endogenous fibrinolysis (lysis time, $LT \geq 3000$ sec) despite DAPT, and this is predictive of recurrent adverse cardiovascular events (HR: 2.52, $p < 0.04$) and cardiovascular death (HR: 4.2, $p = 0.033$) over the subsequent year. with hazard increasing with increasing lysis time (Saraf et al., 2010). More recently, the RISK PPCI study from our group, involving nearly 500 patients with ST-segment elevation myocardial infarction (STEMI) showed that impaired endogenous fibrinolysis ($LT \geq 2500$ s), detected in 14% patients on admission, was strongly related to recurrent major cardiovascular events (HR 9.1, 95% CI 4.28-15.03, $p = 0.001$), driven by cardiovascular death and myocardial infarction (Farag et al., 2019).

Pharmacological modulation of endogenous fibrinolysis

Unlike the enhanced platelet reactivity in these patients which lessened from admission to discharge, presumably reflecting the onset of effect of antiplatelet therapy, fibrinolysis in ACS patients appears unaffected by DAPT (Farag et al., 2019; Sumaya et al., 2018). P2Y₁₂ inhibitors have been shown to have minimal impact on endogenous fibrinolysis (Spinthakis, Farag, et al., 2019). In a small study, compared to baseline tests performed in the absence of

anticoagulation, treatment with non-vitamin K antagonist oral anticoagulation appeared to favourably enhance endogenous fibrinolysis (Farag et al., 2016). The finding of improvement in fibrinolysis through FXa inhibition is translated to clinical outcomes supported by the results of the ATLAS ACS-2 TIMI (Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome–Thrombolysis in Myocardial Infarction) 51 study, showing that addition of VLDR at 2.5 mg twice daily to DAPT comprising of aspirin and clopidogrel in ACS patients significantly reduced the primary efficacy end point of the composite of death from cardiovascular causes, myocardial infarction, or stroke compared to placebo (9.1% vs. 10.7%, $P=0.02$) but increased the risk of major bleeding and intracranial haemorrhage (Mega et al., 2012). In individuals with stable cardiac or vascular disease, the COMPASS (Cardiovascular Outcomes for People Using Anticoagulation Strategies) study demonstrated the benefit of VLDR in addition to aspirin in reducing the composite of cardiovascular death, stroke, or myocardial infarction (4.1 vs 5.4%, $P<0.001$) albeit at a cost of increased bleeding (Eikelboom et al., 2017).

Aim

Study rationale

Measurement of endogenous fibrinolysis appears to identify patients who, despite DAPT, are at increased risk of recurrent adverse cardiovascular events. Additional pharmacotherapy to improve endogenous fibrinolysis may improve outcomes in high risk patients, whilst avoiding unnecessary additional pharmacotherapy and bleeding in low risk patients.

Hypothesis

I hypothesised that in patients with ACS who demonstrate impaired fibrinolysis, use of VLDR, in addition to DAPT, will result in improved fibrinolytic profile, compared to patients taking DAPT alone.

Methods

Study principle and population

The VaLiDate-R study (ClinicalTrials.gov Identifier: NCT03775746, EudraCT: 2018-003299-11) is an investigator-initiated, randomised, open-label, single centre trial comparing the effects of ticagrelor, clopidogrel and clopidogrel combined with VLDR on fibrinolytic status in patients with ACS. Patients admitted to hospital with ACS (including those with STEMI, NSTEMI and unstable angina) who fulfilled the inclusion and exclusion criteria of the study were recruited for screening blood test to assess their fibrinolytic profile, within their index admission.

Inclusion and exclusion criteria

All patients presenting with ACS who meet the inclusion and exclusion criteria as set out in Table 7.1 were approached for participation in the study.

A two-stage consent process was adopted where an eligible patient would first consent to a screening blood test to identify impaired endogenous fibrinolysis before proceeding to a full consent to allow for randomisation and trial procedures. In cases of patients presenting acutely to the hospital with STEMI for primary PCI, a delayed consent approach was undertaken, with ethical permission, whereby screening blood tests for assessment of thrombotic status were taken at the same time as standard of care blood samples on arrival, and patients were consented for the study after the primary PCI procedure following stabilisation, when they were better able to carefully consider the study. This was to ensure the consent process was fully informed (patients presenting with STEMI are unwell and often haemodynamically unstable, thus may not be in a suitable state to give full informed consent) and did not impact on the clinical care (explaining the study and obtaining full consent might cause delay to the emergency primary PCI procedure) whilst not compromising the integrity

of the sampling process (drugs such as heparin given during the procedure may affect the thrombotic status of patients). Patients that declined to participate after the emergency procedure were recorded as a screen-fail and were not included in the study. Patients who consented after the procedure fitting the eligibility criteria were included if written full informed consent was obtained.

Randomisation and study groups

Allocation of patients to one of the three study groups were made by the use of a web-based block randomisation process. In subjects whose screening blood test showed that fibrinolytic status was impaired (LT >2000s), following full written informed consent, the patients were randomised to one of 3 treatment arms in a 1:1:1 ratio: clopidogrel 75 mg daily (Group 1); clopidogrel 75 mg daily plus rivaroxaban 2.5 mg twice daily (Group 2); ticagrelor 90 mg twice daily (Group 3), in addition to standard therapy of aspirin 75 mg daily and other secondary prevention medication as clinically indicated. For patients randomised to a new drug, for example patients initially treated with ticagrelor who were subsequently randomised to clopidogrel, a loading dose of the new medication was given (clopidogrel 300 mg or ticagrelor 180 mg). The duration of rivaroxaban was 30 days, after which rivaroxaban was discontinued and patients continued on clopidogrel only or were switched to ticagrelor (including with loading), as decided by the clinical care team. During follow-up, all other treatments were continued in accordance with standard of care, at the discretion of the clinical team.

Study follow up

Assessment and blood draw were performed at 2 weeks, 4 weeks and 8 weeks post randomisation to assess fibrinolytic status, record any adverse events and evaluate compliance. At each visit, subjects were asked about compliance. Additionally, patients in

the rivaroxaban arm had Factor Xa levels assessed to confirm compliance during Visit 2 and 4, taken during the peak effect of rivaroxaban (between 2-5 hours post-dose). A telephonic follow up was performed at 6 months to assess for further clinical events. Unfortunately, due to the COVID-19 pandemic, a number of patients were unable to return to the hospital for their follow-up appointment and had a telephonic safety follow-up instead.

Study related procedures

A single blood draw was used for tests of thrombosis and thrombolysis. The first 5 ml were used for standard of care blood tests and the next 10 ml blood was used for the GTT assay and TEG.

Global Thrombosis Test

The use of the GTT (Thromboquest Limited, London, UK) has been described in Chapter 2. Briefly, it is a point-of-care test of thrombotic status that utilises native (non-anticoagulated) blood to assess thrombosis (OT) and thrombolysis (LT).

Thromboelastography

Similarly, TEG (Haemonetics Corporation, USA) has been described in detail in Chapter 2. In brief, native venous blood was tested immediately following withdrawal and thrombotic status including platelet independent clot formation (R, K, A, MA, TMA) as well as primary fibrinolytic potential (LY30, LY60 and CLT).

Primary and secondary endpoints

The primary outcome measure was the change in fibrinolytic status, as measured by LT using the GTT, from admission to follow-up visit (Week 2 or 4). Secondary outcome measures were clinical events including re-intervention (further coronary angioplasty), major adverse

cardiac events (composite of heart attack, stroke or death) and bleeding events evaluated by Bleeding Academic Research Consortium (BARC) criteria (Mehran et al., 2011).

Sample size calculation

Sample size was calculated using Stata version 15.1 (StatCorp, College Station, TX, USA). Spinthakis et al. showed that full dose apixaban improved LT by 400s (Spinthakis, Gue, Farag, Srinivasan, et al., 2019) and Farag et al. showed a trend of about 300s improvement with full dose rivaroxaban at 20mg o.d. dose (Farag et al., 2016). Therefore, we postulated that about 90 sec would be seen with 2.5mg b.i.d. Assuming $\alpha=0.05$ for two sided tests, to detect a difference of LT=90s with a power $1-\beta=0.80$, a sample size of 45 in each group is required. Accounting for a 10% drop-out, withdrawal and lost to follow up, we calculated we would need 50 patients in each group, totalling to a sample size of 150. However, due to the COVID-19 pandemic, recruitment was halted and a total of 98 randomised participants had data available for analysis. Power calculation with the current sample size, with an $\alpha=0.05$ to detect a difference of LT=90s showed a power of $1-\beta=0.6702$.

Statistical considerations

The study intended to evaluate the extent to which VLDR (Group 2) reduces LT compared to DAPT alone (Group 1 and Group 3). Separate paired comparisons were used to evaluate univariate effects between the study groups baseline and follow up fibrinolytic status. Binary baseline characteristics were compared using Fisher's exact test and normally distributed continuous variables were compared using ANOVA whereas non-normally distributed variables were compared using Wilcoxon rank sum test (continuous). Fibrinolytic status (OT and LT) was tested for normality using Shapiro-Wilk test. Normally distributed variables were analysed using t-test, ANOVA and paired t-test whereas non-normally distributed variables were analysed using Wilcoxon rank-sum, Kuskal-Wallis and Wilcoxon matched-

pair sign-rank test. Spearman's rank correlation was used to identify correlation between variables.

A sensitivity analysis comparing baseline to 4-week follow-up was performed with patients showing a clinically significant impairment of endogenous fibrinolysis ($LT \geq 2500$ sec) (Farak et al., 2019). Analyses were performed with Stata version 15.1 (StataCorp, College Station, TX, USA) and significance was taken at <0.05 .

Ethical and regulatory aspects

The regulatory and ethical permission are described in Chapter 2 in detail and the relevant approvals are shown in Appendix 2 (Page 260 – 273). A trial steering committee was established to take responsibility for overseeing the good execution and administrative progress of the protocol. The Data Safety Monitoring Board took responsibility for periodically reviewing and evaluating the accumulated study data for participant safety, study conduct and progress, and making recommendations to the steering committee concerning the continuation, modification, or termination of the trial.

Table 7.1 Inclusion and exclusion criteria for VaLiDate-R study

Inclusion	Exclusion
<ol style="list-style-type: none"> 1. Male and female patients aged 18 years or over 2. Have a diagnosis of acute coronary syndrome requiring treatment with dual antiplatelet therapy 3. Be willing and able to understand the Participant Information Sheet and provide informed consent 4. Agree to comply with the drawing of blood samples for the assessments 5. Not meet any of the exclusion criteria 	<ol style="list-style-type: none"> 1. Male and female participants aged < 18 years of age. 2. Patient unwilling or unable to give informed consent 3. Patients who might be pregnant or are breast-feeding 4. Active clinically significant bleeding 5. Patient who, in the opinion of the investigator, has condition considered to be a significant risk for major bleeding (such as current or recent gastrointestinal ulceration, presence of malignant neoplasm at high risk of bleeding, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial haemorrhage, known or suspected oesophageal varices, arteriovenous malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities) 6. Hepatic disease associated with coagulopathy and clinically relevant bleeding risk including cirrhotic patients with Child Pugh B and C 7. Patient with any contraindications to use of antiplatelet agents or anticoagulants 8. Hypersensitivity to the active substance or to any of the excipients listed in section 6.1 of Summary of Product Characteristics (SmPC) of Rivaroxaban 9. Concomitant treatment with any other anticoagulants e.g. unfractionated heparin (UFH), low molecular weight heparins (enoxaparin, dalteparin, etc.), heparin derivatives (fondaparinux, etc.), oral anticoagulants (warfarin, dabigatran etexilate, apixaban etc.) except under specific circumstances of switching anticoagulant therapy or when UFH is given at doses necessary to maintain an open central venous or arterial catheter 10. Concomitant treatment of ACS with antiplatelet therapy in patients with a prior stroke or a transient ischaemic attack (TIA) 11. Patient with ongoing active alcohol or substance abuse or demonstrates signs or clinical features of active substance abuse. 12. Patient with any major bleeding diathesis or blood dyscrasia at baseline (platelets < 70 x 10⁹/l, Hb < 80 g/l, INR > 1.4, APTT > x 2UNL, leucocyte count < 3.5x 10⁹/l, neutrophil count < 1x 10⁹/l) 13. Patient currently enrolled in an investigational drug trial

Results

As reported in Chapter 6, between the period of January 2019 and March 2020, a total of 305 patients who were eligible underwent a screening blood test. Out of those, 98 patients who displayed impaired endogenous fibrinolysis proceeded to full informed consent and were randomised into the study. In total, 33 patients were randomised into Group 1 and 2 each, with the remaining 32 patients in Group 3. A total of 11 patients withdrew from the study (five from Group 1 – 2 was patient's choice and 3 was medical decision due to requirements for anticoagulation post-randomisation, four from Group 2 – 1 was patient's choice and 3 was medical decision and two from Group 3 – 1 was patient's choice and 1 was medical decision). Two patients from Group 3 were lost to follow up and seven patients were not able to attend any follow up appointments due to restrictions during the COVID-19 pandemic (three from Group 1, two from Group 2 and two from Group 3). The remaining patients were included in the final analysis – 25 patients in Group 1, 27 patients in Group 2 and 26 patients in Group 3.

Table 7.2 shows the baseline characteristics of the patients. All the baseline characteristics including demographics and medical history were similar in the 3 groups of patients. Similarly, there was no significant difference in admission observations and blood results (Table 7.3), angiographic findings and management (Table 7.4) in the 3 groups.

Comparison of baseline to 4-week follow-up

As OT was normally distributed, parametric statistic tests were utilised. As shown in Table 7.5, The OT of all 3 groups were comparable at baseline (Group 1: 469.2 ± 173.4 vs. Group 2: 447.6 ± 164.0 vs. Group 3: 498.1 ± 168.9 s, $p=0.5540$). At the follow up visit, Group 1 had the shortest OT whereas Group 2 was longest (Group 1: 476.3 ± 139.6 vs. Group 2: 600.6 ± 136.0 vs. Group 3: 543.3 ± 136.7 , $p=0.0069$). Group 2 displayed an overall significant

increase in OT compared to baseline (delta OT 153 ± 206.9 s, $p=0.0065$) and similarly when compared to Group 1 (7.02 ± 156.5 s, $p=0.0063$) and Group 3 (45.3 ± 126.2 s, $p=0.0269$).

Only Group 2 showed a significant change in OT ($p=0.0007$) with paired comparison of OT from baseline to 4-week follow-up.

Baseline LT was similar amongst the 3 groups. Follow up LT was also similar in the 3 groups. Delta LT was not significant when comparing amongst the 3 groups ($p=0.139$) and between Group 1 and 2 ($p=0.394$) but was significantly more in Group 3 when compared with Group 2 (-800 [-1278 to -484] vs. -496 [-1002 to 238] s, $p=0.042$). However, all 3 groups showed a significant decrease in LT with paired comparison analysis (Group 1: $p=0.0128$; Group 2: $p=0.0116$; Group 3: $p=0.0001$).

Spearman's rank correlation has shown that the delta LT was significantly correlated to baseline LT ($r = 0.6467$, $p<0.0001$). There was no correlation between baseline LT and baseline OT with any of baseline characteristics or blood variables. Baseline LT was split into 2 groups based on previous study defining clinically relevant cut-off, $LT > 2500$ s and $LT < 2500$ s, to further understand the effects of baseline LT on the magnitude of change. Delta LT was significantly lower in the $LT < 2500$ s group (-283 [-602 – 238] vs. -1159 [-1842 – -668] s, $p<0.0001$).

Comparison to 8-week follow-up

Follow up sampling at 8-weeks showed that OT was similar in all 3 groups and when compared separately with Group 2 (Table 7.5). When compared to the OT at baseline, the OT was not significantly different from baseline in all 3 groups. However, the OT from 4-week follow-up decreased significantly in Group 2 with the discontinuation of rivaroxaban (600.6 ± 136.0 vs. 497.6 ± 139.2 , $p=0.0045$)

Similarly, the 8-week LT was comparable amongst the 3 groups. When compared to the LT at baseline, there was significant decrease in all groups which persisted from the 4-week follow-up. However, there was no significant change in LT when compared to the 4-week LT.

Comparison of TEG parameters

Paired comparison of TEG parameters (Table 7.6) showed no difference between measured parameters at baseline and at follow up in any group, except for Group 2 where there was a change in reaction time (R) (5.05 [3.6 – 97] vs. 10.2 [7.7 – 14] s, $p=0.039$) and time to maximum amplitude (TMA) (32.25 [29.2 – 43.2] vs. 42.5 [33.8 – 56.6] s, $p=0.011$).

