The Clinical Efficacy of Oral P2Y12 Inhibitor Antiplatelet Therapies During an Acute Myocardial Infarction in Patients Undergoing Percutaneous Coronary Intervention

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Abstract

Introduction: In patients who present with ST-elevation myocardial infarction (STEMI), primary percutaneous coronary intervention and the administration of dual antiplatelet therapy (aspirin plus a P2Y12 inhibitor) are recommended. This thesis aimed to determine the extent to which oral P2Y12 inhibitors (clopidogrel/prasugrel/ticagrelor) exert their antiplatelet effect during the acute phase of a STEMI compared to non-ST elevation myocardial infarction (NSTEMI).

Methods: The degree and time-course of platelet inhibition following oral administration of P2Y12 inhibitors was determined in 87 patients from their pharmacokinetic (plasma concentration) and pharmacodynamic (degree of platelet inhibition) profiles at 20 minutes, balloon inflation, 60 and 240 minutes using liquid chromatography/mass spectrometry, VerifyNow and VASP-phosphorylation assays.

Results: In STEMI patients, oral P2Y12 inhibitors do not provide adequate levels of platelet inhibition at the time of angioplasty and have a limited effect at 240 minutes. The NSTEMI group displayed a marked and rapid antiplatelet effect at all time points. A significant difference in the acute efficacy of oral P2Y12 inhibitors in STEMI vs NSTEMI patients ($p < 0.001$) was seen.

Conclusion: Oral P2Y12 inhibitors display delayed and attenuated antiplatelet effects in STEMI patients in the immediate period following administration of a loading dose when compared with NSTEMI patients.

Acknowledgements

Firstly, I would like to thank my clinical supervisor, Professor James Cotton for the opportunities he has given me within the Cardiovascular Research Group and I am grateful for his support, guidance and encouragement throughout the duration of my thesis. I would also like to thank Professor Ray Fitzpatrick for his support in allowing me to pursue my interest in research related activities.

Extra special thanks to my family and in particular my mother, who is an amazing person, a true source of inspiration and someone who has always encouraged me to go the extra mile. My brother Jawad has been supportive in every way possible, providing motivation, encouragement, laughter and occasional words of wisdom when necessary.

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List of Abbreviations

AA	Arachadonic acid
ACEI	Angiotensin converting enzyme inhibitor
ACS	Acute coronary syndromes
ADP	Adenosine diphosphate
A & E	Accident and emergency
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
APTT	Activated partial thromboplastin time
ARB	Angiotensin II receptor blocker
ARU	Aspirin response units
AUC	Area under the curve
BARC	Bleeding academic research consortium
BMI	Body mass index
CABG	Coronary artery bypass graft surgery
C-AM	Clopidogrel active metabolite
cAMP	Cyclic adenosine monophosphate
CAD	Coronary artery disease
CARDAS	Coronary artery disease database
CASP	Critical appraisal skills programme
CHD	Coronary heart disease
CHF	Congestive heart failure
COPD	Chronic obstructive pulmonary disease
COX-1	Cyclo-oxygenase enzyme 1
COX-2	Cyclo-oxygenase enzyme 2

Cmax	Maximum plasma concentration
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
CRF	Case report form
CTIMP	Clinical Trial of an Investigative Medicinal Product
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
CYPP450	Cytochrome P450 isoenzyme
DAPT	Dual antiplatelet therapy
DTB	Door to balloon time
EDTA	Ethylenediaminetetraacetic acid
ECG	Electrocardiogram
ESC	European Society of Cardiology
GI	Gastrointestinal
GP IIb/IIIa	Glycoprotein IIb/IIIa receptor
GPI	Glycoprotein IIb/IIIa receptor inhibitor
GTN	Glyceryl trinitrate
Hb	Haemaglobin
HR	Heart rate
HRPR	High residual platelet reactivity
HTPR	High on treatment platelet reactivity
5-HT_2A	Serotinin receptor
IPA	Inhibition of platelet aggregation
IRAS	Integrated Research Applications System
IV	Intravenous

LC-MS/MS	Liquid chromatography in tandem with mass spectometry
LDLC	Low density lipoprotein cholesterol
LMWH	Low molecular weight heparin
LRPR	Low residual platelet reactivity
LTA	Light transmittance aggregometry
MACE	Major adverse cardiovascular event
MACCE	Major adverse cardiovascular and cerebrovascular event
MHRA	Medicines and Healthcare products Regulatory Agency
MI	Myocardial Infarction
MBP	2-bromo-3'-methoxy acetophenone
NICE	National Institute for Health and Care Excellence
NRES	National Research Ethics Service
NSTEMI	Non-ST segment elevation myocardial infarction
NO	Nitrous oxide
ONS	Office for National Statistics
P-AM	Prasugrel active metabolite
PAR1	Proteinase activated receptor 1
PAR4	Proteinase activated receptor 4
PD	Pharmacokinetic
PCI	Percutaneous coronary intervention
PGI2	Prostaglandin I2 (prostacyclin) receptor
PGE2	Prostaglandin E2 receptor
PGH2	Prostaglandin H2 receptor
P-gp	P-glycoprotein
PK	Pharmacokinetic

PLA2	Phospolipase A2 receptor
PO	Per os
PPCI	Primary percutaneous coronary intervention
PRI	Platelet reactivity index
PRISMA-P	Preferred Reporting Items for Systematic review and Meta-Analysis Protocols
PRU	P2Y12 reactivity units
PVD	Peripheral vascular disease
REC	Research ethics committee
RLCCB	Rate limiting calcium channel blocker
RRR	Relative risk reduction
RWH	The Royal Wolverhampton Hospitals NHS Trust
SCAD	Stable coronary artery disease
SBP	Systolic blood pressure
STEMI	ST segment elevation myocardial infarction
TC	Total cholesterol
TIA	Transient ischaemic attack
T-AM	Ticagrelor active metabolite
Tmax	Time taken to reach Cmax
TP	Thromboxane receptor
T-PC	Ticagrelor parent compound
TXA_2	Thromboxane A_2
UA	Unstable angina
UFH	Unfractionated heparin
UK	United Kingdom

VASP	Vasodilator Stimulated Phosphoprotein
VHD	Valvular heart disease
VN	VerifyNow

List of clinical trials

ACCOAST-PCI	A Comparison of prasugrel at the time of percutaneous Coronary intervention Or as pre-treatment At the time of diagnosis in patients with non-ST-segment elevation myocardial infarction.
ATLANTIC-PCI	Administration of Ticagrelor in the cath Lab or in the Ambulance for New ST elevation myocardial Infarction to open the Coronary artery.
ATT	Antithrombotics Trialists Collaboration
CAPRIE	A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events
CLARITY-TIMI 28	Clopidogrel as Adjunctive Reperfusion Therapy -- Thrombolysis in Myocardial Infarction 28
COGENT	Clopidogrel with or without Omeprazole in Coronary Artery Disease
CREDO	Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial.
CURE	Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial
CURRENT OASIS 7	Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes (CURRENT-OASIS 7): a randomised factorial trial.
DISPERSE	Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin
DISPERSE-2	Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome.

GRAVITAS	Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety
HORIZONS-AMI	Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction
JUMBO-TIMI 26	Joint Utilization of Medications to Block Platelets Optimally-Thrombolysis In Myocardial Infarction 26
ISAR-CHOICE	Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect
ISIS-2	Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival**)**
PCI-CLARITY	PCI-Clopidogrel as Adjunctive Reperfusion Therapy (CLARITY) study
PCI-CURE	Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study.
PLATO	Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes
PRINCIPLE-TIME 44	The Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation–Thrombolysis in Myocardial Infarction 44 Trial
TRILOGY-ACS	Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes

Background

Rationale behind thesis and research question

In the UK alone, up to 2.3 million people have a diagnosis of coronary heart disease; of this number approximately 175,000 will experience a heart attack or myocardial infarction. Despite advances in both pharmacological and non-pharmacological management, mortality following a myocardial infarction remains high; 11% of men and 15% of women who are admitted to hospital following a heart attack die within 30 days (BHF 2016).

A heart attack occurs when the arteries that supply oxygen rich blood to the heart muscle become suddenly blocked. Clotting blood cells called platelets become activated and clump together to form a clot (thrombus) within the narrowed vessel. If the blockage in the coronary artery is partial this leads to a non-ST-elevation myocardial infarction (NSTEMI) or minor heart attack. However, if the blockage is complete, this will lead to an ST-elevation myocardial infarction (STEMI) or a major heart attack.