Spearman's rank correlation showed no correlation between any TEG parameters at baseline with baseline LT or OT.

Sensitivity analysis

In the sensitivity analysis using the subgroup of patients with LT > 2500s, there were 14 patients in Group 1, 11 patients in Group 2 and 16 patients in Group 3 (Table 7.7). There was no significant difference in the baseline fibrinolytic profile (OT and LT) amongst the 3 groups. Paired comparison of LT remained significantly different in all 3 groups with no significant difference in delta LT. However, paired OT was no longer significant in Group 2 in this subgroup of patients ($p=0.0559$). When comparing the delta OT and delta LT, there was no longer any significant difference when comparing the 3 groups overall or with comparison between 2 groups.

Table 7.2 Baseline characteristics of patients randomised in VaLiDate-R study

	Group 1 (n=25)	Group 2 (n=27)	Group 3 (n=26)	p-value
Age, years	63 (54 – 72)	62 (53 – 70)	63.5 (55 – 72)	0.723
Male	23 (92)	21 (77.8)	19 (73)	0.214
BMI, kg/m²	26.77 (24.22 – 29.76)	30.08 (24.22 – 32.62)	27.92 (23.99 – 33.88)	0.650
Race				0.376
Caucasian	23 (92)	24 (88.9)	25 (96.2)	0.774
Black	1 (4)	0 (0)	1 (3.8)	0.541
Asian	1 (4)	3 (11.1)	0 (0)	0.263
ACS type				0.240
STEMI	9 (36)	13 (48.2)	9 (34.6)	0.546
NSTEMI	15 (60)	11 (40.7)	17 (65.4)	0.170
Unstable angina	1 (4)	3 (11.1)	0 (0)	0.263
Current smoker	6 (24)	10 (37)	5 (19.2)	0.347
Units of alcohol/week	2 (0 – 5)	0 (0 – 2)	1 (0 – 10)	0.508
Hypertension	7 (28)	12 (44.4)	12 (46.2)	0.367

Diabetes	4 (16)	7 (25.9)	4 (15.4)	0.626
Dyslipidaemia	8 (32)	8 (29.6)	12 (46.2)	0.425
Angina	3 (12)	4 (14.8)	3 (11.5)	1.000
Previous MI	3 (12)	4 (14.8)	3 (11.5)	1.000
Previous PCI	3 (12)	4 (14.8)	4 (15.4)	1.000
Previous CABG	0 (0)	1 (3.7)	3 (11.5)	0.212
CKD	0 (0)	1 (3.7)	1 (3.9)	1.000
Previous CVA	2 (8)	1 (3.7)	1 (3.9)	0.686
PVD, n(%)	0 (0)	1 (3.7)	1 (3.9)	1.000

ACS – acute coronary syndrome, BMI – body mass index, CABG – coronary artery bypass grafting, CKD – chronic kidney disease, MI – myocardial infarction, NSTEMI – non-ST-elevation myocardial infarction, PCI – percutaneous coronary intervention, PVD – peripheral vascular disease, STEMI – ST-elevation myocardial infarction

All binary variables presented as n(%) and continuous variable as median (IQR)

Table 7.3 Admission observations and blood tests

	Group 1 (n=25)	Group 2 (n=27)	Group 3 (n=26)	p-value
Heart rate, bpm	77 (65 – 83)	70 (60 – 79)	73.5 (66 – 83)	0.429
Systolic BP, mmHg	139 (123 – 155)	137 (119 – 154)	139.5 (128 – 160)	0.601
Diastolic BP, mmHg	81 (72 – 92)	85 (70 – 91)	81.5 (71 – 92)	0.969
Haemoglobin, g/dL	147 (135 – 152)	140 (120 – 146)	137 (126 – 150)	0.330
WCC, x10⁹/L	9.3 (7.3 – 11.3)	10.1 (8.7 – 11.6)	10.3 (7.5 – 11.8)	0.538
Neutrophil, x10⁹/L	5.9 (4.8 – 8.6)	6.7 (5.3 – 8.7)	7.6 (5.3 – 8.9)	0.567
Platelet, x10⁹/L	237 (201 – 296)	246 (194 – 299)	257 (205 – 314)	0.582
Albumin	45 (42 – 46)	43 (40 – 45)	43 (40 – 44)	0.509
Creatinine	79 (72 – 88)	86 (75 – 97)	74.5 (65 – 89)	0.070
eGFR	84 (74 – 89)	76 (55 – 90)	82.5 (72 – 90)	0.103
PT, sec	11 (10.4 – 11.3)	10.7 (10.3 -11.2)	10.9 (10.5 – 11.2)	0.714
INR	1 (1 – 1)	1 (1 – 1)	1 (1 – 1.1)	0.756
aPTT, sec	23.1 (21.2 – 25.6)	23.2 (22.1 – 25.2)	24 (22.2 – 25.3)	0.844
Fibrinogen	3.5 (2.7 – 4.1)	3.6 (3.4 – 4.2)	3.7 (3.1 – 4.2)	0.391

CRP	2.5 (1 – 6)	3 (2 – 7)	8 (3 – 11)	0.028
Total cholesterol	5.5 (4.3 – 5.7)	4.4 (4.2 – 5.6)	4.8 (3.8 – 5.2)	0.369
HDL	1.2 (0.9 – 1.5)	1.1 (1 – 1.1)	1.2 (1.0 – 1.5)	0.185
LDL	3.5 (1.9 – 4.2)	2.6 (2.0 – 3.2)	2.8 (2.0 – 3.3)	0.369
Triglycerides	1.7 (1.2 – 3.3)	2 (1.4 – 2.1)	1.6 (1.3 – 1.9)	0.724

WCC – white cell count, eGFR – estimated glomerular filtration rate, PT - prothrombin time, INR – international normalised ratio, aPTT – activated partial thromboplastin time, CRP – C-reactive protein, HDL – high density lipoprotein, LDL – low density lipoprotein

All binary variables presented as n (%) and continuous variable as median (IQR)

Table 7.4 Angiographic findings and intervention details

	Group 1 (n=25)	Group 2 (n=27)	Group 3 (n=26)	p-value
Radial access used	23 (92)	23 (85.2)	23 (88.5)	0.904
No. vessels diseased				0.896
1	11 (44)	11 (40.7)	13 (50)	
2	8 (32)	10 (37)	5 (19.2)	
3	5 (24)	5 (18.5)	7 (26.9)	
PCI performed	21 (84)	19 (70.4)	17 (65.4)	0.312
No. of stents				0.228
1	16 (64)	9 (33.3)	12 (46.2)	
2	5 (20)	8 (29.6)	4 (15.4)	
>2	0 (0)	1 (3.7)	1 (3.9)	

PCI – percutaneous coronary intervention, POBA – plain old balloon angioplasty

All variables presented as n (%).

Table 7.5 Fibrinolytic profile of whole cohort

	Group 1 (n=25)	Group 2 (n=27)	Group 3 (n=26)	3 group comparison p-value	Group 1 & 2 comparison p-value	Group 2 & 3 comparison p-value
<i>Occlusion Time</i>						
Baseline OT	469.2 ± 173.4	447.6 ± 164.0	498.1 ± 168.9	0.5540	0.6458	0.2750
4-week OT	476.3 ± 139.6	600.6 ± 136.0	543.3 ± 136.7	0.0069*	0.0021*	0.1322
Delta OT	7.02 ± 156.5	153 ± 206.9	45.3 ± 126.2	0.0065*	0.0063*	0.0269*
Paired OT[§] (Baseline and 4-week)	0.8244	0.0007*	0.0795			
8-week OT	473.4 ± 162.7	497.6 ± 139.2	516.7 ± 118.0	0.6290	0.6081	0.6446
Paired OT[§] (Baseline and 8-week)	0.3888	0.3025	0.7562			
Paired OT[§] (4-week and 8- week)	0.8052	0.0045*	0.4689			
<i>Lysis Time</i>						
Baseline LT	2507 (2125 – 3301)	2362 (2190 – 2785)	2645 (2370 – 3377)	0.211	0.394	0.071
4-week LT	1928 (1717.1 – 2606)	2034 (1718 – 2599)	1898.5 (1675 – 2316)	0.583	0.840	0.294
Delta LT	-721 (-1245.9 – -58)	-496 (-1002 – 238)	-800 (-1278 – -484)	0.139	0.394	0.042*
Paired LT[#] (Baseline and 4-week)	p=0.0128*	p=0.0116*	p=0.0001*			
8-week LT	1748 (1504 – 2504)	1939 (1621 – 2205)	1840 (1531 – 2238)	0.815	0.538	0.636

Paired LT# (Baseline and 8-week)	p=0.0002*	p=0.025*	p=0.0001*
Paired LT# (4-week and 8- week)	p=0.5901	p=0.5430	p=0.6292

§ Paired comparison using paired t-test; # Paired comparison using Wilcoxon matched-pair signed-rank test;
 LT – lysis time, OT – occlusion time

LT presented as median (IQR) and OT as mean ± standard deviation

Table 7.6 Comparison of TEG parameters

	Baseline	4-week	p-value
Group 1			
Reaction time (R), sec	14.5 (10.8 – 19.3)	6.1 (4.5 – 10.05)	0.075
Kinetics time (K), sec	5.8 (2.4 – 7.8)	4.2 (2.5 – 5.7)	0.059
Angle (A), degrees	29.8 (24.2 – 60.3)	56 (32.3 – 69.2)	0.075
Maximum amplitude (MA), mm	70.6 (60.9 – 77.5)	69.9 (62.7 – 76.4)	0.285
Shear modulus strength (G), Kdynes/sec	12 (7.8 – 17.2)	11.6 (8.4 – 16.2)	0.508
Coagulation index (CI)	1.6 (-1.2 – 3.5)	3.4 (2.8 – 4.7)	0.110
Lysis in 30 minutes (LY30), %	0 (0 – 1)	0 (0 – 0.1)	0.545
Lysis in 60 minutes (LY60), %	0 (0 – 3.9)	0.3 (0 – 3.1)	0.660
Time to maximum amplitude (TMA), sec	39.8 (31.2 – 55)	32.8 (25.2 – 42.7)	0.285
Clot lysis time (CLT), sec	60.2 (59.2 – 61.2)	59.8 (59 – 61.2)	0.307
Thrombodynamic potential index (TPI)	20.6 (12.7 – 33.3)	32 (19.1 – 52.1)	0.386

Group 2

Reaction time (R), sec	5.05 (3.6 – 9.7)	10.2 (7.7 – 14)	0.039*
Kinetics time (K), sec	4.7 (2.8 – 6)	4.3 (3.5 – 5.3)	0.221
Angle (A), degrees	47.3 (32.6 – 70)	45.9 (39.2 – 55.1)	0.553
Maximum amplitude (MA), mm	73 (67.8 – 78.1)	79.6 (74.4 – 82.5)	0.069
Shear modulus strength (G), Kdynes/sec	13.55 (10.5 – 17.8)	19.5 (14.6 – 23.6)	0.064
Coagulation index (CI)	4.3 (3.4 – 5.2)	5 (3.3 – 5.4)	0.534
Lysis in 30 minutes (LY30), %	0 (0 – 0)	0 (0 – 0.6)	0.717
Lysis in 60 minutes (LY60), %	0.4 (0 – 2.5)	0.2 (0 – 1.6)	0.916
Time to maximum amplitude (TMA), sec	32.3 (29.2 – 43.2)	42.5 (33.8 – 56.6)	0.011*
Clot lysis time (CLT), sec	61.1 (60.4 – 62.8)	60.6 (59.2 – 61.4)	0.124
Thrombodynamic potential index (TPI)	36.6 (17.5 – 50.4)	38.9 (23.2 – 62)	0.917

Group 3

Reaction time (R), sec	13.5 (6.65 – 24.1)	8.05 (5.5 – 11.7)	0.308
Kinetics time (K), sec	5.8 (2.8 – 9.9)	3.9 (3 – 4.8)	0.100
Angle (A), degrees	36.4 (25 – 61.5)	44.75 (40.5 – 59.2)	0.308
Maximum amplitude (MA), mm	68.9 (58.3 – 78.8)	73.65 (65.8 – 77.2)	0.209
Shear modulus strength (G), Kdynes/sec	11.1 (7 – 18.6)	13.95 (9.6 – 17)	0.289
Coagulation index (CI)	2.9 (0 – 5.5)	4.3 (2.9 – 5.1)	0.195
Lysis in 30 minutes (LY30), %	0 (0 – 0.5)	0 (0 – 0.8)	0.935
Lysis in 60 minutes (LY60), %	1 (0 – 2.5)	0.45 (0 – 3.1)	0.937
Time to maximum amplitude (TMA), sec	42.6 (31.3 – 57.2)	34.5 (31.1 – 44.5)	0.209
Clot lysis time (CLT), sec	60.95 (60 – 61.4)	60.8 (59.2 – 61.3)	0.182
Thrombodynamic potential index (TPI)	11.5 (4.3 – 40.2)	34.4 (19.1 – 62)	0.117

Values presented as median (IQR).

Table 7.7 Fibrinolytic profile of subgroup (LT>2500)

	Group 1 (n=25)	Group 2 (n=27)	Group 3 (n=26)	3 group comparison p-value	Group 1 & 2 comparison p-value	Group 2 & 3 comparison p-value
Baseline OT	452.4 ± 193.9	431.0 ± 169.1	497.4 ± 184.7	0.6290	0.7742	0.3510
4-week OT	466.9 ± 137.1	559.8 ± 130.0	553.7 ± 153.2	0.1746	0.0992	0.9152
Delta OT	14.5 ± 136.4	128.8 ± 197.5	56.2 ± 142.0	0.2053	0.1005	0.2763
Baseline LT	3290 (2815 – 4642)	2889 (2668 – 3657)	3172 (2715.5 – 4845)	0.676	0.366	0.505
4-week LT	2030 (1811 – 3032)	1969 (1593 – 2238)	1924.5 (1735.5 – 2508)	0.641	0.381	0.657
Delta LT	-1233.45 (-2550 - -228)	-1002 (-1419 - -699)	-1215 (-2272 - -658.5)	0.866	0.743	0.657
Paired OT [§]	p=0.6972	p=0.0559	p=0.1340			
Paired LT [#]	p=0.0092	p=0.0033	p=0.0038			

§ Paired comparison using paired t-test; # Paired comparison using Wilcoxon matched-pair signed-rank test; LT – lysis time, OT – occlusion time

LT presented as median (IQR) and OT as mean ± standard deviation.

Discussion

Effect on fibrinolytic status

The VaLiDate-R study has shown that in patients with ACS and impaired endogenous fibrinolysis, treatment with antithrombotic agents results in significantly improved fibrinolytic status. This is irrespective of the combination of antithrombotic agents i.e. aspirin plus clopidogrel or aspirin plus ticagrelor or combination aspirin, clopidogrel and rivaroxaban. This effect is persistent throughout the monitored period of 8 weeks, stabilised from the first follow up at 4 weeks with no further significant change. This is likely a reflection of the normalisation of the fibrinolytic system back to a baseline level following ACS which has previously been shown by Shantsila *et al.* that fibrinolytic factors such as tPA and PAI-1 normalises to stable CAD level within 30 day follow-up (Shantsila et al., 2012) which may or may not be affected by the antithrombotic treatment. Such a change was not observed in the TEG parameters of fibrinolysis (Ly30, Ly60 and CLT) in the study in any of the 3 groups.

When comparing the effect of the addition of VLDR to clopidogrel only, there was no significant difference in the delta LT albeit a numerical difference identified (clopidogrel - 721 [-1245.9 to -58] s vs. clopidogrel and rivaroxaban -496 [-1002 to -238] s, $p=0.394$). This shows that the addition of VLDR does not significantly enhance LT over and above any effects of clopidogrel. However, when comparing delta LT with ticagrelor (-800 [-1278 to -484] s), there appears to be a statistically significant difference with p-value of 0.042. The difference in the change in LT could in part be explained by the higher baseline LT in the group with ticagrelor, which although was not statistically significantly different to the baseline LT in the other groups. This is firstly supported by the correlation identified between change in LT and baseline LT, with a higher baseline LT showing a significant relation to a

larger magnitude of change ($r = 0.6467$, $p < 0.0001$). Secondly, as shown in the sensitivity analysis in patients with higher LT (> 2500 s), although the paired LT continued to show a significant improvement, the differences in delta LT were no longer significant, irrespective of group allocations. Therefore, this likely reflects that rather than a treatment effect, the initial difference in LT is magnified by the larger number of patients with higher LT within the ticagrelor and clopidogrel group.