The first approach to treatment for a patient who presents with a myocardial infarction (MI) is to administer anti-platelet agents. Aspirin is the first agent used in this context, and a second antiplatelet, such as clopidogrel, prasugrel or ticagrelor (P2Y12 receptor antagonists) is prescribed in combination with aspirin (dual anti-platelet therapy) following a heart attack. These agents are administered in tablet form.

The second mode of treatment for a heart attack is to restore blood flow (reperfusion) as quickly as possible to the affected part of the heart. A patient admitted following a major heart attack (STEMI) is treated with Primary Percutaneous Coronary Intervention (PPCI), a procedure, in which a wire and balloon are used to reopen the coronary artery and then a stent (a slotted meshed metal tube) is placed to keep the artery open. Ninety four percent of all STEMIs are treated with PPCI, the mortality rate or death following a STEMI has fallen from 12.4% to 8.1% following the introduction of PPCI services within the UK (MINAP 2014).

PPCI is an emergency procedure, which should be performed within 90 minutes of a patient's arrival at hospital (MINAP 2014). Data derived from healthy volunteers or those with stable coronary artery disease indicates that the oral P2Y12 inhibitors take at least two hours to exert a therapeutic effect and provide sufficient levels of platelet inhibition (Brandt, Payne et al. 2007, Gurbel, Bliden et al. 2009).

Until recently, the clinical efficacy of oral P2Y12 inhibitors, during the acute phase of a myocardial infarction was largely an under investigated area. In addition to the short timescales involved for PPCI, it is possible that the condition of STEMI itself, with the effects of concomitant treatments might limit the effectiveness of oral P2Y12 inhibitors.

The physiological state of STEMI coupled with effects of severe pain and co-administration of opioid-based analgesia may well lead to a reduction in drug

absorption and metabolism through direct effects on hepatic and splanchnic blood flow and gut motility.

At the time of the conception of my research question and hypothesis there were little clinical human data available exploring the way in which oral P2Y12 inhibitors are handled by the body in the immediate period following a myocardial infarction.

The principle aims and objectives of my thesis were therefore to:

1. Determine the degree and time course of platelet inhibition by clopidogrel, prasugrel and ticagrelor administered acutely prior to emergency PPCI, during the procedure and in the following four hours.
2. Determine whether the state of acute STEMI reduces the absorption and/or subsequent clinical efficacy of oral P2Y12 inhibitors when compared with other acute coronary syndromes.

My research will therefore aim to answer two key questions:

- Is the speed of onset of action of oral P2Y12 inhibitors and the degree of platelet inhibition achieved following administration adequate for patients undergoing PPCI following a STEMI?
- Is drug absorption during an acute MI the same as that during other less acute ACS at presentation e.g, STEMI vs NSTEMI/UA?

Thesis Structure

Chapter 1:

Provides a general introduction into the pathophysiology of acute coronary syndromes as well as describing the intricate relationship between platelet activation and subsequent atherothombosis. This chapter will summarise the role of platelets in ACS, the reperfusion strategies adopted in the management of ACS and recent advances in the armamentarium of antithrombotic and in particular antiplatelet therapies.

Chapter 2:

Will provide a review of all STEMI patients treated at our PPCI centre over a five-year period to assess our local mortality and compare this against national figures. In addition, this work will not only provide context with regards to our local patient demographic and the characteristics of the local population who present with and are treated for STEMI but will also form the basis from which the PK/PD study patient population is recruited in chapter 4. The work undertaken in this chapter will also allow me to map drug utilisation (following the administration of oral clopidogrel, prasugrel and ticagrelor) against clinical outcomes such as in-hospital bleeding episodes (cerebrovascular events, gastrointestinal bleeds and blood transfusions), in-hospital and 30-day mortality. I independently collected all patient specific data, managed and maintained the patient registry and independently completed all statistical analyses of the results generated.

Chapter 3:

I completed a systematic review to evaluate currently available evidence relating to the pharmacokinetics and pharmacodynamics of oral P2Y12 inhibitors during the acute phase of a myocardial infarction; the findings of which further support the need to undertake and complete our research proposal. The systematic review has since been published in a leading peer reviewed journal.

Chapter 4:

The principal aim of my thesis was to determine and quantify the pharmacokinetic and pharmacodynamics profiles of oral P2Y12 inhibitors during the acute phase of a myocardial infarction using both platelet function assays (pharmacodynamics) and liquid chromatography in tandem with mass spectrometry (pharmacokinetics).

This chapter describes the considerations given to the trial protocol to ensure our practice with regards to patient recruitment; sample collection, data storage and analysis were in line with ethical principles. The methodology used to undertake sample collection and subsequent analysis is also described in this chapter. As a member of the Cardiovascular Research Group, I co-assisted with study management, patient identification, recruitment, sample collection, analysis and interpretation. I independently completed all statistical analyses presented in the results sections of chapters 5-8.

Chapters 5-8:

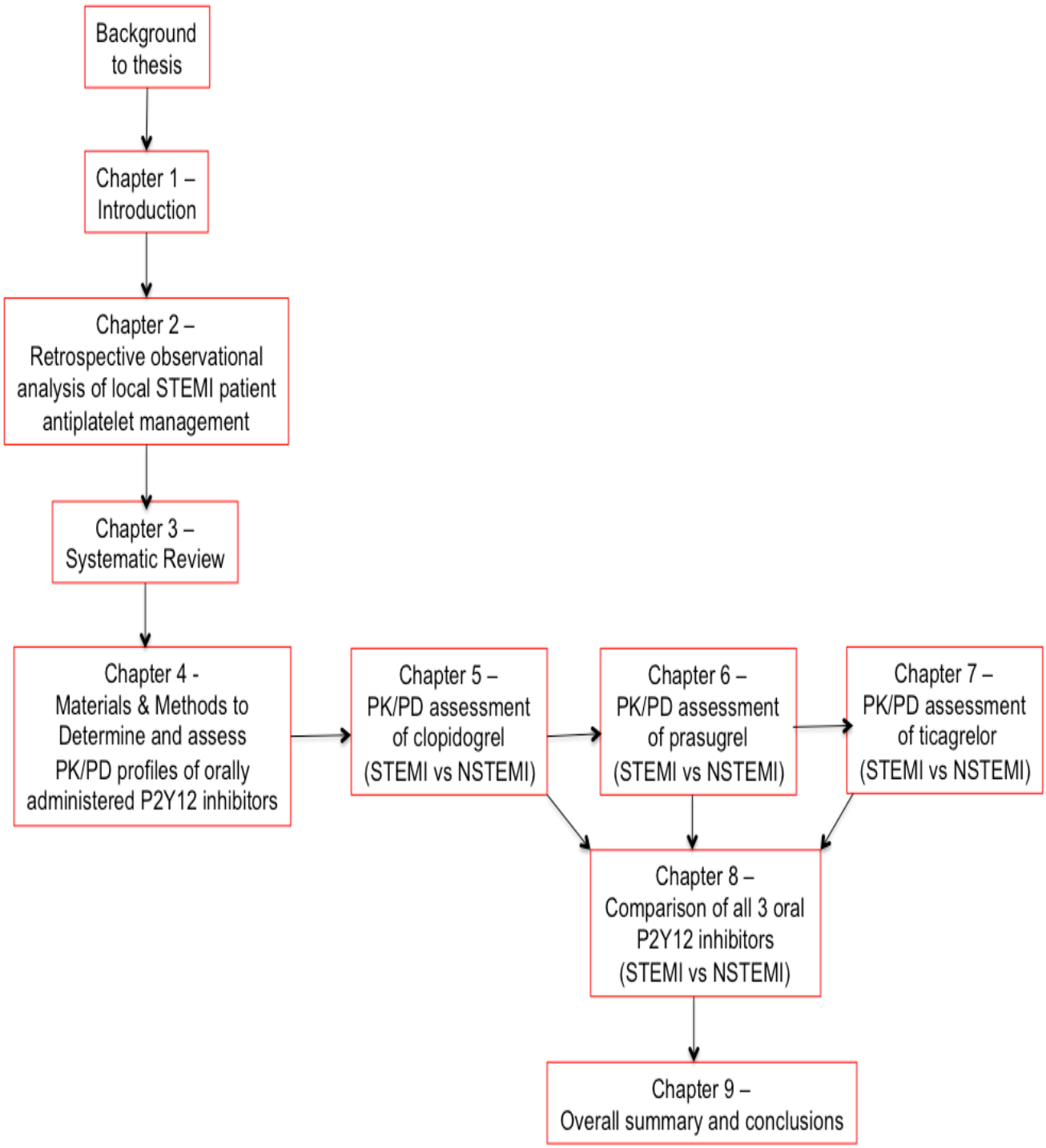
These chapters describe pharmacokinetic and pharmacodynamic analyses and the results generated following the administration of clopidogrel, prasugrel and ticagrelor in STEMI and NSTEMI patients.