Effect on thrombotic status

Although the baseline OT was comparable between the 3 groups, the 4-week OT was significantly different, showing the potent effect of the addition of rivaroxaban on OT. This is evident in delta OT as well, with a significantly higher increase in OT compared to baseline with clopidogrel and rivaroxaban, when compared to clopidogrel alone ($p = 0.0063$), ticagrelor group alone ($p = 0.0269$) and all 3 groups together ($p = 0.0065$). Paired comparison of baseline to 4-week OT also supports the findings above, with a significant p-value (0.0007) only in patients randomised to VLDR with clopidogrel. This difference was lost with the withdrawal of rivaroxaban after the 4-week visit as shown by the significant drop in OT with paired comparison of 4-week and 8-week OT ($p = 0.0045$). As OT did not differ significantly from baseline to 8 weeks post ACS in all 3 groups, except during the period where rivaroxaban was administered in Group 2, it showed that the potent change in OT was driven by the addition of rivaroxaban. It is likely that as the patients were already loaded on DAPT prior to blood draw, the effect of the antithrombotic treatment on OT was therefore not apparent in the study.

With regards to TEG parameters of thrombotic status, only R and TMA were significantly increased in Group 2 but not the other groups, reflecting an improvement towards a less coagulable state, using a low dose anticoagulant.

This study showed that in patients with ACS, the addition of rivaroxaban enhances the reduction in platelet reactivity achieved by P2Y₁₂-inhibitors alone, and this was evidenced by enhanced inhibition of thrombus formation detected in the OT as measured by the GTT, and partially by TEG. However, addition of rivaroxaban did not enhance endogenous fibrinolysis when compared to clopidogrel or ticagrelor.

The longitudinal nature of the study also highlighted that in patients with impaired fibrinolysis, currently approved pharmacotherapeutic agents do promote the improvement or normalisation of endogenous fibrinolysis. However, because we do not have a control group without any pharmaceutical agents, this improvement could be a reflection of change over time related to the disease state, rather than the effect of medication.

Limitations

Due to the COVID-19 pandemic, the analysis was performed prior to completion of recruitment, and a large number of patients who could not attend follow-up due to restrictions in attending hospital. Therefore, the relatively small sample size limits the reliability of the conclusion drawn from the study. It also limits the potential to investigate other potential correlation such as different ACS presentations, time of blood tests which could potentially affect the results. As all the patients were already loaded with DAPT as part of the standard treatment prior to blood draw, we were unable to elicit the true impact of the antithrombotic regime on thrombotic profile.

Secondly, as above, some variables were not considered and could be an unknown confounder although this was partially eliminated through the use of paired comparison analysis by means of the longitudinal data.

Thirdly, although randomisation process was employed and the groups were statistically comparable at baseline, the fibrinolytic status was numerically diverse, and this may have impacted the treatment effect identified. The different timing of baseline blood draw, with some patients such as patients with STEMI who had fibrinolytic testing close to antiplatelet loading whilst other patients may have had blood test taken many hours after antiplatelet loading. This effect may be nullified by the randomisation process but remains a potential area for error.

Regarding follow-up, the compliance of treatment was only performed through questioning of the patients and performing a tablet count on follow-up visits which may not be fully accurate. The compliance with rivaroxaban could be checked through assessment of Factor Xa level but as this was planned to be performed in bulk, it has not been evaluated during the time of this analysis.

Although every effort was made to standardise follow-up date and time, it was still difficult to ensure exact timing post-dose for blood draw. In the same note, the circadian variation was not accounted for patients presenting out-of-hours due to the difficulty and logistics of arranging follow up at similar time of the day.

Lastly, although clinical outcomes were recorded for the study, the number of events (1 thrombotic and 1 bleeding event) at the point of analysis was limited to provide any meaningful discussion and therefore could not provide any clinical correlation to the results discussed.

Conclusion

In the VaLiDate-R study, I have shown that patients with impaired endogenous fibrinolysis showed improvement in fibrinolytic status over the follow up period, irrespective of the antithrombotic regimen prescribed post ACS. The magnitude of improvement of endogenous fibrinolysis appeared similar amongst the regimens compared, and was correlated to the baseline degree of impairment, with no additional benefit of VLDR over and above DAPT. The addition of low-dose rivaroxaban did convey additional effect on inhibition of thrombus formation beyond that achieved with P2Y₁₂-inhibitors and may be of use in patients who are more pro-thrombotic. A larger cohort study to correlate these findings with clinical outcomes is required.

Chapter 8. Assessing the outcome and prognosis in patients with out- of-hospital cardiac arrest

Abstract

Out-of-hospital cardiac (OHCA) arrest carries an extremely high mortality rate, with the majority of cases caused by a primary coronary event. Many variables play an important role in determining the prognosis in this group of patients. A good prognostification tool should take into account different components contributing to the outcome and be able to predict outcomes reliably.

NULL-PLEASE is a novel ‘futility’ score developed to assess prognosis in patients presenting with an OHCA. The score assigns 2 points to each of the initial arrest characteristics (Nonshockable rhythm, Unwitnessed arrest, Long no-flow or Long low-flow period) and 1 point to each patient characteristic (blood PH <7.2, Lactate >7.0 mmol/L, End-stage kidney disease on dialysis, Age \geq 85 years, Still resuscitation, and Extra-cardiac cause). I performed a multicentre, retrospective cohort study to assess the ability of the score to predict survival in patients with OHCA.

A total of 700 patients’ medical records were assessed with data extracted. In-hospital mortality occurred in 52.6% of this cohort of patients. The NULL-PLEASE score performed well with c-statistic of 0.873 (95% CI 0.848 – 0.898) in predicting in-hospital mortality. Patients with a score of \geq 3 had a 24-fold increased risk of in-hospital death (OR 23.59; 95%CI 14.87 – 37.40, $p < 0.0001$) when compared to patients with lower scores. Within the subgroup of patients with cardiac aetiology, the NULL-PLEASE score was a strong predictor of survival with c-statistic of 0.836 (95% CI 0.80 – 0.87).

The study shows that the NULL-PLEASE score is a simple, clinically friendly scoring system with good predictive values which can allow for early prognostification. This may assist in helping clinicians assess prognosis and guide family and friends on the likelihood of survival.

Introduction

Out-of-hospital cardiac arrest (OHCA) affects 84 per 100,000 individuals and in 28% of these, there is return of spontaneous circulation and of these, only 10% survive to 30 days or hospital discharge (Gräsner et al., 2016). The cause of OHCA varies but a cardiac cause, in particular coronary artery disease, remains the commonest (Lombardi et al., 1994; Myat et al., 2018). As such, the ESC guidelines recommend the consideration of urgent angiography with a view to PCI, in patients with ST-elevation on the ECG or high index of suspicion of ongoing infarction (Ibanez et al., 2018). Patients presenting to a heart attack centre (HAC) following an OHCA belong to an extremely high-risk cohort with high mortality, not only in the immediate post-arrest period but further along during their in-hospital stay.

The post-cardiac arrest syndrome, comprising of possible brain injury, myocardial dysfunction, systemic ischaemia/reperfusion response, and the persistent precipitating pathology, often requires resource-intensive monitoring and lengthy treatment in the intensive care unit (Nolan et al., 2015). The post-arrest changes in the fibrinolytic pathway (Wada, 2017) and limited routes for administration of medications also produce unique challenges to the routine antithrombotic therapy used during and after invasive coronary angiography and PCI (Gorog et al., 2020).

Risk stratification in OHCA

Despite the numerous ethical issues which may be involved (Bossaert et al., 2015), an accurate prognostic assessment early in the pathway may be helpful to aid in decision making, guide families, and to allow allocation of resources to those that are likely to benefit most, in an objective fashion.

An accurate predictive score in this context should have high sensitivity to predict patients with poor prognosis and high specificity to ensure all patients with potentially good outcomes

are treated (Gold et al., 2014; Sunde et al., 2007). Through the years, several risk scoring tools of varying complexity and practical application have been developed and validated but there is currently no recommended system for routine clinical use. This is highly important as such a risk stratification tool may be beneficial to healthcare professionals and relatives/friends in providing objective, realistic and non-emotive prognostication.

Current risk stratification tools available

Table 8.1 shows a list of risk scores to assess prognosis in patients with OHCA in the literature. These risk scores to predict mortality have important limitations. The OHCA Score integrates arrest-related and biochemical variables without patient-specific characteristics (Adrie et al., 2006), with a c-statistic of 0.88. However, its main limitation is the difficulty to calculate, including complex weighting of characteristics and calculation of the natural logarithm of 3 characteristics, making it unpracticable, and it has only been assessed in small cohorts.

The ACLS score, developed more than 30 years ago, is difficult to calculate and has relatively poor performance, with an area under the ROC curve, AUC of 0.786 (Eisenberg et al., 1981).

Similarly, the Graphic Model is very difficult to compute, requires data that are frequently not available (such as minutes to start of cardiopulmonary resuscitation [CPR] or defibrillation) and has not been externally validated (Larsen et al., 1993).

The Prediction Tool is also complex and cumbersome to calculate, and not externally validated (Aschauer et al., 2014).

Most scores have only been evaluated in small cohorts (Adrie et al., 2006; Thompson et al., 1998), some not prospectively assessed (Eisenberg et al., 1981; Larsen et al., 1993; Thompson et al., 1998), not externally validated (Aschauer et al., 2014; Ishikawa et al., 2013;

Larsen et al., 1993; Sladjana, 2011; Thompson et al., 1998), and some only predict survival to 1 month, but not in the hospital setting (Adrie et al., 2006; Aschauer et al., 2014; Ishikawa et al., 2013; Thompson et al., 1998).

The NULL-PLEASE score

The NULL-PLEASE score is a relatively new “futility” score to help identify patients who are unlikely to survive following OHCA (Ahmad et al., 2016). The score is simple to calculate but has only been validated to predict death in the emergency room, with a c-statistic of 0.658 (Potpara et al., 2017). Its usefulness for predicting in-hospital mortality and survival has not been assessed.

Table 8.1 OHCA risk scores for predicting mortality

Risk Score	Categories included	Components of score	Calculation of score	Outcome predicted	Development / Validation Cohort	No. of patients
ACLS Score (Eisenberg et al., 1981)	Arrest characteristics	Arrest Witnessed Cardiac Rhythm Lay bystander CPR Speed (response time of paramedic unit)	80% - (40% if not witnessed) - (40% if not VF or VT) - (20% if not bystander-initiated CPR) - (speed in minutes X 5%) = % likelihood of discharge alive.	Survival to discharge AUC 0.33	External validation (Haukoos et al., 2003)	575
				Survival to discharge AUC 0.786	External validation (Sladjana, 2011)	591
Graphic Model (Larsen et al., 1993)	Arrest characteristics	Time to CPR Time to defibrillation Time to ACLS	67% - 2.3% per minute to CPR - 1.1% per minute to defibrillation - 2.1% per minute to ACLS	Survival to discharge	Development (Larsen et al., 1993)	1667
Cardiac arrest score (Thompson et al., 1998)	Arrest characteristics Patient clinical status	Time to ROSC Initial ED systolic BP Initial neurologic status	<25 mins =1, ≥25 mins = 0 ≥90 =1, <90 = 0 Unresponsive patients or having only simple reflexes (comatose) = 0, others = 1.	In-hospital mortality	Development (Thompson et al., 1998)	127
Decision Tree Model (Haukoos et al., 2004)	Arrest characteristics	Witnessed arrest or paramedic response time < 6 minutes Arrest in nursing home Initial rhythm PEA	Decision tree model	In-hospital mortality	Development (Haukoos et al., 2004)	754

OHCA Score (Adrie et al., 2006)	Arrest characteristics	Initial rhythm VF/VT	- 13 if VF/VT	Mortality at 30 days	External validation (Choi et al., 2018)	173
	Initial test results	No-flow interval(min)	+ 6 x ln(noflowinterval)	AUC: 0.740		
		Low-flow interval (min)	+ 9 x ln(lowflowinterval)	Mortality at discharge	External validation (Hunziker et al., 2011)	128
		Serum creatinine	- 1434/serum creatinine	AUC 0.85		
		Arterial lactate	+ 10 x lactate			
SR-QOLs (Sladjana, 2011)	Arrest characteristics	Bystander CPR	15	Survival to discharge	Development (Sladjana, 2011)	591
	Patient clinical status	Witnessed arrest	8	AUC 0.882		
		Shockable rhythm	20			
		CPR within 4 mins	12			
		Pupil photoreaction	6			
Simple prognostication score (Ishikawa et al., 2013)	Arrest characteristics	Witnessed arrest	1 each (total 10)	Mortality at 1 month	Development (Ishikawa et al., 2013)	750
	Patient clinical status	Bystander CPR				
	Initial test results	Shockable initial cardiac rhythm				
	Aetiology of arrest	ROSC before arrival at hospital				
		Light reflex present				
		Cardiac aetiology				
		GCS ≥ 5 on arrival				
		Arterial pH ≥ 7				
		Potassium ≤ 5 mEq/L				
		CRP ≤ 0.3 mg/dl				

Prediction tool (Aschauer et al., 2014)	Arrest characteristics Patient characteristics	Age group Adrenaline administered Minutes until ROSC Shockable rhythm	> 80 =32, >70= 27, >50 =23, >60 = 20, >40 =16, ≤40 = 11 >10mg = 24, >5mg =12, >4mg = 7, >3mg = 5, >2mg = 4, >1mg = 2, >0mg = 1, 0mg = 0 >100min = 35, >50min = 21, >40min = 13, >30min = 10, >20min = 7, >0 min = 1, 0min = 0 Yes -15, no 0	Mortality at 30 days AUC 0.81	Development and internal validation (Aschauer et al., 2014)	Development 1068 Internal validation 291	
NULL-PLEASE (Ahmad et al., 2016)	Arrest characteristics Patient characteristics Initial test results Aetiology of arrest	Non-shockable rhythm Unwitnessed arrest Long no-flow period Long low-flow period Blood pH<7.2 Lactate >7 End stage CKD on dialysis Age ≥ 85 Still on-going CPR Extra-cardiac cause of arrest	2 2 2 2 1 1 1 1 1 1		In-hospital mortality	Development (Ahmad et al., 2016)	56
Polish Hypothermia Risk Registry Score (PHR-RS) (Kołtowski et al., 2019)	Arrest characteristics Patient characteristics	Age Mild Therapeutic Hypothermia (1 point each) <ul style="list-style-type: none"> • Cardiac arrest to CPR >10 mins • Time from cardiac arrest to ROSC >20 mins • In-hospital cardiac arrest • Unwitnessed cardiac arrest • Non-shockable rhythm 	$[(\text{age [years]} \times 0.003) + (\text{score in MTH Scale} \times 0.11) - 0.25] \times 100\%$	In-hospital mortality Area under ROC: 0.74	Development (Kołtowski et al., 2019)	376	

rCAST score (Nishikimi et al., 2019)	Arrest characteristics Patient clinical status Initial test results	Initial rhythm Witness/until ROSC pH Lactate GCS motor score	Shockable:0, non:1 <20min:0, ≥20min:1, No witness:2 ≥7.31:0, 7.3-7.16:1, 7.15-7.01:2, ≤7:3 ≤5:0, 5.1-10:1, 10.1-14:2, ≥14.1:3 ≥2:0, 1:1	Mortality at 30 days AUC 0.832 Mortality at 90 days AUC 0.827	Development (Nishikimi et al., 2019)	460
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ACLS – advanced cardiac life support, AUC – area under receiver operator curve, BP – blood pressure, CKD – chronic kidney disease, CPR – cardiopulmonary resuscitation, CRP – C-reactive protein, ED – emergency department, EMS – emergency medical services, GCS – Glasgow Coma Scale, ICU – intensive care unit, OHCA – out-of-hospital cardiac arrest, PEA – pulseless electrical activity, ROSC – return of spontaneous circulation, TTM – Targeted Temperature Management, VF – ventricular fibrillation, VT – ventricular tachycardia.

Aim

It was the aim of this study to assess and provide external validation of the NULL-PLEASE score for prediction of in-hospital survival, in a large cohort of patients with OHCA including patients with primary cardiac aetiology.