Chapter 9:

Provides a concise overview of the conclusions that can be drawn from the results generated and presented throughout the thesis write-up. Limitations to the study design and the methods used to generate results are also discussed in addition to future research opportunities and proposed changes to clinical practice.

Thesis layout

The pictorial outline below describes the structure of this thesis.



Publications

The pace of change in terms of clinical and pharmacological developments in cardiology is so rapid, that there was a necessity to publish elements of my work prior to completion of my thesis. As such the following sections of my thesis have been published and presented in various pharmacy and cardiology settings: Chapter 1 (general review articles), chapter 3 (systematic review), chapter 4 (research protocol which was registered with Research Ethics Committee), chapters 5-9 (the findings of this thesis have been presented at a national cardiology meeting and published as part of the systematic review).

Chapter 1 – Introduction

1.1 Incidence and Prevalence of Cardiovascular Disease

Cardiovascular disease (CVD), an overarching term that encompasses coronary heart disease and stroke, remains the leading cause of death worldwide (Bhatnagar, Wickramasinghe et al. 2015). Although survival from myocardial infarction (MI) has improved in England, data from the Global Burden of Disease Study indicates that CVD was responsible for 30% of all deaths worldwide and is the most common cause of death in the UK (Nichols, Townsend et al. 2014). Health statistics data from 2016 indicates that in the UK alone, 2.3 million people have a current diagnosis of CHD, up to 188,000 individuals will experience a heart attack, and approximately 11% of men and 15% of women who are admitted to hospital following a heart attack will die within 30 days of presentation (BHF 2016). The higher mortality observed in women is attributable to their older age at presentation in addition to the presence of a greater number of co-morbidities and risk factors, for example, diabetes, that may well contribute to their cardiovascular disease burden (Maas and Appelman 2010).

CHD death rates vary according to age, gender, socioeconomic status and UK geographic location and ethnicity; people of South Asian origin have almost a 50% higher death rate compared to the general population (BHF 2014).

Despite advances over the last decade in both the interventional and pharmacological management of patients who present with coronary heart

disease (CHD), there is still significant morbidity and mortality associated with this condition. Compared to 2003 where 30-day mortality for patients following an MI was 13% the mortality at 30 days in 2014 is 8% (MINAP 2014).

The economic burden associated with CHD is already considerable and continues to grow, predominantly because of improvements in the availability of and access to interventional strategies to manage CHD and greater uptake of secondary prevention measures, more and more people are surviving their heart attacks and living longer. In addition, as a population the incidence of diabetes and obesity and their associated complications are also increasing, all of which contribute towards an individual's cardiovascular disease burden. The economic burden of CHD relates not only to the direct healthcare related costs, but also to the provision of formal and informal care of patients and the loss of productivity that may occur following an adverse cardiac event (Leal, Luengo-Fernandez et al. 2006).

1.2 Risk Factors

The underlying cause of CVD is the presence of atherosclerosis, which refers to the build up of atheromatous plaques in the walls of blood vessels that supply the brain, heart and peripheral vasculature. As described in table 1, a number of risk factors can contribute to and directly influence the development of atherosclerosis and in turn an individuals CVD burden.

Table 1. Modifiable and non-modifiable risk factors for coronary artery disease.

Modifiable	Non-modifiable
Smoking	Increasing age
Dyslipidaemia	Male sex
Diabetes mellitus	Family history
Hypertension	Ethnic origin
Obesity and the metabolic syndrome	Chronic kidney disease
High calorie, high fat diet	
Physical inactivity	

1.2.1 Modifiable risk factors:

Although current UK statistics indicate that the prevalence of smoking in adults has reduced from 46% in the early seventies to 19% in 2014, it is still thought to be the single most notable contributor to the development of CHD (HSCIC 2016). Smoking leads to a significant 50% increase in risk of developing CHD and mortality from any CVD is thought to be 60% higher in smokers (BHF 2014). Smoking cessation is associated with almost immediate benefit and as such individuals with established atherosclerosis or at an increased risk of developing atherosclerosis should be advised to stop smoking.

Obesity is known to adversely affect an individuals cardiovascular health and increase their risk of developing CHD (Lavie, Milani et al. 2009). The prevalence of obesity is increasing rapidly on a global scale; in the UK alone, adult obesity has increased by more than 50% in less than 10 years (BHF 2016). In addition, childhood obesity is on the rise also; this will in turn

exacerbate the problem in adulthood and will adversely contribute to an individuals CVD burden.

Studies have demonstrated that overweight and obese people tend to be less physically active and often tend to consume a lower quality diet, which contributes further to their atherogenic risk (Buttar, Li et al. 2005). A balanced healthy diet and exercise should be considered first line interventions together with careful surveillance for and aggressive management of diabetes, hypertension and dyslipidaemia to mitigate against the development and progression of CVD.

Diabetes, is known to significantly increase an individuals risk of developing CVD, since hyperglycaemia and insulin resistance contribute directly to the development of atherosclerosis and it's associated complication. A strong positive correlation is also observed between the onset of type 2 diabetes and obesity, this, in turn triggers a series of complex pathways involving inflammatory mediators, which ultimately leads to insulin resistance (Paneni, Beckman et al. 2013). The development of insulin resistance secondary to obesity and type 2 diabetes leads to endothelial dysfunction and platelet aggregation thereby contributing to the development of atherothrombosis and the clinical manifestations of CVD (Lavie, Milani et al. 2009).

Dyslipidaemias, and disorders of lipid metabolism, which result in an increase in low density lipoprotein cholesterol (LDLC) and total cholesterol (TC) levels and their subsequent contribution to the development of atherosclerosis, have

long been recognised as important risk factors in the development of CVD (Miller 2009).

Hypertension is also known to increase an individuals CAD and stroke risk; uncontrolled and persistently elevated blood pressure (>140/90 mmHg) is known to cause adverse changes in peripheral and coronary vasculature in addition to changes in the cardiac conduction system and myocardial structure and function. These changes eventually manifest as cardiac dysrhythmias such as atrial fibrillation, systolic or diastolic dysfunction of the myocardium or coronary artery disease in the form of unstable angina or myocardial infarction. The latter association between hypertension and myocardial infarction is well documented in that persistently elevated blood pressures are known to not only initiate, but also accelerate the process of atherosclerosis, causing endothelial dysfunction and subsequent atherosclerotic plaque rupture (Dunn 1983, Rakugi, Yu et al. 1996).

Often time, modifiable risk factors tend to present simultaneously in an individual; modification of risk factors through implementing dietary and lifestyle changes and optimising the management of co-morbidities can lead to a significant reduction in an individuals CVD burden.

1.2.2 Non-modifiable risk factors.

Atherosclerosis is a natural consequence of aging, and aging is a major risk factor for atherosclerotic disease. As such, advancing age is considered to be an important determinant of an individual's risk of developing CVD. This is in part due to the cumulative impact of a worsening risk factor profile in

combination with the degenerative processes that are associated with aging (National Institute for Health and Care Excellence 2014). Gender is also significant in terms of risk; CAD is more common and often presents earlier in men than in women. The incidence of CAD in women increases rapidly once they become menopausal, at which point their risk profile is similar to that seen in men (BHF 2016). An individual's family history is considered to be significant when atherosclerotic disease and/or an adverse cardiac event manifests in a first-degree male relative before the age of 55 years or female relative before the age of 65 years. Such an individual then has a positive family history of premature CAD.

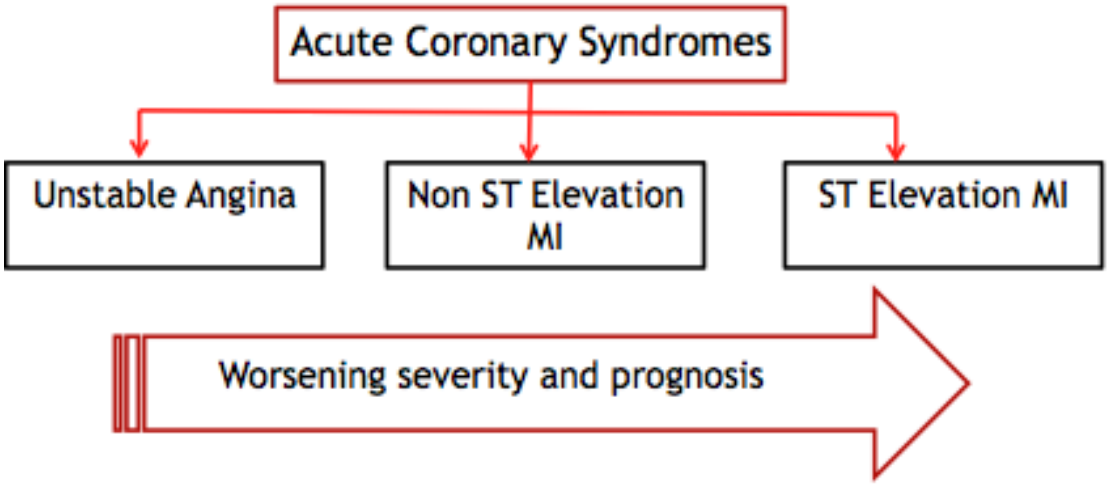
Ethnicity is also known to impact an individuals CVD risk; mortality from CAD is 50% higher in individuals from South Asian origin living in the UK. Afro-caribbeans on the other hand have a lower prevelance of CHD, but greater incidence of stroke and end-stage renal failure. Although increased prevalence of risk factors, including dyslipidaemias, insulin resistance and reduced physical activity explain much of this risk, genetic factors in both ethnicities and are thought to contribute significantly to their CVD risk (BHF 2016).

1.3 Pathophysiology of ACS

Cardiovascular disease can present as a variety of clinical syndromes that are either cerebrovascular or coronary in origin. Cardiovascular manifestations of CHD are described using the umbrella term of the acute coronary syndromes (ACS), which refers to a spectrum of clinical presentations of the same

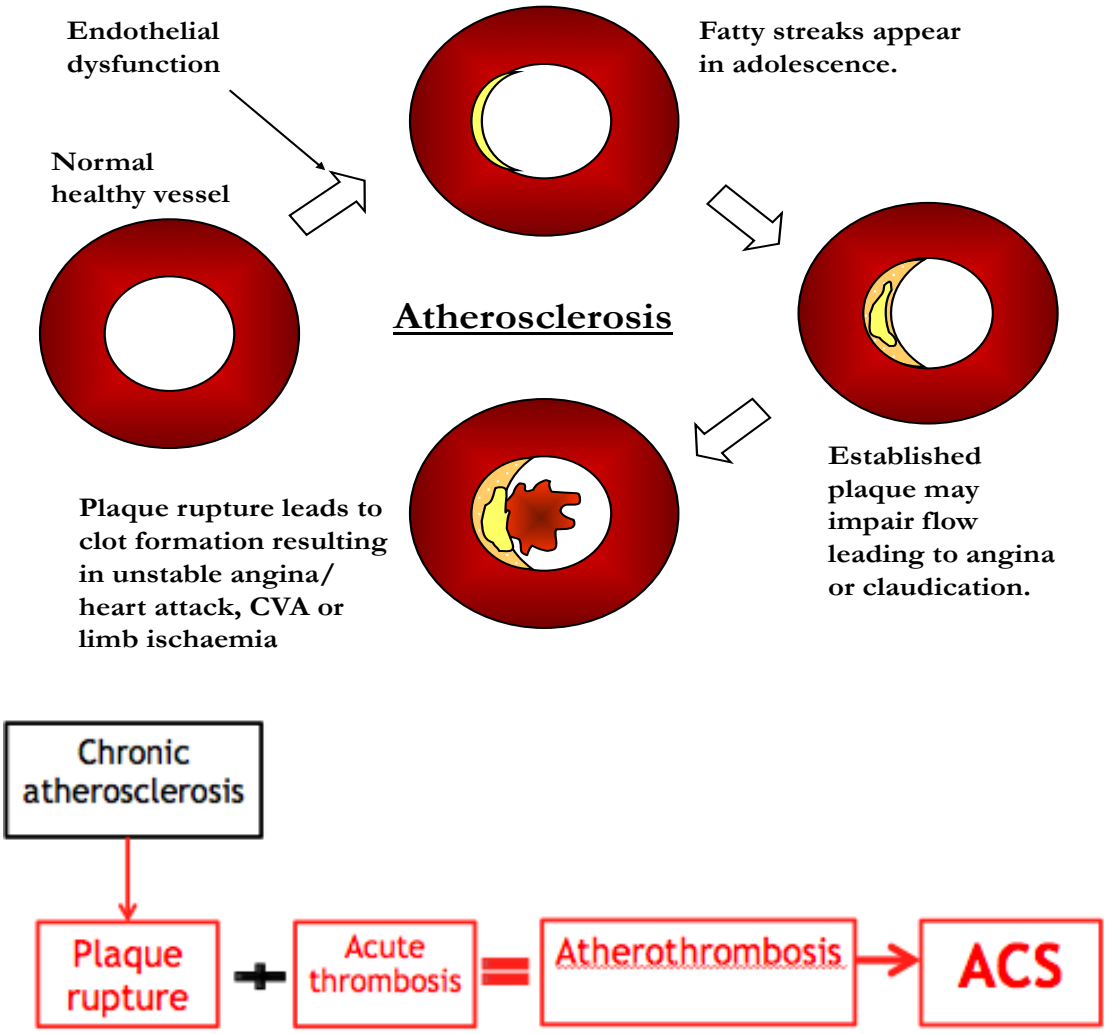
underlying disease process (atherosclerosis) and encompasses conditions such as unstable angina (UA), non ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) as described in figure 1. Disease severity and prognosis often worsens as we progress from UA through to STEMI.

Figure 1. Clinical manifestations of the acute coronary syndromes.



Atherosclerosis is the common underlying cause of ACS, the onset of which and speed of progression will vary between individuals and will be influenced by the presence of risk factors such as smoking, family history and presence of co-morbidities such as diabetes. As the atheromatous plaques grow in size they can become unstable and rupture; the subsequent endothelial and vascular damage that occurs acts as a stimulus to platelet adherence, activation and aggregation, leading to thrombus formation at the site of damage (figures 2 and 3) (Hall and Mazer 2011).

Figure 2. Process of atherothrombosis



The development of atherosclerosis is associated with significant morbidity and mortality in patients with cardiovascular disease (Alexander and Peterson 2010). The exact nature of the relationship between atherosclerosis, platelets and thrombosis was first described in the early 70's (Steele, Weily et al. 1973). Steele et al proposed the concept of "platelet survival time" as a measure of the interaction of platelets with a vascular surface; the idea being that a reduction in platelet survival time would occur in the following scenarios; rupture of an atherosclerotic plaque, in response to turbulent blood

flow induced by an irregular lumen (endothelial dysfunction) or due to excessive circulating catecholamines (Steele, Weily et al. 1973). Platelet exposure to such endothelial dysfunction will lead to platelet aggregation and subsequent thrombus formation; which can lead to a partial obstruction (NSTEMI) or complete obstruction of a coronary artery (STEMI). The corresponding reduction in the delivery of oxygenated blood to the area of the myocardium usually supplied by the infarcted artery leads to myocardial cell death/necrosis and is characterised by ECG changes and the presence of cardiac biomarkers that are released as a result of myocardial cell death/necrosis.

1.4 Reperfusion Strategies for Acute Myocardial Infarction/STEMI

The principal aim of treatment for patients who present following a myocardial infarction is to ensure timely, rapid and complete restoration of blood flow to the affected section of the myocardium to limit the extent and degree of myocardial cell death and thereby limit infarct size, preserve left ventricular function and ultimately improve long-term survival (Keeley, Boura et al. 2003).

1.4.1 Thrombolysis

The role of thrombolytic agents such as streptokinase became firmly established as a reperfusion strategy in the early 90's following publication of the landmark ISIS-2 study. As part of ISIS-2, 17,187 participants with suspected acute myocardial infarction were randomised to treatment with either streptokinase monotherapy, aspirin monotherapy, a combination of streptokinase and aspirin or placebo. The results of ISIS-2 demonstrated that

in the context of acute MI, administration of streptokinase or aspirin was associated with a significant reduction in mortality of 25% and 24% respectively, however, the combination was associated with a statistically significant reduction in mortality of 42% (P<0.001) (ISIS-2CollaborativeGroup 1988).

Whilst thrombolysis proved to be an effective treatment option, the risk of early reinfarction following its administration is associated with adverse outcomes and increased mortality (Barbash, Birnbaum et al. 2001). In addition, there are a number of contra-indications, which must be considered prior to its use; recent trauma, gastro-instestinal bleed within the last month, ischaemic stroke within the last 6 months, recent intracranial haemorrhage (Lyengar and Godbole 2011).