Methods

Study design and population

A multicentre retrospective observational study was carried out with approval from the Health Research Authority and local research and development boards as shown in Appendix 2 (Page 254 - 259). Consecutive patients presenting with OHCA to three NHS Trusts in England (East and North Hertfordshire NHS Trust, Royal Brompton and Harefield Hospitals NHS Trust and Royal Papworth Hospital, Cambridge) from September 2015 to December 2018, were identified by the Principal Investigators and their clinical teams. Eligible patients were allocated a study ID, medical records were accessed and recorded anonymously in an electronic CRF.

Data collection

Demographics, descriptive data pertaining to the arrest, initial blood results including pH and lactate, cause of arrest (or presumed cause), length of hospital stay and outcomes were retrieved from medical notes and recorded onto the CRF.

NULL-PLEASE score

Using the data collected, the NULL-PLEASE score was calculated for each patient. The NULL-PLEASE score assigns 2 points to each of the initial arrest characteristics (Nonshockable rhythm, Unwitnessed arrest, Long no-flow or Long low-flow period) and 1 point to each patient characteristic (blood PH <7.2, Lactate >7.0 mmol/L, End-stage kidney disease on dialysis, Age \geq 85 years, Still resuscitation, and Extra-cardiac cause). Definitions of individual components of the score are shown in Table 8.2. As a number of patients did not have lactate or pH measured on arrival, the performance of a modified version of the scoring system excluding these variables, namely the NULL-EASE score, was also assessed.

Outcome

The primary outcome was in-hospital death or survival to discharge from hospital. The secondary outcome was length of stay.

Statistical analysis

Categorical variables were summarised as proportion (number and percentage) and continuous variables as median with IQR. The association of the NULL-PLEASE score components with the primary outcome was examined using univariate logistic regression analysis. Components that were significantly associated were added to a multivariate model. OR with 95% CI and p-values were obtained for each component and the score as a whole. The predictive ability of the NULL-PLEASE score for the primary outcome was tested using Area under receiver operator curve (AUC) analysis and the c-statistic reported. The same analyses were repeated for patients in whom only the NULL-EASE score was available.

A subgroup analysis was performed in patients who had return of spontaneous circulation following the initial arrest, exploring in particular, the secondary outcome of length of stay in hospital in relation to the NULL-PLEASE score.

Further analyses were performed in the subgroup of patients with myocardial infarction as the presumed cause of arrest. Significance was taken as <0.05 . Statistical analyses were performed using Stata 15 software (StataCorp, College Station, Texas, USA).

Table 8.2 Definition of components of NULL-PLEASE score

Non-shockable rhythm	Initial rhythm on first medical respondent is non-shockable i.e. not VF or pulseless VT
Unwitnessed arrest	No clear account of circumstances leading to arrest
Long low-flow period	Duration of CPR > 30 minutes
Long no-flow period	No bystander CPR performed prior to arrival of medical respondent
PH	Initial blood pH < 7.2
Lactate	Initial blood lactate > 7
End-stage renal failure on dialysis	Past medical history of end-stage renal failure on dialysis
Age	Age > 85 years old
Still resuscitation	Ongoing CPR (no ROSC) on arrival to hospital emergency department or cardiac catheterisation laboratory
Extra-cardiac cause	Non-cardiac related cause (e.g. trauma or pulmonary embolism)

CPR – cardiopulmonary resuscitation defined as initiation of chest compression with/without mechanical ventilation; ROSC – return of spontaneous circulation defined as resumption of perfusing cardiac output with respiratory effort post arrest; VT – Ventricular tachycardia; VF – Ventricular fibrillation

Results

A total of 700 patients were included in the study. The median age of the entire cohort was 64 years with 77% male patients. Admission patient characteristics are shown in Table 8.3.

Blood pH results were unavailable in 196 patients and lactate levels were unavailable in 233 patients.

The commonest cause of OHCA was myocardial infarction with 454 patients (64.9%). Other common causes include pulmonary embolism (2.9%), cerebrovascular accident (0.4%), haemorrhage (0.9%) and trauma (1.3%). In 91 patients, no clear cause was identifiable through their medical records. The median length of hospital stay was 5 days. The primary outcome of in-hospital mortality occurred in 368 (52.6%) patients.

When comparing survivors with non-survivors, survivors were significantly younger and more likely to be male. Past medical history appears similar between the 2 groups although a higher proportion of patients who died suffered from chronic obstructive pulmonary disease. When comparing their initial blood results, survivors have statistically significantly higher haemoglobin and pH, lower white cell count and lactate, than those who died.

All components of the NULL-PLEASE score were significantly lower in survivors except end-stage renal disease on dialysis. The median NULL-PLEASE score was also significantly higher in patients that died (4 [2 – 6] vs. 0 [0 – 1], $p < 0.0001$).

Logistic regression analysis

On univariate logistic regression analysis (Table 8.4), most components of the score were individually significantly associated with in-hospital mortality, except for end-stage renal failure which was under-represented within the cohort. Amongst the components, ongoing resuscitation on arrival to the hospital carries the highest odds ratio (OR 37.75; 95% CI 13.75 – 103.65). On multivariate regression involving the components that were significantly

associated with mortality, only non-shockable rhythm (OR 6.23; 95% CI 2.84 – 13.69, $p < 0.0001$), long low flow time (OR 5.78; 95% CI 3.17 – 10.53, $p < 0.0001$) and pH < 7.2 (OR 7.64; 95% CI 3.07 – 19.03, $p < 0.0001$) remained significantly associated.

Predictive value of NULL-PLEASE score

The NULL-PLEASE score was a strong predictor of in-hospital death (c-statistic 0.873; 95% CI 0.848 – 0.898). A NULL-PLEASE score ≥ 3 was chosen as the optimal cut-point to predict mortality, with sensitivity 65.8% and specificity 92.5% (Figure 5.1), with a positive predictive value (PPV) of 90.6% for in-hospital death and negative predictive value (NPV) of 70.9% for survival. Although a score ≥ 2 had the best combined sensitivity (82.9%) and specificity (80.1%), the cut-point of 3 was chosen to improve specificity, to ensure almost all patients with potentially good outcomes are treated, whilst preserving reasonable sensitivity. Patients with a score ≥ 3 had a 24-fold increased risk of in-hospital death (OR 23.59; 95%CI 14.87 – 37.40, $p < 0.0001$) compared to patients with lower scores.

The modified NULL-EASE score

Similarly, the modified NULL-EASE score was a strong predictor of death (c-statistic 0.849; 95% CI 0.822 – 0.876) (Figure 5.2). A score ≥ 3 had a sensitivity of 57.3% and specificity of 93.4%, PPV and NPV of 90.6% and 66.4% respectively. Patients with a NULL-EASE score ≥ 3 had a 19-fold increased risk of in-hospital death (OR 18.84; 95% CI 11.73 – 30.58, $p < 0.0001$) compared to patients with lower scores.

NULL-PLEASE score and length of stay

In patients who achieved return of spontaneous circulation following the initial arrest, the median length of stay was 6 days (IQR 3-12). Among these, length of stay was significantly longer in patients who survived compared to those who died in hospital (9 [IQR 4-16] vs. 4 [IQR 2-7] days, $p < 0.00005$). Using Spearman rank correlation, the NULL-PLEASE score

showed weak positive correlation with length of stay in survivors ($r = 0.248$, $p < 0.0005$) and moderate negative correlation in patients who died ($r = -0.472$, $p < 0.0005$).

Subgroup of patients with OHCA secondary to myocardial infarction

MI was the cause of arrest in 454 patients and 249 (55%) survived to discharge. The score performed well in this group (AUC 0.836, 95% CI 0.80 – 0.87). Amongst these patients, those with a NULL-PLEASE score ≥ 3 had a 19-times higher risk of death (OR 19.6; 95% CI 10.3 – 37.1, $p < 0.0005$) compared to those with lower scores.

Table 8.3 Baseline, admission characteristics, length of stay and NULL-PLEASE score

	Overall (n=700)	Discharged (n=332)	Died (n=368)	p-value
Age, years	64 (53 – 74.5)	61 (53 – 71)	67 (54 – 78)	0.0002*
Female	158 (22.6)	59 (17.8)	99 (26.9)	0.005*
Medical History				
Hypertension	232 (33.1)	118 (35.5)	114 (31.0)	0.228
Diabetes	103 (14.7)	41 (12.4)	63 (16.9)	0.109
Previous myocardial infarction	101 (14.4)	41 (12.4)	60 (16.3)	0.161
COPD	38 (5.4)	10 (3.0)	28 (7.6)	0.007*
Chronic kidney disease	27 (3.9)	8 (2.4)	19 (5.2)	0.076
Admission blood count				
Haemoglobin	137 (122 – 148)	140 (129 – 150)	131 (112 – 144)	0.0412*
White cell count	13.9 (10.9 – 18.5)	13.3 (10.9 – 17.7)	14.7 (10.9 – 19.3)	<0.0001*
Platelet count	223 (179 – 274)	228 (187 – 270)	215 (167.5 – 283)	0.1203
pH (504 patients)	7.26 (7.08 – 7.33)	7.32 (7.26 – 7.38)	7.16 (6.94 – 7.27)	<0.0001*
Lactate (467 patients)	4 (2.1 – 7.55)	2.41 (1.61 – 4.2)	6.01 (3.31 – 9.80)	<0.0001*
NULL-PLEASE parameters				
<u>N</u> on-shockable rhythm	180 (25.7)	20 (6.0)	160 (43.5)	<0.0001*
<u>U</u> nwitnessed arrest	55 (7.9)	9 (2.7)	46 (12.5)	<0.0001*
<u>L</u> ong no-flow	78 (11.1)	16 (4.8)	62 (16.9)	<0.0001*
<u>L</u> ong low flow	227 (32.5)	24 (7.3)	203 (55.2)	<0.0001*
<u>p</u> H <7.2 (504 patients)	169 (34.2)	17 (7.8)	152 (53.3)	<0.0001*
<u>L</u> actate >7.0 mmol/L (467 patients)	127 (27.2)	18 (8.8)	109 (41.4)	<0.0001*
<u>E</u> nd-stage kidney disease on dialysis	2 (0.3)	1 (0.3)	1 (0.3)	1
<u>A</u> ge ≥85 years	46 (6.6)	12 (3.6)	34 (9.2)	0.003*
<u>S</u> till resuscitation	120 (17.1)	4 (1.2)	116 (31.5)	<0.0001*
<u>E</u> xtra-cardiac cause	126 (18)	18 (5.4)	108 (29.4)	<0.0001*
NULL-PLEASE score	2 (0 – 4)	0 (0 – 1)	4 (2 – 6)	<0.0001*
NULL-PLEASE score ≥3	267 (38.1)	25 (7.5)	242 (65.8)	<0.0001*
NULL-EASE score	2 (0 – 3)	0 (0 – 0)	3 (2 – 5)	<0.0001*
Length of hospital stay, days	5 (2 – 10)	9 (4 – 16)	0 (0 – 6)	<0.0001*

Table 8.4 Univariate logistic regression analysis of variables

	OR (95% CI)	p-value
Age	1.02 (1.01 – 1.03)	0.001*
Female	1.70 (1.18 – 2.45)	0.004*
Medical History		
Hypertension	0.81 (0.59 – 1.12)	0.200
Diabetes	1.44 (0.94 – 2.20)	0.095
Previous myocardial infarction	1.38 (0.90 – 2.12)	0.138
COPD	2.65 (1.27 – 5.55)	0.010*
Chronic kidney disease	2.20 (0.95 – 5.11)	0.065
NULL-PLEASE parameters		
<u>N</u> on-shockable rhythm	12 (7.30 – 19.72)	<0.0001*
<u>U</u> nwitnessed arrest	5.13 (2.47 – 10.65)	<0.0001*
<u>L</u> ong no-flow time	4.00 (2.26 – 7.09)	<0.0001*
<u>L</u> ong low-flow time	15.74 (9.90 – 25.02)	<0.0001*
<u>p</u> H <7.2 (504 patients)	13.58 (7.86 – 23.47)	<0.0001*
<u>L</u> actate >7.0 mmol/L (467 patients)	7.14 (4.19 – 12.14)	<0.0001*
<u>E</u> nd-stage kidney disease on dialysis	0.90 (0.06 – 14.48)	0.942
<u>A</u> ge ≥85 years	2.71 (1.38 – 5.34)	0.004*
<u>S</u> till resuscitation	37.75 (13.75 – 103.65)	<0.0001*
<u>E</u> xtra-cardiac cause	7.25 (4.29 – 12.25)	<0.0001*
NULL-PLEASE score	2.41 (2.12 – 2.75)	<0.0001*
NULL-PLEASE score ≥3	23.59 (14.88 – 37.40)	<0.0001*
NULL-EASE score	2.62 (2.27 – 3.03)	<0.0001*