1.4.2 Primary Percutaneous Coronary Intervention (PPCI)

Pecutaneous coronary intervention (PCI) refers to a process in which the infarct related artery is viewed via angiography and a wire and balloon are used to reopen the artery (angioplasty) and a coronary artery stent is implanted to maintain artery patency and prevent re-occlusion.

Primary PCI (PPCI) is defined as “an emergent percutaneous coronary intervention in the setting of a STEMI” (Steg, James et al. 2012). In order to ensure the maximum amount of myocardial salvage and ensure optimal benefit in terms of survival, PPCI should be completed within 120 minutes of the onset of chest pain (Steg, James et al. 2012).

A quantitative review conducted by Keeley et al found that PPCI when compared with thrombolysis was shown to be associated with clear benefits in terms of reducing the incidence of major adverse cardiovascular events (MACE), such as non-fatal reinfarction and stroke, as well as improving both short and long term survival/mortality. The combined end point of death, non-fatal MI and stroke was statistically significantly lower when patients were treated with PPCI compared to thrombolytic therapy with a reduction of 8% and 14% respectively ($p < 0.0001$) (Keeley, Boura et al. 2003).

1.5 The Role of Platelets in ACS

Platelets are anucleate cells produced in the bone marrow, and have a life span of approximately 10 days (Papp, Kenyeres et al. 2013). They are important components in the process of normal haemostasis as well as pathological thrombus formation (Michelson 2011).

Platelet adhesion, activation and subsequent aggregation, secondary to endovascular injury or plaque rupture are key stages that contribute to the underlying pathophysiology of the acute coronary syndromes (Libby 2013). The content of the atherosclerotic plaque contains both inflammatory cells and thrombogenic materials such as tissue factor, a potent procoagulant molecule which stimulates thrombin generation. Vascular injury secondary to atherosclerotic plaque rupture exposes subendothelial collagen and von Willebrand factor, in addition to releasing tissue factor. All three acts as powerful agonists, which stimulate the process of platelet adhesion and subsequent activation (Libby 2013, Papp, Kenyeres et al. 2013). In addition, a complex series of signal transduction/activation pathways that complement

this initial process (figure 3), also occur, for example adenosine diphosphate (ADP) binds to P2Y1 and P2Y12 receptors, thromboxane A2 binds to the thromboxane receptor (TP), thrombin binds to the proteinase-activated receptor, PAR1 and PAR4, serotonin binds to the 5-HT_2A receptor and epinephrine via the alpha-adrenergic receptor (Papp, Kenyeres et al. 2013). As shown in figure 3, the binding of these agonists to their corresponding receptors results in an increase in intracellular calcium, which in turn reduces cyclic AMP levels, causing the activation of phospholipase A2. Phospholipase A2, cleaves arachadonic acid from membrane lipids, which will first be converted to prostaglandin H2 by the ubiquitous cyclo-oxygenase 1 enzyme and then to thromboxane A2 (TXA2) by thromboxane synthase (Jennings 2009, Papp, Kenyeres et al. 2013). TXA2 is a potent vasoconstrictor and platelet agonist, which amplifies the process of platelet activation through releasing intracellular granules, which stimulate the binding of other agonists to receptors on the platelets surface. In addition, this process results in a rapid change in platelet morphology such that the platelet itself changes from a smooth disk like structure into an irregular spheroid, thereby facilitating the process of platelet aggregation and perpetuating thrombus formation (Davi and Patrono 2007, Papp, Kenyeres et al. 2013).

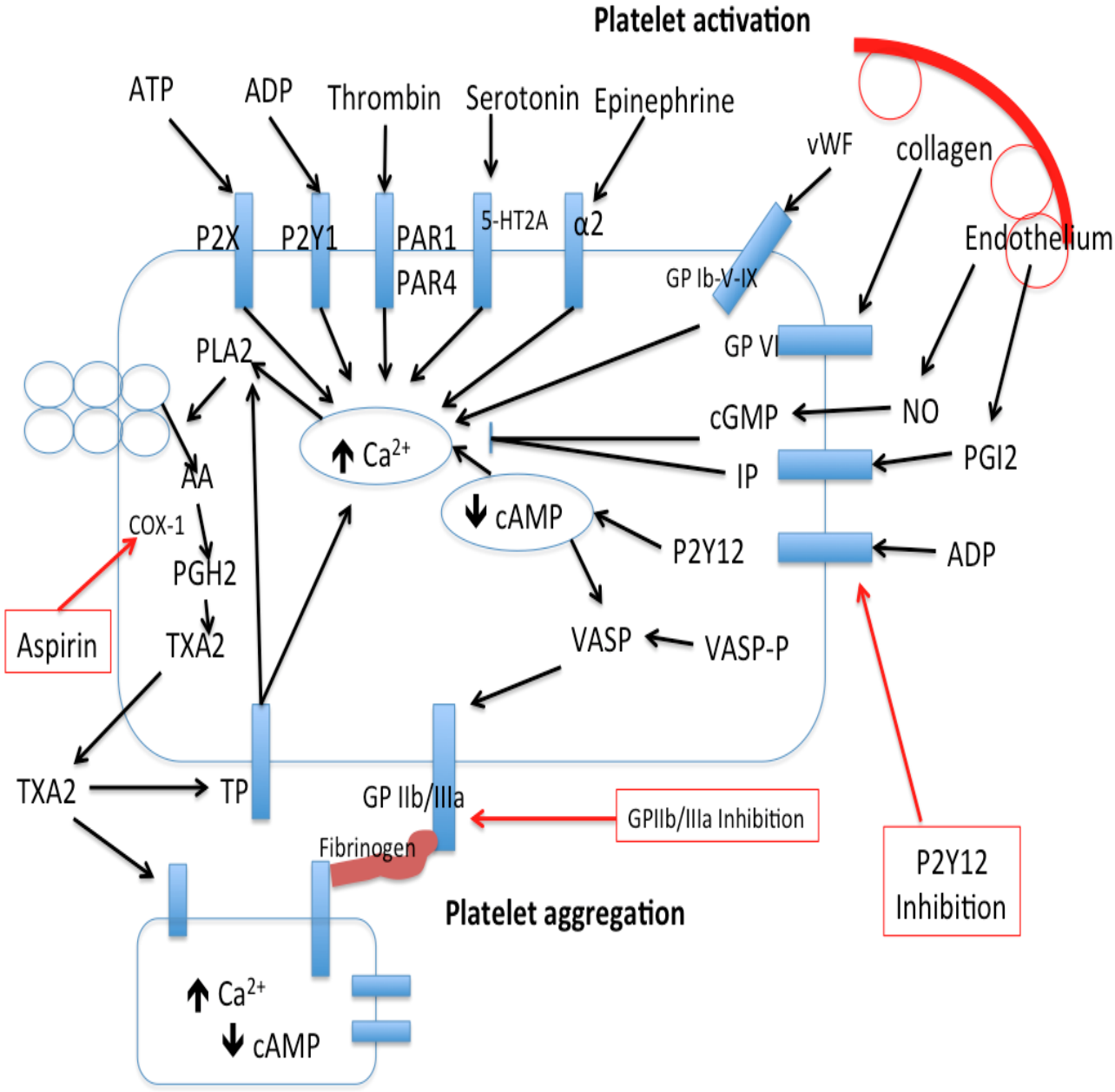
The binding of agonists such as ADP, thrombin and serotonin to their corresponding extracellular receptors on the platelet surface, stimulates an increase in intracellular calcium, which in turn decreases levels of cAMP, resulting in a decrease in the phosphorylation of vasodilator activated phosphoprotein (VASP). Dephosporylated VASP facilitates conformational

changes that lead to the activation of the integrin GP IIb/IIIa receptor (Papp, Kenyeres et al. 2013). The activated GP IIb/IIIa receptor binds fibrinogen which allows for bridging and cross-linking between activated platelet and allows for further thrombus formation as well as maintaining the stability of the thrombus formed (Davi and Patrono 2007, Jennings 2009). This is a haemostatic mechanism designed to limit the degree of vascular injury/damage and allow for vascular repair. However, within the confines of the affected coronary artery, this healing process leads to partial/complete coronary occlusion and subsequent myocardial cell death/necrosis, which manifests as a myocardial infarction.

The role of ADP as a potent platelet agonist is well established and is the target of pharamacological intervention. ADP is known to stimulate two G-protein coupled receptors; P2Y1 and P2Y12. While the P2Y12 receptor is the main target of drug therapy, co-activation of both P2Y1 and P2Y12 receptors is necessary for ADP-induced platelet aggregation to occur (Jennings 2009).

The complex molecular and signaling pathways involved in the process of platelet adhesion, activation and subsequent aggregation are described in figure 3 (Papp, Kenyeres et al. 2013) .

Figure 3. A pictorial description of the complex molecular and signaling pathways involved in platelet adhesion, activation and aggregation. (Adapted from Papp, Kenyeres et al 2013).



1.6 Role of Antiplatelet Therapy

In view of the underlying pathophysiology of ACS and significant contribution of platelets, antiplatelet agents are an integral component of the pharmacological management of patients who present following an ACS.

Antithrombotic therapies and in particular antiplatelet therapies play a pivotal role in preventing further ischaemic complications and major adverse cardiovascular and cerebrovascular events (MACCE) secondary to atherothrombosis as well as reducing the likelihood of stent thrombosis following PCI (Angiolillo, Guzman et al. 2008). Stent thrombosis, although rare with an incidence of 1-2%, is associated with a mortality of 40% and is considered to be a potentially fatal complication of the procedure which can be mitigated against through the appropriate use of antithrombotic therapies (Curzen 2012).

1.6.1 Aspirin

Aspirin is the most commonly prescribed antiplatelet agent in clinical practice, and when prescribed at low doses (75-150mg daily) irreversibly inhibits cyclo-oxygenase-1, which subsequently inhibits thromboxane A2 (TXA2), which is a potent platelet activator (Angiolillo, Guzman et al. 2008). The mechanism of action of aspirin is outlined in figures 3 and 5; when administered at low doses (75mg – 150mg once daily) it acts as an indirect inhibitor of the cyclo-oxygenase 1 (COX-1) enzyme, which in turn inhibits the production of thromboxane A2 (TXA2) a potent platelet agonist stimulating platelet activation (Angiolillo, Guzman et al. 2008).

The clinical benefit and efficacy of aspirin as an antiplatelet became apparent following the publication of the landmark ISIS-2 trial, in which aspirin monotherapy was associated with a 23% reduction in mortality in the context of AMI (ISIS-2CollaborativeGroup 1988), this positive finding was further

supported by the results of a meta-analysis undertaken by the Antithrombotics Trialists Collaboration, which demonstrated that aspirin was associated with a 25% reduction in serious adverse events without any increase in bleeding events (Antithrombotic Trialists 2002).

In view of the significant benefits in terms of reduction in cardiovascular and cerebrovascular events, aspirin is now recommended for long-term use in patients who present with atherosclerotic vascular disease and, as such, the place of aspirin in the context of secondary prevention is well defined. This recommendation is further supported by the National Institute for Health and Care Excellence (NICE) guidelines for secondary prevention following an MI in which it is stipulated that all patients should be offered aspirin following a heart attack and that it should be continued indefinitely (NICE CG 167 2013).

In terms of primary prevention, aspirin is no longer recommended in patients with a low-to-moderate cardiovascular risk; that is those with a calculated lifetime risk of <20% over 10 years (Antithrombotic Trialists' (ATT) Collaboration 2009). In this patient group, aspirin results in only a 1.5% reduction in the incidence of vascular events but is associated with a two-to-three fold increase in bleeding risk, such that the marginal cardiovascular benefit seen is far outweighed by the increase in the risk of adverse effects, particularly that of gastrointestinal bleeds/irritation (Antithrombotic Trialists' (ATT) Collaboration 2009).

Whilst a robust body of evidence supports the use of aspirin in the context of ACS, prescribing aspirin alone will not be sufficient to prevent against further

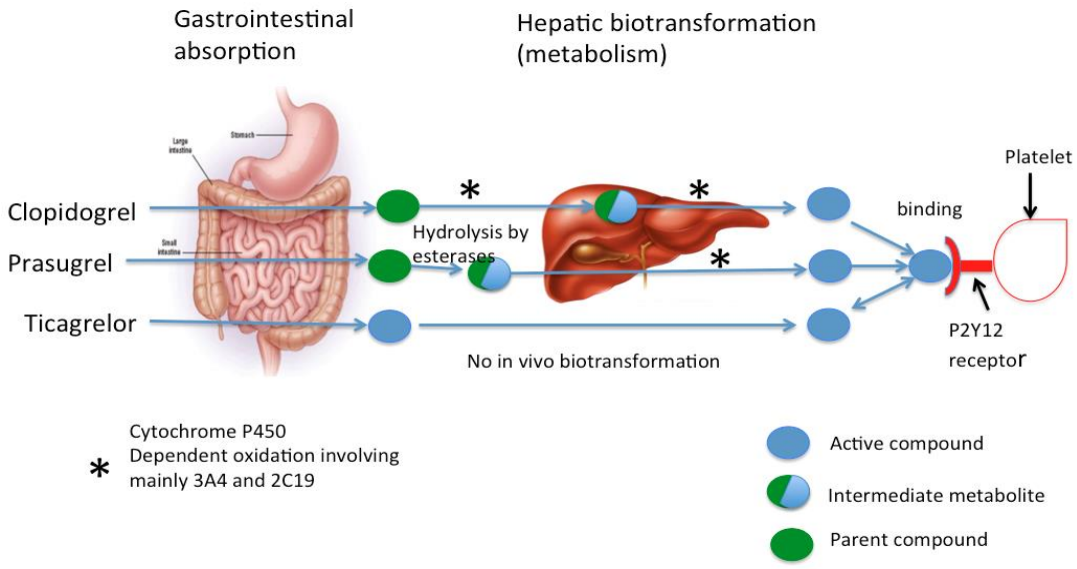
adverse cardiac events during the acute of phase of an ACS or following coronary artery stent implantation, since multiple pathways are involved in the process of platelet activation (figure 3) (Libby 2013).

The need to target these alternative platelet signaling pathways resulted in the development of the thienopyridines; a group of agents that inhibit the process of platelet activation and aggregation through blockade of the ADP/P2Y12 receptor found on the platelet surface (Gurbel and Tantry 2009). Preventing the binding of ADP to its receptor on the platelet surface stops activation of the glycoprotein IIb/IIIa complex and thereby inhibits platelet aggregation (Gurbel and Tantry, 2009).

1.6.2 Thienopyridines

The thienopyridines are a class of antiplatelet agents, which exert their therapeutic effect through blockade/inhibition of the platelet P2Y12 receptor, as shown in figures 3 and 4.

Figure 4. Absorption, biotransformation and mechanism of action of clopidogrel, prasugrel and ticagrelor. (Adapted from (Schomig 2009)



1.6.2.1 Clopidogrel

Clopidogrel, a second-generation thienopyridine, is a selective and irreversible inhibitor of the P2Y12 receptor and is indicated for the prevention of atherothrombotic events in patients with peripheral arterial disease, following an ischaemic stroke, a myocardial infarction or after elective PCI (Khan 2015). In the context of secondary prevention following an ACS or following coronary artery stent implantation, dual antiplatelet therapy (aspirin in addition to clopidogrel) has been the pharmacological standard of care since the early nineties. Numerous clinical trials have demonstrated a significant reduction in MACCE and in particular stent thrombosis in patients who receive a coronary artery stent following NSTEMI and STEMI (Mehta, Yusuf et al. 2001, Yusuf, Zhao et al. 2001, Sabatine, Cannon et al. 2005, Sabatine, Morrow et al. 2005).

1.6.2.1.1 Clopidogrel – Clinical Efficacy

CAPRIE, a randomised, blinded, international trial, was the first to demonstrate long-term benefits of clopidogrel administration in comparison to aspirin in terms of reducing combined risk of myocardial infarction, ischaemic stroke, or vascular death in patients with atherosclerotic vascular disease (Committee 1996). Whilst the outcomes of CAPRIE were able to support long-term safety and efficacy of clopidogrel, its benefits in terms of pre-treatment and maintenance therapy following a myocardial infarction became apparent following publication of CLARITY-TIMI 28. CLARITY-TIMI 28 was able to demonstrate that pre-treatment with clopidogrel in addition to aspirin and thrombolytic therapy, in patients under the age of 75 years, was associated with a significant reduction in cardiovascular death, recurrent myocardial infarction and stroke, without any increase in bleeding complications (Sabatine, Morrow et al. 2005). PCI-CLARITY; a planned pre-specified sub-group analysis of CLARITY-TIMI 28, was the first study to provide clinical justification for pre-treatment with clopidogrel prior to PCI in STEMI patients. Pre-procedural administration of a clopidogrel 300mg loading dose was associated with a reduction in cardiovascular death and ischaemic events prior to and after PCI (Sabatine, Cannon et al. 2005).