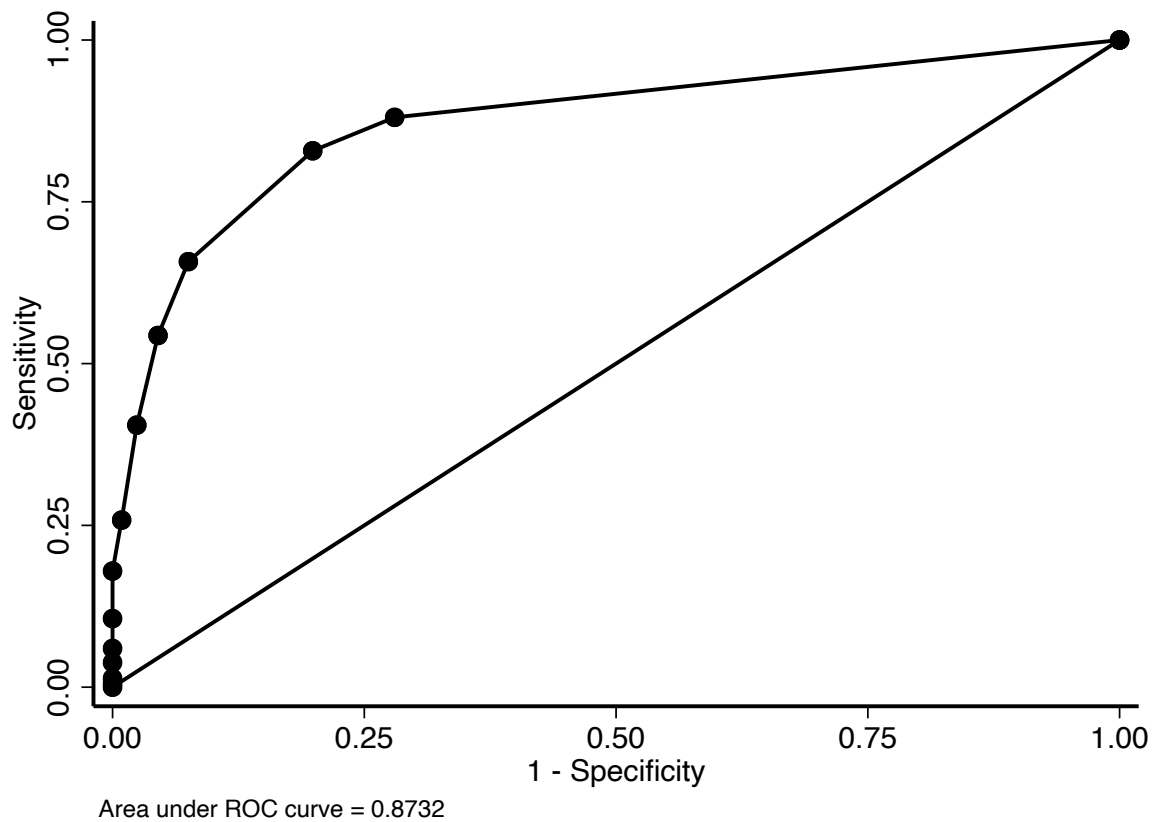


Figure 8.1 Area under the receiver operator curve for NULL-PLEASE score

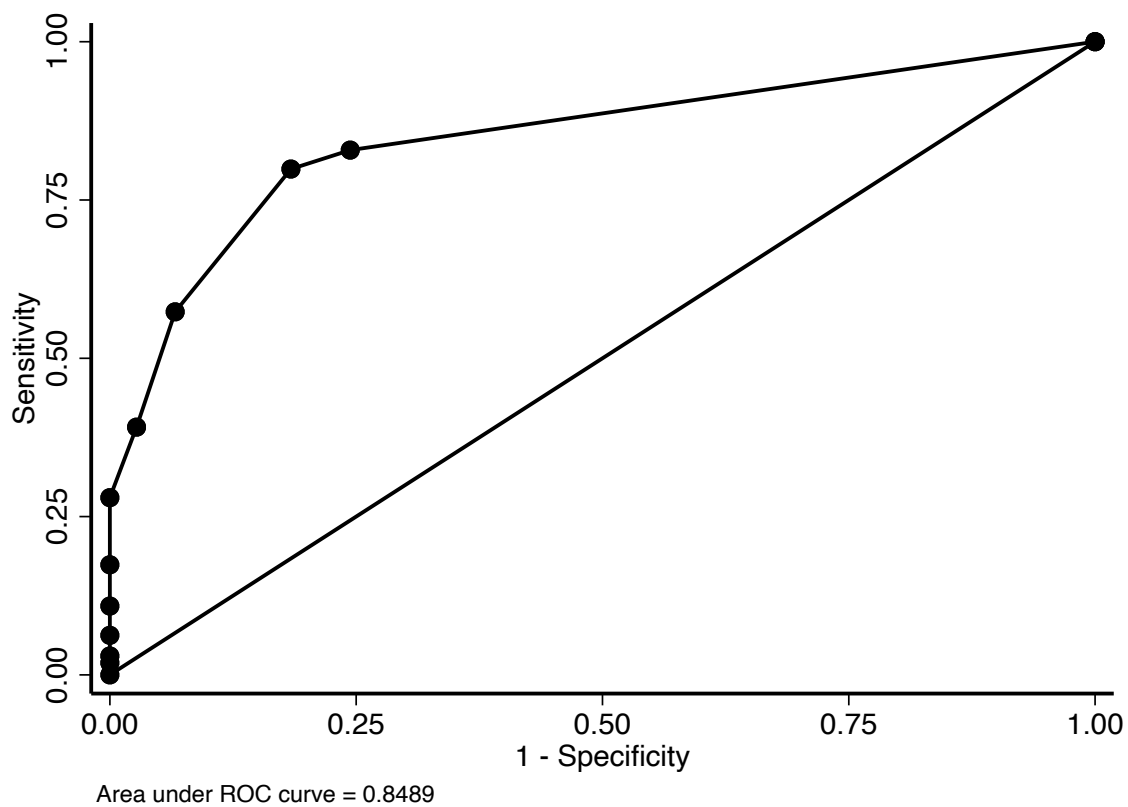


Figure 8.2 Area under the receiver operator curve for NULL-EASE Score

Discussion

The study has shown that OHCA carries a high in-hospital mortality rate of 52.6%, although this is much lower than the reported global and UK mortality rates of slightly higher than 90% (British Heart Foundation, 2016; Yan et al., 2020). This could be explained by the cohort of patients being assessed in the study. The reported survival to hospital admission in patients with OHCA within the UK is approximately 25% (Barnard et al., 2019) and as the study only focuses on patients admitted to hospital, there was a selection bias within the dataset. This indicates that with every patient that was included in the study, there were 3 other patients that did not survive to be admitted. With this extrapolation, the mortality rate within the cohort would increase to approximately 88% (Figure 8.3). Out of the 3 hospitals included in the study, 2 of them were primarily receiving cardiac caused admissions i.e. suspected myocardial infarction as the cause of the arrest and hence, this would introduce further selection bias as these patients historically have better outcomes when compared to other causes of OHCA (Barnard et al., 2019). These factors could potentially explain the “better” mortality rate within the study when compared to the national and global level.

NULL-PLEASE score

When exploring the predictive ability of the NULL-PLEASE score, the study has shown it to be a strong predictor of in-hospital mortality in OHCA, with a c-statistic of 0.873 (95% CI 0.848 – 0.898) and can help identify patients who are unlikely to survive within this cohort. Individuals with a score ≥ 3 had a 24-fold increased risk of death compared to those with a score of 0 to 2, with relatively good predictive value when compared to other similar scoring systems currently available in the literature. Other externally validated models within the literature have documented c-statistics ranging from 0.33 to 0.85 (Choi et al., 2018; Haukoos et al., 2003; Hunziker et al., 2011; Sladjana, 2011).

A NULL-PLEASE score ≥ 3 had a specificity of 92.5%, ensuring most patients with potentially good outcomes are not disadvantaged, with a PPV for in-hospital death of 90.6% with sensitivity 65.8%. In comparison to other scoring systems, an OHCA score ≥ 32.5 has specificity of 85%, PPV of 94% and sensitivity of 80%. (Adrie et al., 2006). The strength of the NULL-PLEASE score is not only its strong prognostic value, but its simplicity and ease-of-use. It can be calculated on the spot and is easy to interpret. In comparison, both the OHCA (Adrie et al., 2006) and CAHP (Martinell et al., 2017) scores are difficult to calculate, needing advanced calculator functions, or nomograms, and are neither easy to calculate, nor clinically-friendly.

These results support and extend the findings of the initial validation of the NULL-PLEASE score for death in the emergency room in a small cohort (Potpara et al., 2017) to now predict survival to hospital discharge. The excellent predictive value is likely because of the combination of different factors incorporated into the score (Sasson et al., 2010). The different elements within the NULL-PLEASE score can be subdivided into arrest characteristics (initial rhythm, bystander CPR, arrest location, arrest duration and absence of return of spontaneous circulation or ROSC), blood parameters (pH and lactate), patient characteristics (renal failure and age) and aetiology of arrest.

Regression analysis has shown all the components contributes significantly to mortality although at different weightage. Ongoing resuscitation on arrival to hospital appears disproportionately weighted compared to the other components with an odds ratio of 37.75 (95% CI 13.75 – 103.65). Pre-hospital ROSC has previously been shown to be associated with better outcomes (Y. Goto et al., 2013; Gregers et al., 2018). Therefore, patients arriving into the hospital without ROSC are more likely to have cardiopulmonary resuscitation (CPR) terminated when following the termination of resuscitation guidelines (Dinning et al., 2017). This would therefore confound the data presented with ongoing resuscitation having a higher impact on mortality.

Although some parameters within the score are correlated – such as patients who had longer CPR or absence of bystander CPR would consequently have worse lactate and pH levels (biochemical evidence of prolonged ischemia), and may appear duplicated, they could potentially provide a more objective measure of the effectiveness of CPR and accurate reflection the condition of the patients. Details pertaining to the circumstances of the OHCA and resuscitation are based on documentation and approximation during or post-event, which may be commonly inaccurate due to recall bias (Maupain et al., 2016; Sundermann et al., 2015). This may also explain the more heavily weighted blood results when compared to other arrest parameters in the univariate and multivariate logistic regression model.

Not only can the score be used to predict mortality, there was also a correlation between the score and length of hospital stay. Patients who survived to discharge tended to stay in hospital longer with higher scores, echoing a more complicated recovery compared to patients with lower scores. On the other hand, patients who died had a shorter hospital stay (i.e. earlier mortality) when they had a higher admission NULL-PLEASE score, showing a moderate negative correlation. This implies that there potentially could be a role for the score to assess severity of each patient's condition.

The length of stay is short compared to a recent UK cohort managed on the intensive care unit (Petrie et al., 2015), reporting a median stay of 12 days. This is likely due to the unselected nature, whereas Petrie *et al.* reviewed only patients admitted to the intensive care unit. Even though our median stay is shorter, it still reflects the very significant health economic burden that patients with OHCA place on healthcare systems. When resources are limited, the appropriate allocation of resources to patients that are most likely to survive may be useful.

NULL-EASE score

Although routine blood gas analysis is recommended in patients with OHCA, it is frequently not performed upon arrival, due to the pressures of manpower or time and competing priorities in an emergency situation. The sensitivity analysis using the modified NULL-EASE score showed a PPV of 90.6% for a score ≥ 3 , similar to that of the NULL-PLEASE score, although sensitivity was lower at 57.3% and NPV only at 66.4%. This highlights the importance of measuring pH and lactate upon arrival to optimise the performance of the score.

NULL-PLEASE in myocardial infarction

Since some 55% of OHCA are attributable to a cardiac cause (Berdowski et al., 2010), the strong performance of the score in this subgroup is highly pertinent. The study shows that NULL-PLEASE was equally strong in predicting mortality within the MI cohort and could potentially be used, in conjunction with other clinical data, to assist in decision making. In patients with ST-segment elevation post arrest or with high suspicion of coronary event, the guidelines support urgent angiography with a view to PCI (Ibanez et al., 2018). However, in the latter scenario, unfavourable pre-hospital arrest and resuscitation parameters should be considered and may argue against an initial invasive strategy (Ibanez et al., 2018; Noc et al., 2014). A recent report shows that the use of a prognostication score may permit avoidance of unnecessary procedures in patients with minimal chances of survival, but also reinforces the association between an early invasive strategy and good outcome in patients with preserved neurological function (Bougouin et al., 2018). The NULL-PLEASE score may therefore be very useful in this setting as an additional tool in guiding clinical management.

Clinical use of score

Importantly, no risk score calculator will be 100% accurate. Experienced clinicians will recognise that not infrequently, patients defy expectations and those thought to have no

chance have recovered, whilst some of those predicted to do well, have succumbed. Therefore, such a scoring system can at best serve as an adjunct to decision-making and cannot be used to make decisions on withdrawal of life-supporting treatment in individual patients. It can, however, be used to guide and explain prognosis to relatives who may find that being quoted an objective survival rate based on the score may help better prepare them for the future. Currently, information given to relatives is often varied, being frequently both emotive and subjective (for example, wishing to convey hope even in perhaps hopeless scenarios, or predicting gloom to avoid unrealistic expectations by relatives and to prepare them for the worst), and varying with the seniority and experience of the clinician. Providing a simple and objective manner of translating this information to them would be very welcomed, both by clinicians and relatives alike.

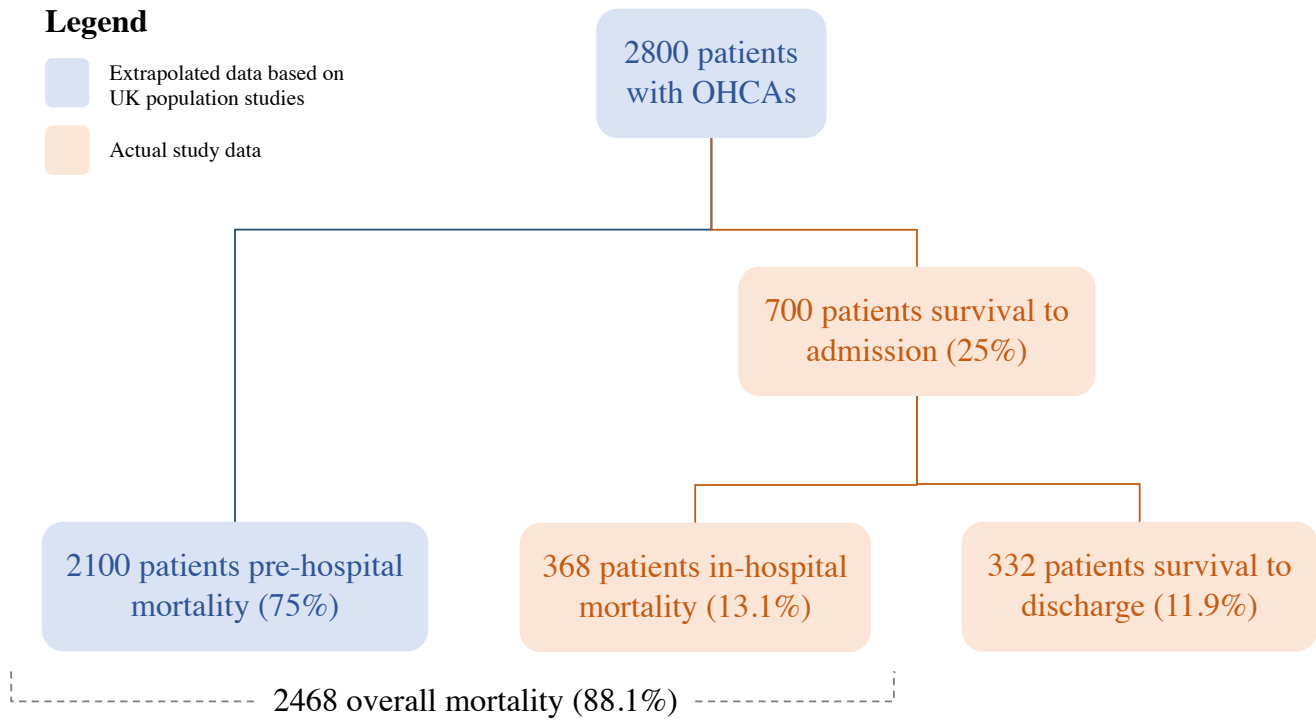


Figure 8.3 Extrapolated mortality rate of current cohort

Limitations

There was inherent selection bias in the studied population as mentioned in the discussion – individuals already survived to reach hospital, and those who died pre-admission were excluded. Although the mortality rate, following extrapolation of the data, appears in keeping with nationally-reported levels, these patients who account for the majority of OHCA within the UK are not being accounted for in the score. However, one can argue that the score should be utilised mainly for in-hospital assessment of prognosis and therefore including pre-hospital mortality may reduce the accuracy of the score.

Secondly, with the difference in admission criteria between the 3 centres, 2 being primarily tertiary cardiac centres, the data is skewed towards patients with OHCA attributable to primary cardiac aetiology. There are intrinsic differences in traumatic versus non-traumatic causes for cardiac arrest in terms of mortality and as there is very low representation of traumatic OHCA within the cohort, drawing conclusions with regards to such underrepresented causes of OHCA may be limited.

Being a retrospective observational study, details pertaining to the circumstances of the OHCA and resuscitation are based on documentation and approximation during or post-event. Therefore, recall bias and documentation error would be unavoidable. This could have limitations on the accuracy of data entered and collated.

Individually, each component poses different limitations for the study. The score incorporates aetiology, namely “E – extra cardiac cause”, which in practical terms is frequently not available. Furthermore, the cause of death was presumed in many cases, without definitive tests, especially in those who died shortly after admission as post-mortems are not routinely performed. Hence, the cause of death will be determined by clinicians based on likelihood, given presentation and comorbidities, which may be commonly inaccurate. 'L – Long no-flow period' is defined as no bystander CPR prior to arrival of emergency medical services.

However, there are no defined time periods for the no-flow period, it could therefore range from a few to many minutes. Furthermore, patients with end-stage kidney disease on dialysis were under-represented in the study, with only 1 patient in each group, hence no useful conclusions can be drawn about this particular component. Blood results such as lactate and pH were not always available, and the score appears to perform less well without inclusion of these. However, this also accurately reflects real-life scenarios where these measurements are not always available at the time of decision making and hence having the modified NULL-EASE score available provides another way to utilise available parameters to provide prognostication.

Conclusion

Patients presenting to hospital following an OHCA belong to a high-risk group with high mortality, with the majority of cases caused by a primary coronary event. Understanding different components that contribute to risk of mortality allows more accurate risk stratification and prognostication in this cohort of patients. A good risk-stratification tool should take into account different components contributing to the outcome which in OHCA includes arrest characteristics, patient characteristics and initial blood parameters. The use of a simple and objective clinical scoring system incorporating these components with the NULL-PLEASE score allows for early prognostication, which may assist in helping clinicians to assess the likelihood of survival and to provide objective and realistic guidance on survival likelihood to family and friends of the affected patient.

Chapter 9. Conclusion and future directions

Conclusion

The role of endogenous fibrinolysis as a risk predictor in ACS has been established over the last few years but there remain a multitude of unknowns, including uncertainty about its role in relation to the severity of coronary disease. My dissertation was aimed towards trying to provide a deeper understanding of the endogenous fibrinolytic process, its variation with time and with differences in coronary disease severity and potential for pharmacological modulation.

In Chapter 3, within the limitations discussed, I showed that there was no evidence of variation of overall thrombotic and thrombolytic profile, both with circadian rhythm and week-to-week variation over a 4-week duration in healthy volunteers. This finding together with previous studies reporting diurnal variation of opposing fibrinolytic factors such as tPA and PAI-1, point towards a well-maintained equilibrium and therefore no variation in the overall thrombotic profile of healthy individuals (Figure 9.1 [A]). Similarly, differences in fibrinolytic factors between genders, identified in diseased states where the thrombolytic system is under stress but not in healthy individuals lends further support to the findings in my study showing no difference of overall fibrinolytic profiles between healthy males and females.

In Chapter 4, I showed that endogenous fibrinolysis was not correlated to overall coronary plaque burden but does show an inverse association with the degree of maximal stenosis. This provides further support to the findings above where, in diseased states, such as with a flow-limiting coronary artery stenosis generating a more pro-thrombotic environment, could relate to the occurrence of impaired endogenous fibrinolysis and may contribute to the risk of occlusive thrombotic events such as myocardial infarction. (Figure 9.1 [B]).

In Chapter 5, I showed that patients with NSTEMI displayed the most impaired endogenous fibrinolysis amongst the different presentations of ACS. However, the difference was

nullified in the analysis with a matched cohort implicating that the difference was reflective of co-morbidities rather than type of presentation. This again supports the hypothesis of a more impaired endogenous fibrinolysis in more advanced diseased states (Figure 9.1 [B]). However, it indicates that the different ACS presentations may result from the differences in the magnitude of the thrombotic drivers, rather than differences in the fibrinolytic status.

In Chapter 6, the case series comparing MINOCA STEMI and MI-CAD STEMI patients highlighted that some MINOCA presentations could, in part, be explained by an effective endogenous fibrinolytic system.

In Chapter 7, I showed through the VaLiDate-R study, that patients with ACS and impaired fibrinolysis displayed an improvement in fibrinolytic status over the follow up period irrespective of antithrombotic regimens (clopidogrel, ticagrelor or VLDR plus clopidogrel) prescribed post-ACS. The magnitude of improvement was correlated to baseline degree of impairment and not significantly different amongst the 3 regimes assessed, with no additional benefit of VLDR over and above DAPT. This reflects the “normalisation” of the fibrinolytic system from an unstable, prothrombotic environment during ACS (Figure 9.1 [C]) to a more stable, less thrombotic state several weeks after the acute event (Figure 9.1 [D]), potentially improved with the addition of antithrombotic agents. The study highlights that assessment of endogenous fibrinolysis should be taken in the context of the clinical state of the patient and is a more clinically meaningful assessment during the period of acute hospitalisation.

In summary, I have shown that

1. the overall fibrinolytic status, in particular, endogenous fibrinolysis does not display significant diurnal and short-term variation,
2. Impairment of endogenous fibrinolysis is not correlated to overall plaque burden but has an association with degree of maximal coronary stenosis,

3. there was no difference in endogenous fibrinolysis when comparing different presentations of ACS after accounting for pre-morbid status, and

4. in patients with impaired endogenous fibrinolysis, the addition of VLDR does not confer additional benefit in modulating endogenous fibrinolysis. The use of antithrombotic medications during and after an acute thrombotic event may improve global thrombotic status, including fibrinolysis or the disease may settle with reduction in thrombotic drivers in response to potent antithrombotic medication.

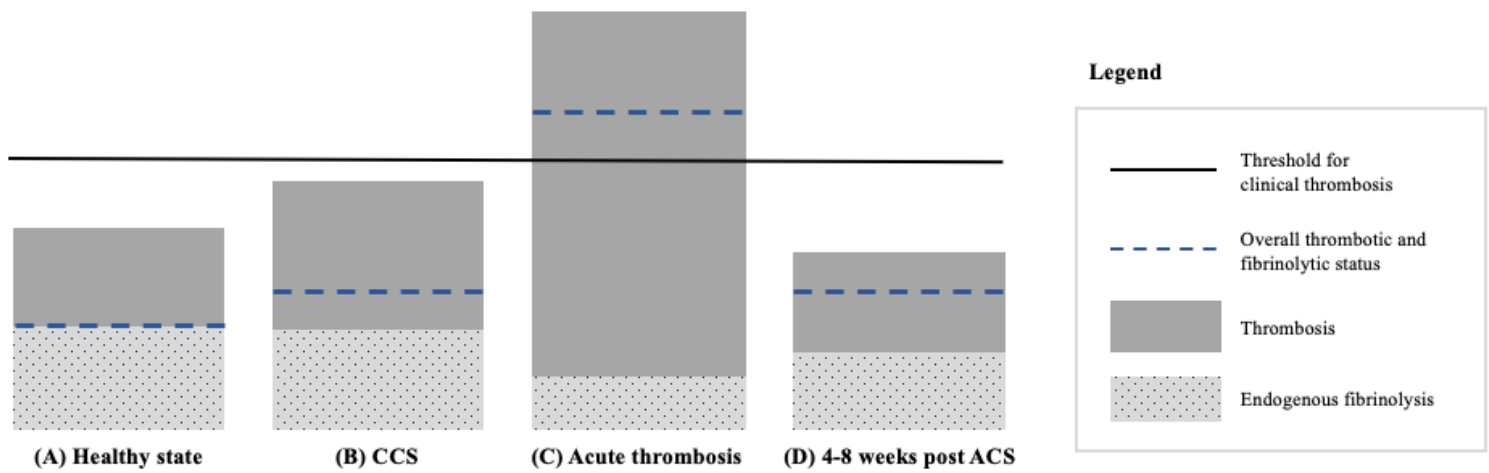


Figure 9.1 Endogenous fibrinolysis in different states of health

In healthy individuals (A), there may be variation in the thrombotic and fibrinolytic factors, but as the overall fibrinolytic potential remains constant as any fluctuations towards thrombosis is buffered by the fibrinolytic potential. In the presence of CCS (B) such as with flow-limiting coronary artery disease where there is an increase in pro-thrombotic environment, there is an overall deterioration of fibrinolytic status.

Acute thrombosis (C) occurs during an overwhelming thrombotic stimulus resulting in occlusive coronary thrombosis resulting in ACS. Measurement of endogenous fibrinolysis during the event shows impairment of this protective mechanism and the inability of the fibrinolytic response to cope with the level of insult. With the resolution of thrombosis and implementation of anti-thrombotic therapy (D), the thrombotic status and platelet reactivity are returned to baseline, and the equilibrium is restored back to previous levels.

Future directions

Larger cohort studies, with more frequent sampling, would be helpful to confirm whether there is any diurnal variation in endogenous fibrinolysis in both healthy individuals and also in patients with established coronary disease. Furthermore, a larger cohort study would be useful to further characterise the relationship between the severity of the coronary stenosis and the global thrombotic status. A large cohort population level prospective study with the measurement of endogenous fibrinolysis at baseline and performing clinical follow up over a long-term period, identifying patients who suffer from cardiovascular adverse events, could potentially test if the assessment of endogenous fibrinolysis can be used as a predictor of future cardiovascular events. If so, the measurement of the thrombotic profile could be a biomarker used to risk stratify patients and provide guidance to clinicians on providing advice to reduce future cardiovascular events and tailor medical advice based on an objective measure.

With regards to pharmacological modulation of endogenous fibrinolysis, the use of Factor Xa inhibitor remains an option. This may require a higher dose, namely full anticoagulation dose, to demonstrate an effect on fibrinolysis, instead of the very low dose used in the VaLiDate-R study. However, this will correspondingly increase the risk of bleeding and therefore, to achieve net clinical benefit, careful selection of patients with very high thrombotic risk is required. This could potentially be assessed in a smaller mechanistic study first. Another potential would be to alter the combination of antiplatelet agents, namely dropping the aspirin, and perhaps combine only full anticoagulation with a P2Y₁₂ inhibitor such as clopidogrel, to utilise the separate mechanisms of action of these two antithrombotic agents to achieve maximal antithrombotic effect whilst reducing the bleeding risks associated with triple therapy.

Appendix 1: Abbreviations

A: Angle

A2AP: α -2-antiplasmin

ACS: Acute coronary syndrome

ADP: Adenosine diphosphate

AHA: American Heart Association

ANOVA: Analysis of variance

AUC: Area under receiver operator curve

BMI: Body mass index

CACS: CT coronary artery calcium scoring

CAD: Coronary artery disease

cAMP: Cyclic adenosine monophosphate

CCS: Chronic coronary syndrome

CI: Confidence interval

CLT: Clot lysis time

COVID-19: Coronavirus disease 2019

CPR: Cardiopulmonary resuscitation

CRF: Case report forms

CTCA: Computed tomography coronary angiogram

CV: Coefficients of variation

CVD: Cardiovascular death

DAPT: Dual antiplatelet therapy

DSMB: Data safety monitoring board

DTI: Direct oral thrombin inhibitor

ECG: Electrocardiogram

ELISA: Enzyme-linked immunosorbent assay

ESC: European Society of Cardiology

EudraCT: European Union Drug Regulating Authorities Clinical Trials Database

FFR: Fractional flow reserve

FXa: Factor Xa

FXIII: Factor XIII

GCP: Good clinical practice

GTT: Global thrombosis test

GUSTO: Global Utilisation of Streptokinase and TPA for Occluded arteries

HAC: Heart attack centre

HR: Hazard ratio

HTPR: High on-treatment platelet reactivity

ICH: Intracranial haemorrhage

IQR: Interquartile range

IRAS: Integrated Research Application System

K: Kinetics

LAD: Left anterior descending

LCx: left circumflex

LDL: Low-density lipoprotein

LMS: Left main stem

Lp(a): Lipoprotein (a)

LT: Lysis time

LTA: Light transmittance aggregometry

LV: Left ventricle

Ly30: Lysis 30

Ly60: Lysis 60

MA: Maximum amplitude

MACE: Major adverse cardiovascular event

MESA: Multi-Ethnic Study of Atherosclerosis

MHRA: Medicines and Healthcare products Regulatory Agency

MI-CAD: Myocardial infarction with coronary artery disease

MI: Myocardial infarction

MINOCA: Myocardial infarction with non-obstructive coronary arteries

NICE: National Institute of Health and Care Excellence

NPV: Negative predictive value

NRES: National Research Ethics Service

NSTEMI: Non-ST-segment elevation myocardial infarction

NVAF: Non-valvular atrial fibrillation

OHCA: Out-of-hospital cardiac arrest

OR: Odds ratio

OT: Occlusion time

PAI: Plasminogen activator inhibitor

PAR: Protease-activated receptors

PCI: Percutaneous coronary intervention

PPV: Positive predictive value

R: Reaction Time

RCT: Randomised controlled trials

ROSC: Return of spontaneous circulation

RR: Rate ratio

RWMA: Regional wall motion abnormality

STEMI: ST-segment elevation myocardial infarction

TAFI: Thrombin-activatable fibrinolysis inhibitor

TCFA: Thin cap fibroatheroma

TEG: Thromboelastography

TIMI: Thrombolysis In Myocardial Infarction

TMA: Time to maximum amplitude

tPA: Tissue plasminogen activator

TSC: Trial steering committee

TT: Triple therapy

TxA₂: Thromboxane A₂

UA: Unstable angina

uPA: Urokinase plasminogen activator

VF: Ventricular fibrillation

VKA: Vitamin K antagonist

VLDR: Very low dose rivaroxaban

VT: Ventricular tachycardia

vWF: von Willebrand factor

Appendix 2: Ethical Approval Documents

Research & Development Department
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Tel: 0203 826 2068/2069

Wednesday 8th November 2017

Professor Diana Gorog
East and North Hertfordshire NHS Trust
Lister Hospital
Coreys Mill Lane
Stevenage
Hertfordshire
SG1 4AB

Research and Development Steering Group (RDSG)

Dear Professor Gorog,

Re: RD2017-82

Serum Calcific Potential: A tool to predict vascular calcification and its progression in subjects with coronary artery disease

Thank you for your application to the East and North Hertfordshire NHS Trust Research and Development Steering Group (RDSG).

The following documents have been reviewed:

Document Title	Version	Date
Protocol	1.0	1 st September 2017
Participant Information Sheet	1.0	1 st September 2017
Informed Consent Form	1.0	1 st September 2017
GP Letter	1.0	1 st September 2017
Participant Invitation Letter	1.0	1 st September 2017

Further to scientific review by the RDSG, I am pleased to advise you that the Committee have approved the application for the above study and granted East & North Hertfordshire NHS Trust sponsorship in principle.

The Committee has not requested any further amendments to the above documentation therefore a submission to the relevant research ethics committee may be made.

A full Sponsorship Agreement outlining the responsibilities of the Sponsor and Chief Investigator will also follow in due course.

For non-commercially sponsored multicentre studies where tissue is being provided and sent outside East & North Hertfordshire NHS Trust please ensure that you have a Material Transfer Agreement in place.

You are reminded that your project must be conducted in accordance with the Research Governance Framework for Health and Social Care and that all members of the research team must be aware of and understand their responsibilities under this Framework.

Please be aware that this letter is part of the R&D process but is not the final confirmation that the site has capability and capacity to run the study. **Please remember that you must have received the full R&D confirmation email before you can recruit patients and commence this study.**

Yours sincerely

A handwritten signature in black ink, appearing to read 'R. Bhatti', written in a cursive style.

Rishma Bhatti

Research and Development Manager

(Signed on behalf of the Research and Development Steering Group)

London - Fulham Research Ethics Committee

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20 December 2017

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Corey's Mill Lane
SG14AB

Dear Professor Gorog

Study title: Serum calcific potential: a tool to predict vascular calcification and its progression in subjects with coronary artery disease

REC reference: 17/LO/2067

Protocol number: RD2017-82

IRAS project ID: 235341

The Research Ethics Committee reviewed the above application at the meeting held on 11 December 2017.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1. The Committee would like the information sheet revised to:
 - a. Amend the first sentence under the heading 'What is the purpose of the study?' to '-Many diseases of the heart such as a heart attack are related **to** narrowing of the heart arteries.'
 - b. Amend the sentence under the heading 'Will my taking part in the study be kept confidential?' to 'All those involved with the study will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside of the research team.'

- c. Amend the sentence under the heading 'What if something goes wrong?' to 'However, the normal NHS complaints mechanism is available to **you** if you wish to complain about any aspect of the way you are approached or treated during the course of this study.'

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Summary of discussion at the meeting

Ethical issues raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting

The Chair welcomed Dr Ying Gue and thanked him for attending the meeting to discuss the study.

Recruitment arrangements and access to health information, and fair participant selection

The Committee were unclear whether the research team would be accessing medical records or identifiable data before consent had been obtained. Dr Gue explained that the research team was the clinical team as well, so would have access to data via their clinical role and would identify possible participants themselves.

The Committee noted that expenses would not be paid and queried if there would be any extra visits. Dr Gue explained that they would try to incorporate the study visit with a normal routine visit so participants did not have to pay any extra for travel. He explained that patients were very positive and happy to take part in research.

The Committee noted that poor kidney function was an exclusion criterion, but noted that diabetes was not and queried this with the researcher. Dr Gue explained that potential participants who have long standing diabetes would most likely have poor kidney function and so would be excluded under the kidney function exclusion criteria.

The Committee were satisfied with the above clarifications and had no further issues.

Informed consent process and the adequacy and completeness of participant information

The Committee noted there a number of minor corrections required for the information sheet as detailed in the decision below.

Please contact the REC Manager if you feel that the above summary is not an accurate reflection of the discussion at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Contract/Study Agreement template [MTA]		
GP/consultant information sheets or letters [GP letter]	1.0	01 September 2017
IRAS Application Form [IRAS_Form_15112017]		15 November 2017
IRAS Application Form XML file [IRAS_Form_15112017]		15 November 2017
IRAS Checklist XML [Checklist_15112017]		15 November 2017
Letter from sponsor [Sponsor Letter]		08 November 2017
Letters of invitation to participant [Invitation]	1.0	01 September 2017
Other [Co-CI CV]		
Participant consent form [Consent]	1.0	01 September 2017

Participant information sheet (PIS) [PIS]	1.0	01 September 2017
Research protocol or project proposal [Protocol]	1.0	01 September 2017
Summary CV for Chief Investigator (CI) [CI CV]		
Summary CV for student [Sub-Investigator CV]		

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: <http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

17/LO/2067	Please quote this number on all correspondence
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With the Committee’s best wishes for the success of this project.