Further benefits of clopidogrel pre-treatment in addition to long-term maintenance therapy were proven in the PCI-CURE study (a sub-group analysis of the CURE trial) and subsequent CREDO study.

In PCI-CURE, the primary study end-point, a composite of CV death, MI or urgent target vessel revascularisation at 30 days was significantly lower (p =

0.03) in the clopidogrel arm compared with placebo (Mehta, Yusuf et al. 2001). However, it should be noted that the patient cohort recruited to PCI-CURE consisted of NSTEMI patients and that they received treatment with clopidogrel for a median of 6 days prior to PCI (Mehta, Yusuf et al. 2001).

This strategy is not reflective of current UK based practice, since a loading dose is administered immediately prior to PCI and the recommended time from diagnosis to mechanical reperfusion for NSTEMIs is now 24 to 72 hours and for STEMIs 120 minutes (MINAP 2014). The benefits of pre-treatment and long-term therapy post-PCI were again demonstrated in the CREDO trial, in which dual antiplatelet therapy (clopidogrel plus aspirin) for a 12-month duration of treatment was associated with a 26.9% relative reduction in a composite of death, MI and stroke (Steinhubl, Berger et al. 2002). With regards to pre-treatment, a loading dose of 300mg was utilised during the study, and the investigators were able to provide some insights into the optimal timing of administration. A pre-specified sub-group analysis of patients who received clopidogrel 6 hours before PCI was able to demonstrate a RRR of 38.6% compared with no reduction in primary end-point when administered less than 6 hours before PCI.

In view of such robust and unequivacol clinical trial data, the place in therapy of clopidogrel in combination with aspirin is firmly established and spans the entire spectrum of ACS, inclusive of patients who are medically managed as well as though who undergo revascularisation with PCI. As such, DAPT has become the cornerstone of management for patients who present with ACS

(Mehta, Yusuf et al. 2001, Yusuf, Zhao et al. 2001, Steinhubl, Berger et al. 2002).

However, despite this overwhelming evidence of efficacy following the administration of clopidogrel in addition to aspirin, up to 20% of patients will experience recurrent ischaemic events (Curzen and Sambu 2011). This apparent treatment failure has been extensively investigated and attributed to not only physiological changes that occur during a STEMI, but also secondary to suboptimal characteristics of clopidogrel.

As described in figures 4 and 5, clopidogrel is an inactive prodrug which itself does not effect platelet inhibition (Hall and Mazer 2011). Following a two-step hepatic metabolic conversion process, dependent on the cP450 3A4 and 2C19 enzymes, it is converted to an active metabolite which exerts an antiplatelet effect through preventing binding of ADP to the P2Y12 receptor on the platelet surface (Gurbel and Tantry 2009).

However, owing to significant heterogeneity in the activity of the 2C19 allele in the general population, this process of biotransformation is subject to marked inter-patient variability in terms of the degree of platelet inhibition achieved (Hall and Mazer 2011). Up to 30% of patients will have defective 2C19 alleles (genetic polymorphisms) and will be unable to convert clopidogrel to its active form, such patients are at increased risk of further MACCE and atherothrombotic/ischaemic complications such as stent thrombosis (Contractor and Ruparelia 2012). Whilst genetic testing might provide insights

into those who may have a defective 2C19 allele prior to treatment with clopidogrel, the test requires considerable expertise and skill to carry out, is expensive and time consuming, all of which render its use impractical in a clinical setting (Curzen and Sambu 2011).

Clopidogrel is also relatively slow in terms of its speed of onset; even after administration of a 600mg loading dose in healthy volunteers, only approximately 40% inhibition of platelet aggregation (IPA) is seen after 4-6 hours (Tapp, Shantsila et al. 2010), which is a particular disadvantage in the context of STEMI managed by PPCI, since optimal levels of platelet inhibition are required at the time of the procedure.

ST-segment elevation myocardial infarction (STEMI) is a highly prothrombotic state such that there is an increase in intrinsic platelet reactivity even before the administration of clopidogrel contributes to the high platelet reactivity seen while on treatment, which is an occurrence known as, high residual platelet reactivity (HRPR) (Frelinger, Michelson et al. 2011). This intrinsic platelet reactivity in conjunction with the limitations faced with clopidogrel are responsible for the recurrent ischaemic complications seen particularly following PCI (Aradi, Vorobcsuk et al. 2010, Alexopoulos 2013).

A number of strategies have been investigated in an attempt to overcome the limitations experienced with clopidogrel and address the issue of HRPR.

As demonstrated by the outcomes of the GRAVITAS study, increasing the maintenance dose of clopidogrel to compensate for HRPR did not yield any increase in the degree of IPA (Price, Angiolillo et al. 2011). Furthermore, the findings of ISAR-CHOICE indicate that increasing the loading dose of clopidogrel beyond 600mg did not offer any additional IPA due to the limited absorption of clopidogrel from the gut (von Beckerath, Taubert et al. 2005).

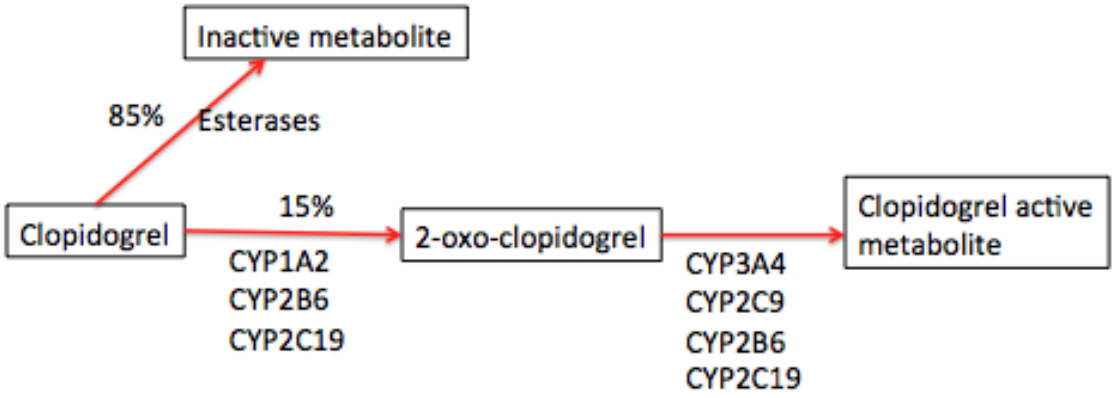
The outcomes of CURRENT-OASIS 7 demonstrated that alternative treatment regimes (600mg loading followed by 150mg daily for 7 days then 75mg daily therafter) may result in a significant reduction in the incidence of stent thrombosis, but at the expense of increased bleeding (Mehta, Tanguay et al. 2010). Whilst increasing the loading and maintenance doses proved to be beneficial in terms of achieving slightly faster and moderately greater degrees of IPA, these advantages were not apparent in those patients in whom clopidogrel could not be converted to its active form i.e. the clopidogrel non-responders.

1.6.2.1.2 Clopidogrel – Mechanism of Action

Clopidogrel selectively and irreversibly binds to the P2Y12 receptor and prevents the binding of the agonist adenosine diphosphate (ADP) and thereby inhibits further platelet aggregation (Park, Franchi et al. 2015). Clopidogrel is an inactive prodrug, of which, following oral ingestion and subsequent gastrointestinal absorption, approximately 85% of the parent compound undergoes enzymatic degradation by esterases into an inactive carboxylic acid derivative (Heestermans, van Werkum et al. 2008, Frelinger, Bhatt et al.

2013). As a result only 15% of the orally ingested parent compound is available to undergo the two-step metabolic biotransformation process (dependent upon the cytochrome P450 3A4 and 2C19 isoenzymes) to generate the active thiol metabolite that is capable of binding to the ADP P2Y12 receptor to exert its therapeutic effect leading to inhibition of platelet aggregation (figure 5).

Figure 5 Pathway leading to the formation of clopidogrel active metabolite



In terms of absorption, in vitro experiments undertaken using colonic adenocarcinoma (Caco-2) cell lines have demonstrated that the absorption of clopidogrel via intestinal epithelial cells is dependent upon the P-glycoprotein (P-gp) efflux transporter (Floyd, Passacquale et al. 2012). Variability in the expression of P-gp within the small intestine can affect intestinal permeability and subsequent oral bioavailability of clopidogrel. This in combination with genetic polymorphisms of the CYP isoenzymes can have a profound effect on the ability of an individual to convert the inactive parent compound into the active metabolite.