Yours sincerely



The Rev'd Nigel Griffin
Chair

E-mail: nrescommittee.london-fulham@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

“After ethical review – guidance for researchers”

Copy to: Rishma Bhatti, East and North Hertfordshire NHS Trust

London - Fulham Research Ethics Committee

Attendance at Committee meeting on 11 December 2017

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Mr Keith Berelowitz	Director of Operations Richmond Pharmacology	No	
Dr Anthony Farrant	Lecturer in Healthcare Law and Ethics	Yes	
Dr Shaun Griffin	Communications Manager	Yes	
The Rev'd Nigel Griffin (Chair)	Parish Priest	Yes	
Miss Lesley Honeyfield	Principal Research Radiographer for the Imaging Department	No	
Dr Akil Jackson	Research Fellow	Yes	
Dr Mays Jawad	Research Governance Operations Manager	No	
Mr Greg Kyle-Langley	Private Banker	Yes	
Ms Monsey McLeod	Pharmacist	No	
Dr Frank Miskelly	Physician	Yes	
Mrs Elizabeth Reeves	Clinical Trials Training Executive	No	
Mrs Gillian Sichau	Retired Occupational Therapist	No	
Miss Onyinyechi Uwasomba	Medical Technologist	No	
Mrs Marney Williams	Teacher	Yes	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Miss Anna Bannister	REC Manager

Research & Development Department

Mount Vernon Cancer Centre

Mount Vernon Hospital

The Clock Tower

Rickmansworth Road

Northwood

Middlesex HA6 2RN

Tel: 0203 826 2068/2069

Friday 13th October 2017

Professor Diana Gorog
East and North Hertfordshire NHS Trust
Lister Hospital
Coreys Mill Lane
Stevenage
Hertfordshire
SG1 4AB

Research and Development Steering Group (RDSG)

Dear Professor Gorog,

Re: RD2017-66

INSIGHT - Assessing individual variation in fibrinolytic status in healthy volunteers

Thank you for your application to the East and North Hertfordshire NHS Trust Research and Development Steering Group (RDSG).

The following documents have been reviewed:

Document Title	Version	Date
Poster	2.0	21 st September 2017
Participant Information Sheet	1.0	17 th August 2017
Participant Invitation letter	1.0	17 th August 2017
Protocol	1.0	17 th August 2017
Informed Consent Form	1.0	17 th August 2017

Further to scientific review by the RDSG, I am pleased to advise you that the Committee have approved the application for the above study and granted East & North Hertfordshire NHS Trust sponsorship in principle.

The Committee has not requested any further amendments to the above documentation therefore a submission to the relevant research ethics committee may be made.

A full Sponsorship Agreement outlining the responsibilities of the Sponsor and Chief Investigator will also follow in due course.

For non-commercially sponsored multicentre studies where tissue is being provided and sent outside East & North Hertfordshire NHS Trust please ensure that you have a Material Transfer Agreement in place.

You are reminded that your project must be conducted in accordance with the Research Governance Framework for Health and Social Care and that all members of the research team must be aware of and understand their responsibilities under this Framework.

Please be aware that this letter is part of the R&D process but is not the final confirmation that the site has capability and capacity to run the study. **Please remember that you must have received the full R&D confirmation email before you can recruit patients and commence this study.**

Yours sincerely

A handwritten signature in black ink, appearing to read 'Rishma Bhatti', written in a cursive style.

Rishma Bhatti

Research and Development Manager

(Signed on behalf of the Research and Development Steering Group)

London – Camden and King’s Cross Research Ethics Committee

Health Research Authority
Skipton House
80 London Road
London
SE1 6LH

Telephone: 020 7972 2561

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

23 April 2019

Prof Diana Gorog
East and North Hertfordshire NHS Trust
Cardiology Department
The Lister Hospital
Coreys Mill Lane
Stevenage
SG14AB

Dear Prof Gorog

Study title:	Assessing individual variation in fibrinolytic status in healthy volunteers
REC reference:	17/LO/1885
Protocol number:	RD2017-66
Amendment number:	Substantial Amendment 1, 07/01/2019
Amendment date:	11 February 2019
IRAS project ID:	230520

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation, for a further blood sample 8-12 hours after the first visit to check for intra-individual diurnal variability,

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering letter on headed paper [Covering Letter]		07 January 2019
Notice of Substantial Amendment (non-CTIMP) [Notice of Substantial Amendment]	Substantial Amendment 1, 07/01/2019	11 February 2019
Participant consent form [Participant Consent Form]	1.2 - Tracked Changes	07 January 2019
Participant information sheet (PIS) [Participant Information Sheet]	1.2 - Tracked Changes	07 January 2019
Participant information sheet (PIS) [Participant Information Sheet]	1.2	07 January 2019
Research protocol or project proposal [Protocol]	1.2 - Tracked Changes	07 January 2019
Research protocol or project proposal [Protocol]	1.2	07 January 2019

Membership of the Committee

The members of the Committee who took part in the review are listed below.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: <https://www.hra.nhs.uk/planning-and-improving-research/learning/>

17/LO/1885:	Please quote this number on all correspondence
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Yours sincerely



pp
Mrs Rosie Glazebrook
Chair

E-mail: nrescommittee.london-camdenandkingscross@nhs.net

Copy to: *Prof Phillip Smith*

London - Camden & Kings Cross Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 05 April 2019

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Dr Emily Cadman	Senior Registrar	Yes	
Mrs Rosie Glazebrook	Consumer Marketing	Yes	



Health Research
Authority

London - Camden & Kings Cross Research Ethics Committee

Jarrow Business Centre
Rolling Mill Road
Jarrow
NE32 3DT

Telephone: 0207 104 8086

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

04 December 2017

Prof Diana Gorog
East and North Hertfordshire NHS Trust, Cardiology Department
The Lister Hospital, Coreys Mill Lane
Stevenage
SG14AB

Dear Prof Gorog

Study title: Assessing individual variation in fibrinolytic status in healthy volunteers
REC reference: 17/LO/1885
Protocol number: RD2017-66
IRAS project ID: 230520

The Research Ethics Committee reviewed the above application at the meeting held on 20 November 2017. Thank you to Dr Ying Gue for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1. Removal of reference to contacting a participant's GP from the participant information sheet, consent form and protocol.
2. Amendment to the study poster to state that blood samples would be required between 8 and 12 hours apart on the same day.
3. Amendment to the participant information sheet as following:
 - a. Revision under the "what will I have to do" heading to state "Also, you should be healthy with no major medical conditions, you should not be smoking or using any type of tobacco products, drinking alcohol above the recommended safe levels or taking any medications which would affect the "stickiness" of your blood e.g. the oral contraceptive pill"

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be

permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Summary of discussion at the meeting

Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.

The Chair welcomed Dr Ying Gue to the meeting and thanked him for attending.

The Chair noted that there were two observers present. The applicant confirmed that the observers could remain in the room for the discussion of this application.

Social or scientific value; scientific design and conduct of the study

The Committee was unclear as to whether the participants GP would be informed of their involvement in the study and sought clarification.

Dr Gue explained that this depended on the participants wishes, some participants would like their GP to be informed as standard. He commented that if there were incidental findings then the GP would be informed.

After discussion, the Committee agreed that it would not be necessary to inform GP's as standard as this study would involve simple blood tests. It was noted that participants could inform their GP in discussion if they wished to. References to informing the GP should be removed from the participant information sheet and consent form.

Clarification as to whether the sample would be stratified and whether results would be compared was required.

Dr Gue confirmed that the sample would not be stratified. He explained that baseline demographics would be considered when undertaking the analysis but the results would not be compared as the intention of the research would be to look at the individual participant.

The Committee accepted this response.

The REC sought clarification as to whether blood samples would be required and the beginning and the end of the same day, and sought confirmation as to how many hours should elapse between sampling.

Dr Gue confirmed that sampling would be required on the same day and they should be between 8 and 12 hours apart. He commented that the participant's willingness to come back to for the second sample would depend on their availability. For example, if the participant was the family member of a patient with cancer and receiving chemotherapy, they may wish to stay at the hospital with the patient all day. Dr Gue commented that the study would likely be a lot more accessible to staff members as the timings would work around their shift patterns.

The Committee requested that the requirements for same day blood sampling and the time between each sample be made clear on the recruitment poster as this should be transparent to all people considering taking part.

Dr Gue agreed to amend the poster as requested.

Recruitment arrangements and access to health information, and fair participant selection

Confirmation as to whether only staff participants would be included was required. The Committee agreed that the study would be more simplistic if this were the case.

Dr Gue confirmed that any person who could see the posters would be eligible, including staff. He detailed that patient groups and family members were often keen to take part in research.

The Committee accepted this response.

The REC sought clarification as to how a participant's status as a 'healthy' volunteer would be ascertained.

Dr Gue asserted that this would be determined by the self-declared information from the participant and confirmed that no background checks would be undertaken.

The Committee accepted this response.

Clarification of the location of the posters was required.

Dr Gue confirmed that they would be in outpatient departments in the reception area.

The Committee accepted this response.

Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)

The REC discussed that the blood sampling could be burdensome on participants given they would be required on the same day several hours apart. Members queried whether any travel expenses or a voucher as a token of thanks could be given to accommodate this.

Dr Gue detailed that unfortunately there was no funding to give a voucher or travel expenses, and he envisioned that people would want to take part out of kindness and to be able to give something back to the NHS.

The Committee accepted this response.

Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity

The Committee sought clarification as to how the samples would be anonymised.

Dr Gue explained that samples would be given a participant ID number and they would be analysed and stored anonymously.

The Committee accepted this response.

The Committee noted that there may be further issues raised in correspondence.

The applicant left the meeting

Please contact the REC Manager if you feel that the above summary is not an accurate reflection of the discussion at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants [Poster]	2.0	21 September 2017
IRAS Application Form [IRAS_Form_13102017]		13 October 2017
Letter from sponsor [Letter from sponsor]		13 October 2017
Letters of invitation to participant [Invitation Letter]	1.0	17 August 2017
Participant consent form [Consent Form]	1.0	17 August 2017
Participant information sheet (PIS) [PIS]	1.0	17 August 2017
Research protocol or project proposal [Protocol]	1.0	17 August 2017
Summary CV for Chief Investigator (CI) [CV]		04 October 2016

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

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HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

17/LO/1885

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely



pp

Mrs Rosie Glazebrook
Chair

E-mail: nrescommittee.london-camdenandkingscross@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

"After ethical review – guidance for researchers"

Copy to: Prof Phillip Smith, East and North Hertfordshire NHS Trust
Mrs Rishma Bhatti, East & North Hertfordshire NHS Trust

London - Camden & Kings Cross Research Ethics Committee

Attendance at Committee meeting on 20 November 2017

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Dr Emily Cadman	Senior Registrar	Yes	
Ms Heidi Chandler (Vice Chair)	Deputy Research Delivery Manager	Yes	
Mrs Julia Crenian	Volunteer with Home-Start	Yes	
Mrs Rosie Glazebrook (Chair)	Consumer Marketing	Yes	
Mrs Julia King	Consultant in human rights	Yes	
Mrs Elizabeth Landers	Tutor	Yes	
Dr Lorraine Ludman	Chartered Psychologist	Yes	
Mrs Georgia Mannion-Krase	Research Assistant	Yes	
Dr Jacqueline Maxmin	Retired GP	Yes	
Dr Andy Petros	Consultant Paediatric Intensivist	No	
Ms Petra Shroff	Paediatric Nurse	Yes	
Mr Jonathan Simons	Investment Manager	No	
Mr Ashley Stratton-Powell	PhD Doctoral Training Centre for Tissue Engineering and Regenerative Medicine	Yes	
Ms Eleni Yerolaki	Specialist Counsellor	No	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Therese Bidder	Clinical Research Nurse (observer)
Maysze Chang	Paediatric Research Nurse (observer)
Miss Christie Ord	REC Manager

Research & Development Department
Mount Vernon Cancer Centre
Mount Vernon Hospital
The Clock Tower
Rickmansworth Road
Northwood
Middlesex HA6 2RN

Tel: 0203 826 2068/2069

Monday 4th June 2018

Professor Diana Gorog
East and North Hertfordshire NHS Trust
Lister Hospital
Coreys Mill Lane
Stevenage
Hertfordshire
SG1 4AB

Research and Development Steering Group (RDSG)

Dear Professor Gorog,

Re: RD2018-33 **OHCA Risk Scoring** – A simple risk scoring system to predict outcome in patients with out-of-hospital cardiac arrest

Thank you for your application to the East and North Hertfordshire NHS Trust Research and Development Steering Group (RDSG).

The following documents have been reviewed:

Document Title	Version	Date
Protocol	1.0	9 th April 2018

Further to scientific review by the RDSG, I am pleased to advise you that the Committee have approved the application for the above study and granted East & North Hertfordshire NHS Trust sponsorship in principle.

The Committee has not requested any further amendments to the above documentation therefore a submission to the relevant research ethics committee may be made.

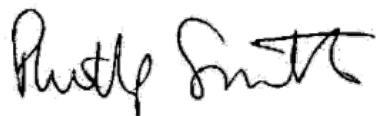
A full Sponsorship Agreement outlining the responsibilities of the Sponsor and Chief Investigator will also follow in due course.

For non-commercially sponsored multicentre studies where tissue is being provided and sent outside East & North Hertfordshire NHS Trust please ensure that you have a Material Transfer Agreement in place.

You are reminded that your project must be conducted in accordance with the UK Policy Framework for Health and Social Care Research and that all members of the research team must be aware of and understand their responsibilities under this Framework.

Please be aware that this letter is part of the R&D process but is not the final confirmation that the site has capability and capacity to run the study. **Please remember that you must have received the full R&D confirmation email before you can recruit patients and commence this study.**

Yours sincerely

A handwritten signature in black ink, appearing to read 'Phillip Smith', written in a cursive style.

Phillip Smith
Associate Director of Research and Development
(Signed on behalf of the Research and Development Steering Group)



Health Research Authority

South Central - Berkshire B Research Ethics Committee

Whitefriars
Level 3, Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 0207 104 8241

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

28 June 2018

Professor Diana Gorog
Cardiology Department
The Lister Hospital
Corey's Mill Lane
SG1 4AB

Dear Professor Gorog

Study title:	A simple risk scoring system to predict outcome in patients with out-of-hospital cardiac arrest
REC reference:	18/SC/0355
Protocol number:	RD2018-33
IRAS project ID:	246483

Thank you for your letter of 24 June 2018, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

A Research Ethics Committee established by the Health Research Authority

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with

A Research Ethics Committee established by the Health Research Authority

prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” above).

Approved documents

The documents reviewed and approved by the Committee are:

<i>Document</i>	<i>Version</i>	<i>Date</i>
IRAS Application Form [IRAS_Form_08062018]		08 June 2018
IRAS Checklist XML [Checklist_24062018]		24 June 2018
Letter from sponsor [Sponsor Letter]	1.0	04 June 2018
Other [ResponseLetter]	1.0	21 June 2018
Other [Protocol clean]	1.1	21 June 2018
Other [Tracked Protocol]	1.1	21 June 2018
Summary CV for Chief Investigator (CI) [CV]	1.0	04 October 2016
Summary CV for student [Student CV]	1.0	27 April 2017
Summary CV for supervisor (student research) [Supervisor CV]	1.0	04 October 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:

<http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance>

We are pleased to welcome researchers and R & D staff at our RES Committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

18/SC/0355

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely



PP

Dr John Sheridan
Chair

Email: nrescommittee.southcentral-berkshireb@nhs.net

Copy to: *Professor Phillip Smith*
Professor Diana Gorog
Ms Rishma Bhatti, East and North Hertfordshire NHS Trust

Research & Development Department
Mount Vernon Cancer Centre
Mount Vernon Hospital
The Clock Tower
Rickmansworth Road
Northwood
Middlesex HA6 2RN

Tel: 0203 826 2068/2069

Friday 24th August 2018

Research and Development Steering Group (RDSG)

Dear Professor Diana Gorog

Re: RD2018-41 VaLiDate-R – Can Very Low Dose Rivaroxaban in addition to dual antiplatelet therapy (DAPT) improve thrombotic status in Acute Coronary Syndrome (ACS) ACS

Thank you for your application to the East and North Hertfordshire NHS Trust Research and Development Steering Group (RDSG).

The following documents have been reviewed:

Document Title	Version	Date
Protocol	1.1	23 rd June 2018
Participant Information Sheet	1.0	28 th July 2018
Consent Form - Main	1.0	28 th July 2018
Consent Form - Screening	1.0	10 th April 2018
GP Letter	1.0	28 th July 2018
SmPC – Clopidogrel		
SmPC – Rivaroxaban		
SmPC - Ticagrelor		

Further to scientific review by the RDSG, I am pleased to advise you that the Committee have approved the application for the above study and granted East & North Hertfordshire NHS Trust sponsorship in principle.

The Committee has not requested any further amendments to the above documentation therefore a submission to the relevant research ethics committee may be made.

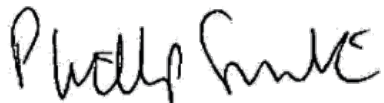
A full Sponsorship Agreement outlining the responsibilities of the Sponsor and Chief Investigator will also follow in due course.

For non-commercially sponsored multi-centre studies where tissue is being provided and sent outside East & North Hertfordshire NHS Trust please ensure that you have a Material Transfer Agreement in place.

You are reminded that your project must be conducted in accordance with the UK Policy Framework for Health and Social Care Research and that all members of the research team must be aware of and understand their responsibilities under this Framework.

Please be aware that this letter is part of the R&D process but is not the final confirmation that the site has capability and capacity to run the study. **Please remember that you must have received the full R&D confirmation email before you can recruit patients and commence this study.**

Yours sincerely

A handwritten signature in black ink that reads "Phillip Smith". The signature is written in a cursive style with a large initial 'P'.

Phillip Smith
Associate Director of Research and Development
(Signed on behalf of the Research and Development Steering Group)



Health Research Authority

London - Hampstead Research Ethics Committee

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 02071048127

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

11 March 2019

Professor Diana Gorog
Consultant Cardiologist and Clinical Lead for Research
The Lister Hospital
Corey's
Mill Lane
SG1 4AB

Dear Professor Gorog

Study title:	Can Very Low Dose Rivaroxaban (VLDR) in addition to dual antiplatelet therapy (DAPT) improve thrombotic status in acute coronary syndrome (ACS)
REC reference:	18/LO/1608
Protocol number:	RD2018-41
EudraCT number:	2018-003299-11
Amendment number:	1
Amendment date:	14 January 2019
IRAS project ID:	246526

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering letter on headed paper [Cover Letter]		04 January 2019
Notice of Substantial Amendment (CTIMP) [NoSA]	1	14 January 2019
Participant consent form [ICF]	1.2	14 January 2019
Participant consent form [Screening ICF]	1.2	14 January 2019
Participant information sheet (PIS) [PIS (tc)]	1.2	14 January 2019
Participant information sheet (PIS) [PIS]	1.2	14 January 2019
Research protocol or project proposal [Protocol (tc)]	1.2	14 January 2019
Research protocol or project proposal [Protocol]	1.2	14 January 2019

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

18/LO/1608:	Please quote this number on all correspondence
--------------------	---

Yours sincerely



Signed on behalf of
Miss Stephanie Ellis, BEM
Chair

E-mail: nrescommittee.london-hampstead@nhs.net

Enclosures: List of names and professions of members who took part in the

review

Copy to: Ms Rishma Bhatti, East and North Hertfordshire NHS Trust

London - Hampstead Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 01 March 2019

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Miss Stephanie Ellis, BEM	Former Civil Servant	Yes	
Dr Kanagasabai Ganeshaguru	Retired Scientist	Yes	

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>
Mr Matt Rogerson	REC Manager



Health Research Authority

London - Hampstead Research Ethics Committee

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 02071048127

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

17 October 2018

Professor Diana Gorog
Consultant Cardiologist and Clinical Lead for Research
East and North Hertfordshire NHS Trust
Coreys Mill Lane
The Lister Hospital
Corey's Mill Lane
SG1 4AB

Dear Professor Gorog

Study title:	Can Very Low Dose Rivaroxaban (VLDR) in addition to dual antiplatelet therapy (DAPT) improve thrombotic status in acute coronary syndrome (ACS)
REC reference:	18/LO/1608
Protocol number:	RD2018-41
EudraCT number:	2018-003299-11
IRAS project ID:	246526

The Research Ethics Committee reviewed the above application at the meeting held on 10 October 2018. Thank you for attending to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at <http://www.rdforum.nhs.uk>.

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Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites listed in the application taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

Non NHS sites

The Committee has not yet completed any site-specific assessment(s) (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Extract of the meeting minutes

The Committee invited Professor Diana Gorog and Dr Ying Gue into the meeting. The researchers were made aware of the presence of an observer, and were happy for him to remain.

Social or scientific value; scientific design and conduct of the study

The Committee thanked the researchers for an exceptionally well written application, which enabled it to understand the design of the study with ease. This was a particularly high quality application, and the Committee wished to convey this to the researchers.

The researchers thanked the Committee for its kind words.

The Committee asked to hear, in the researchers’ own words, what was the new component of this study.

Professor Gorog explained that they currently knew that thrombosis requires treatment and that the treatment usually involves blood-thinning medication. What, Professor Gorog considered, they did not know was if they could identify particular blood-thinning medications for particular people. Professor Gorog expressed a desire to have a particular biomarker to help replace clinical intuition and judgment in ensuring the least aggressive (but most effective) treatment option could be given to avoid unnecessary bleeding.

Recruitment arrangements and access to health information, and fair participant selection

The Committee asked the researchers to describe the consent process.

Professor Gorog explained the initial approach to patients would be on the acute cardiac unit, usually the morning after they had reached the unit. The initial approach would be made by the unit staff. If patients agree, the research team would already be insitu on the unit and would make their approach.

The Committee asked how long participants would be given to decide.

Professor Gorog explained that they would not be given long to decide, due to the nature of their condition the 1st 30 days was deemed to be crucial, and that if potential participants

remain uncertain after a thorough explanation, they would not be able to be recruited onto the trial. Professor Gorog went on to explain the withdrawal process.

The Committee considered the recruitment and withdrawal process to be appropriate given the nature of the condition and the nature of the study.

Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity

The Committee noted that the Trial Safety Committee would meet every six months and report to the sponsor. The Committee asked the researchers to comment upon this.

Professor Gorog explained that, because all three treatments were licensed for the condition and in the combinations being used, the MHRA had considered this to be adequate.

Informed consent process and the adequacy and completeness of participant information

The Committee considered whether there should be a short explanation in the participant Information Sheet to make it clearer to participants that the treatment combinations were being used to ascertain the right combination for the right patient.

Professor Gorog explained that it had been difficult to phrase it to not make it sound coercive, that there is an element of risk involved and that they do not ultimately know if patients will do better on specific treatment combinations or not. Professor Gorog explained that she would be happy to be guided on re-phrasing the PIS.

In private discussion, the Committee considered the wording in the PIS, and considered several potential alternatives. In conclusion, the Committee decided not to ask Professor Gorog to rephrase the wording.

Suitability of the applicant and supporting staff

The Committee considered Professor Gorog and Dr Gue to be experts in their fields and perfectly placed to undertake this study.

In private discussion, the Committee noted Professor Gorog to be one of the most personable, patient and pleasant Professors to have presented to the Committee. The Committee wished to thank Professor Gorog for her pleasant disposition and clear efforts to explain elements of the research to the Committee in lay language.

Recommendation – The Committee wished to draw the researchers' attention to the following sentence in the Participant Information Sheet:

"However, despite two blood thinners combined, some patients still go on to have another clot (heart attack or stroke or death) and this can be life threatening."

The Committee considered there may be a better way to word this, as to suggest that death might be considered life threatening was perhaps not the best way to describe it. The Committee would welcome the researchers to revise this sentence (perhaps omitting the words "or death" as the explanation that follows explains that these are life-threatening events.

This could be considered a non-substantial amendment and therefore would not be notifiable to the Research Ethics Service.

Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.

Please contact the REC Manager if you feel that the above summary is not an accurate reflection of the discussion at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>	
GP/consultant information sheets or letters [GP Letter]	1.0	28 July 2018	
IRAS Application Form [IRAS_Form_24082018]		24 August 2018	
Letter from sponsor [Sponsor Letter]	1.0	24 August 2018	
Other [Screening Consent Form]	1.0	10 April 2018	
Other [Response Document]		05 September 2018	
Other [CTA Notification Acceptance]		30 August 2018	
Participant consent form [Consent Form]	1.0	28 July 2018	
Participant information sheet (PIS) [PIS]	1.0	28 July 2018	
Research protocol or project proposal [Protocol]	1.1	23 June 2018	
Summary CV for Chief Investigator (CI) [Summary CV]		04 October 2016	
Summary CV for student [CV Student]	1.0	27 April 2017	
Summary CV for supervisor (student research) [Summary CV]	1.0	04 October 2016	

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

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The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: <http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

HRA Training

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18/LO/1608

Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project.

Yours sincerely



Signed on behalf of
Miss Stephanie Ellis, BEM
Chair

E-mail: nrescommittee.london-hampstead@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

“After ethical review – guidance for researchers”

*Copy to: Professor Phillip Smith
Ms Rishma Bhatti, East and North Hertfordshire NHS Trust*

Lead Nation HRA.Approval@nhs.net

London - Hampstead Research Ethics Committee

Attendance at Committee meeting on 10 October 2018

Committee Members:

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>	
Dr Waheeb Atia	Retired Consultant Physician	Yes		
Dr Victoria Chapman	Consultant Child and Adolescent Psychiatrist	Yes		
Dr Rahul Chodhari	Consultant Paediatrician	No		
Dr Christine Ellis	Clinical Trial Pharmacist	No		
Miss Stephanie Ellis, BEM (in the Chair)	Former Civil Servant	Yes		
Dr Alicia Isabel Etchegoyen Holiday	Psychiatrist	Yes		
Dr Kanagasabai Ganeshaguru	Retired Scientist	Yes		
Mr Paul Hardiman	Consultant Gynaecologist/Senior Lecturer in Obstetrics and Gynaecology	No		
Dr Michael Jacobs	Retired Academic Pharmacologist	Yes		
Miss Monica Jefford	Retired Midwife	Yes		
Miss Mina Karamshi	Specialist Sister in Radiology	Yes		
Dr Jane Lees-Millais	General Practitioner	No		
Ms Anna Osadcow	Clinical Trials Research Pharmacist	Yes		
Ms Ann Rosenthal	Literary Consultant (Retired)	Yes		
Mrs Arlene Renee Seaton	Retired Medical Publisher	Yes		
Dr Francesca Silverton	Statistics Teacher	Yes		
Dr Latha Weston	Consultant Psychiatrist	No		
Ms Harriet Wood	REC Assistant	No		

Also in attendance:

<i>Name</i>	<i>Position (or reason for attending)</i>	
Mr Enrico Pagani	Observer	
Mr Matt Rogerson	REC Manager	



Medicines & Healthcare products
Regulatory Agency



MHRA

10 South Colonnade
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gov.uk/mhra

Mr P Smith
EAST AND NORTH HERTFORDSHIRE NHS TRUST
LISTER HOSPITAL
COREY'S MILL LANE
STEVENAGE
SG1 4AB
UNITED KINGDOM

05/09/2018

Dear Mr P Smith

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our reference: 31057/0006/001-0001
Eudract Number: 2018-003299-11
Product: Rivaroxaban
Protocol number: RD2018-41

NOTICE OF ACCEPTANCE

I am writing to inform you that the Licensing Authority accepts your request for a clinical trial authorisation (CTA), received on 28/08/2018.

REMARK-

The Sponsor is reminded that women of child-bearing potential should avoid becoming pregnant during treatment with rivaroxaban. Trial participants must be made aware of this advice, as per the SmPC for rivaroxaban.

For further information contact Dr Lisa Campbell by email; lisa.campbell@mhra.gov.uk

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed.

Yours sincerely,



Medicines & Healthcare products
Regulatory Agency



Clinical Trials Unit
MHRA

Appendix 3: Publications arising from and relating to this thesis

Original articles

1. **Gue YX**, Sayers M, Whitby B, Kanji R, Adatia K, Smith R, Davies W, Perperoglou A, Potpara T, Lip GYH, Gorog DA. Usefulness of the NULL-PLEASE Score to predict survival in out-of-hospital cardiac arrest. 2020 May 07.
2. **Gue YX**, Kanji R, Wellsted DM, Srinivasan M, Wyatt S, Gorog DA. Rationale and design of “Can Very Low Dose Rivaroxaban (VLDR) in addition to dual antiplatelet therapy improve thrombotic status in acute coronary syndrome (VaLiDate-R)” study. *Journal of Thrombosis and Thrombolysis*. 2019 Dec 23;49(2):192-8.
3. **Gue YX**, Inoue N, Spinthakis N, Takahara H, Otsui K, Lip GY, Gorog D. Thrombotic Profile and Oral Anticoagulation in Asian and Non-Asian Patients With Nonvalvular Atrial Fibrillation. *Journal of the American College of Cardiology*. 2019 Dec 3
4. **Gue YX**, Corballis N, Ryding A, Kaski JC, Gorog DA. MINOCA presenting with STEMI: incidence, aetiology and outcome in a contemporaneous cohort. *Journal of thrombosis and thrombolysis*. 2019 Nov 1;48(4):533-8.
5. Spinthakis N, **Gue YX**, Farag M, Srinivasan M, Wellsted D, Arachchilage DR, Lip GY, Gorog DA. Apixaban enhances endogenous fibrinolysis in patients with atrial fibrillation. *EP Europace*. 2019 Sep 1;21(9):1297-306.
6. Spinthakis N, Farag M, **Gue YX**, Srinivasan M, Wellsted DM, Gorog DA. Effect of P2Y12 inhibitors on thrombus stability and endogenous fibrinolysis. *Thrombosis research*. 2019 Jan 1;173:102-8.
7. Farag M, Spinthakis N, **Gue YX**, Srinivasan M, Sullivan K, Wellsted D, Gorog DA. Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study. *European heart journal*. 2019 Jan 14;40(3):295-305.

Review Articles

1. **Gue YX**, Kanji R, Gati S, Gorog DA. MI With Non-Obstructive Coronary Artery Presenting With STEMI: A Review Of Incidence, Aetiology, Assessment And Treatment. 2020 April 30.

2. **Gue YX**, Gorog DA. Importance of endogenous fibrinolysis in platelet thrombus formation. International journal of molecular sciences. 2017 Sep;18(9):1850.

Moderated posters and oral presentations

1. **Gue YX**, Mutch N, Kanji R, Farag M, Gorog DA. Correlation between plasma clot properties, thrombin generation and whole blood fibrinolytic assays in patients presenting with STEMI. **Accepted as moderated poster for ESC Congress 2020, Amsterdam.**
2. Kanji R, **Gue YX**, Dinarvand D, Gorog DA. No difference in thrombotic profile of patients with ACS with obstructive CAD and MINOCA. **Accepted as moderated poster for ESC Congress 2020, Amsterdam.**
3. **Gue YX**, Farag M, Spinthakis N, Wellsted D, Srinivasan M, Gorog DA. Diurnal variation in thrombolytic status in patients presenting with STEMI. **Accepted as moderated poster for Frontiers in Cardiovascular Biomedicine 2020, Budapest.**
4. **Gue YX**, Kanji R, Wellsted D, Srinivasan M, Wyatt S, Gorog DA. A randomised trial on modulating endogenous fibrinolysis in patients with acute coronary syndrome – VaLiDate-R. **Accepted as moderated poster for Frontiers in Cardiovascular Biomedicine 2020, Budapest.**
5. **Gue YX**, Spinthakis N, Topping W, Patel J, Baydoun A, Farrington K, Farag M, Gorog DA. Relationship between coronary stenosis severity and high shear thrombosis assessment in vitro. **Accepted as moderated poster for Frontiers in Cardiovascular Biomedicine 2020, Budapest.**
6. **Gue YX**, Spinthakis N, Markides V, Wong T, Gorog DA. Patients with atrial fibrillation exhibit a systemic prothrombotic state attributable to impaired endogenous fibrinolysis. **Moderated poster at ESC Congress 2019, Paris.**
7. **Gue YX**, Gorog DA. Modulating endogenous fibrinolysis in patients with acute coronary syndrome. **Oral presentation University of Hertfordshire Life and Medical Sciences annual conference 2019, Hatfield.**
8. Spinthakis N, **Gue YX**, Lip GYH, Farag M, Gorog DA. Platelet reactivity, clot formation and fibrinolysis with oral antithrombotic agents. **Moderated poster at 11th Asian Pacific Heart Rhythm Society Scientific Session 2018, Taipei.**

9. **Gue YX**, Spinthakis N, Nobukata I, Asumi T, Hirouki T, Kanuzori O, Junichiro Y, Gorog DA. Comparative effects of apixaban and warfarin in Japanese and Western patients. **Moderated poster at 11th Asian Pacific Heart Rhythm Society Scientific Session 2018, Taipei.**
10. **Gue YX**, Spinthakis N, Farag M, Ali M, Potpara TS, Lip GYH, Gorog DA. Usefulness of the NULL-PLEASE Score to predict survival in out-of-hospital cardiac arrest – **Moderated poster at Eurothrombosis 2018, Barcelona**
11. **Gue YX**, Farag M, Spinthakis N, Anwar M, Srinivasan M, Gorog DA. Incidence of MINOCA in patients presenting with STEMI for PPCI – applying the criteria of the ESC working group position paper on MINOCA to a contemporary cohort – **Moderated poster at ESC Congress 2018, Munich**

References

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