In summary, the non-uniform and inconsistent levels of platelet inhibition observed following the administration of clopidogrel are attributable to several sub-optimal characteristics; inter-individual variability in response following oral administration, secondary to impaired gastric absorption and hepatic biotransformation as well as genetic polymorphisms of the 2C19 allele are known to limit the clinical efficacy of clopidogrel in practice. To address these limitations, alternative therapeutic agents were investigated.

1.6.2.2 Prasugrel

Prasugrel, a third generation thienopyridine, is a selective and irreversible inhibitor of the P2Y12 receptor and its mechanism of action is outlined in figures 4 and 6. Like clopidogrel it is also a prodrug that requires metabolic conversion to its active form, however, this process relies on a single step only, making it less prone to genetic polymorphism and inter-individual variability in response. Consequently, prasugrel is able to achieve far more rapid (80% IPA achieved within 30 minutes), greater and more consistent levels of IPA compared to clopidogrel (Payne, Li et al. 2007, Wiviott, Braunwald et al. 2007) (Brandt, Payne et al. 2007).

1.6.2.2.1 Prasugrel – Clinical Efficacy

Following the positive outcomes of the TRITON-TIMI 38 study, in which prasugrel demonstrated superiority over clopidogrel in terms of reducing MACCE and ischaemic complications such as ST, the National Institute for Health and Care Excellence (NICE) and the European Society of Cardiology (ESC) recommend the use of prasugrel in patients who present following a STEMI and are suitable for PPCI. In addition, based on the outcomes of

TRITON-TIMI 38, NICE also recommend the use prasugrel in diabetic NSTEMI patients who receive a coronary artery stent and in those who present with stent thrombosis whilst receiving treatment with clopidogrel (Wiviott, Braunwald et al. 2007, NICE TAG 317 2014).

While prasugrel demonstrates favourable outcomes in STEMI patients undergoing PPCI and those who are diabetic NSTEMIs treated with PCI, a post-hoc sub group analysis of TRITON-TIMI 38 identified three patient groups in whom the administration of prasugrel resulted in little net clinical benefit and increased bleeding risk; those aged over 75 years and under 60 kg and in those with a history of previous stroke or TIA. In this latter group, prasugrel administration resulted in harm and is therefore contraindicated (Wiviott, Braunwald et al. 2007).

In order to determine the safety and efficacy of prasugrel in patients who are medically managed, the TRILOGY-ACS study was conducted. The findings of TRILOGY-ACS demonstrate that the administration of prasugrel to patients presenting with unstable angina (UA)/NSTEMI who were to be managed without revascularization, does not lead to improved clinical outcomes when compared with clopidogrel and was associated with a similar bleeding risk (Roe, Armstrong et al. 2012). The findings of TRILOGY-ACS in combination with TRITON-TIMI 38 very much inform the place in therapy of prasugrel and restrict its use to only in those who undergo mechanical reperfusion with PCI.

In line with standard practice, some cardiac centres pretreat UA/NSTEMI patients with prasugrel prior to PCI. However, findings from the recently

published ACCOAST-PCI study indicate that pretreatment with prasugrel does not reduce the rate of major ischaemic events but does increase the incidence of major bleeding complications (Montalescot, Collet et al. 2014). As a consequence, the manufacturer's advice and recommendation from NICE regarding the management of NSTEMI patients who are scheduled to undergo PCI has been updated to reflect these findings and indicate that the loading dose for UA/NSTEMI patients should be given at the time of PCI and not beforehand (NICE TAG 317 2014, eMC 2016a).

Whilst the place in therapy of clopidogrel became firmly established following the publication of a number of clinical trials demonstrating efficacy across the spectrum of ACS, limitations associated with its use, as described earlier led to the development of alternative antiplatelet agents. Prasugrel, a third generation thienopyridine is a suitable alternative to clopidogrel in certain clinical settings providing rapid, greater and more consistent levels of platelet inhibition (Wiviott, Braunwald et al. 2007).

The current dosing strategy for prasugrel was determined following completion of the JUMBO-TIMI 26 study; a randomised phase II dose finding study in which various loading and maintenance doses of prasugrel were compared with clopidogrel in order to determine the most effective dose and assess the safety of prasugrel in patients undergoing PCI (Wiviott, Antman et al. 2005). This was not an outcome driven study, but rather one in which the incidence of TIMI minor or major bleeding complications and adverse cardiac events were assessed after 30 days of treatment. With regards to safety, there were no differences in terms of bleeding complications between

prasugrel and clopidogrel groups, in addition, a numerically lower incidence of complications such as myocardial infarction, clinical target vessel thrombosis and recurrent ischaemia was observed in prasugrel treated patients (Wiviott, Antman et al. 2005). The findings of JUMBO-TIMI 26 formed the foundation of the pivotal phase III TRITON-TIMI 38 clinical trial in which the safety and efficacy of prasugrel was assessed.

The subsequent adoption and uptake into clinical practice of prasugrel was driven by the outcomes of the landmark TRITON-TIMI 38 in which prasugrel was found to be superior to clopidogrel in patients undergoing PCI leading to statistically significant reductions in myocardial infarction, urgent target vessel revascularisation and stent thrombosis (Wiviott, Braunwald et al. 2007). The primary efficacy end-point of non-fatal MI was found to be statistically significantly lower in the prasugrel group compared with the clopidogrel group; 7.3% vs 9.5% ($p < 0.001$), however, there was no significant difference in the rates of death (2.4% vs 2.1% $p = 0.31$) or stroke (1% vs 1% $p = 0.93$). The incidence of stent thrombosis was found to be statistically significantly lower in the prasugrel group compared with the clopidogrel group (1.1% vs 2.4%, $p < 0.0001$). Whilst the reduction in non-fatal MI and stent thrombosis were beneficial clinical outcomes, their occurrence was accompanied by an increase in serious and non-fatal bleeding episodes particularly in prasugrel treated patients (Wiviott, Braunwald et al. 2007). Whilst TRITON-TIMI 38 provides insights into the superiority of prasugrel, it should be noted that the comparative loading dose of clopidogrel was 300mg administered after angiography.

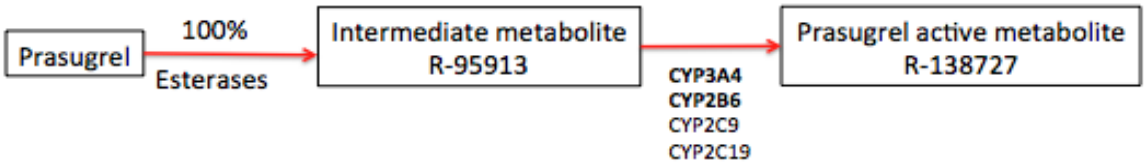
The PRINCIPLE-TIMI 44 study, undertaken in healthy subjects or patients with stable CAD (SCAD) was designed to assess the degree of platelet inhibition of prasugrel 60mg against clopidogrel 600mg in patients undergoing PCI. The findings showed superiority of prasugrel in terms of platelet inhibition when compared with even a 600mg loading and 150mg daily maintenance dose of clopidogrel, indicating that, increasing the maintenance and loading doses of clopidogrel is still not able to overcome inherent limitations such as inter-individual variability in response, genetic polymorphisms of CYP2C19 or the slow and variable onset of action (Wiviott, Trenk et al. 2007).

Whilst the outcomes of TRITON-TIMI 38 were compelling enough to lead to a change in national and international guideline recommendations regarding its place in therapy, a major limitation to it use lies in the fact that its superiority is restricted to a niche group of patients; STEMI or diabetic NSTEMIs undergoing PCI or those who present with stent thrombosis (Steg, James et al. 2012). As such, prasugrel is a drug that is mainly restricted for use in the context of PCI only and not in those who are to be medically managed.

1.6.2.2.2 Prasugrel – Mechanism of Action

Prasugel, a third generation thienopyridine prodrug, which following conversion to its metabolically active form results in irreversible inhibition of the platelet P2Y12 receptor and subsequent inhibition of platelet aggregation.

Figure 6. Pathway leading to the formation of prasugrel active metabolite:



Although prasugrel is a prodrug like clopidogrel, the pathways leading to the generation of the active metabolite differ between the two drugs. As described in figure 6, hydrolysis by intestinal esterases result in a large proportion of orally administered clopidogrel (85%) being converted into an inactive metabolite (Floyd, Passacquale et al. 2012). In contrast, however, intestinal esterases are essential for the conversion of all orally administered prasugrel to its active metabolite, as described in figure 20 above.

Following oral administration, prasugrel undergoes rapid and complete gastrointestinal absorption, hydrolysis via intestinal esterases leads to the generation of an inactive intermediate compound (R-95913), which then undergoes rapid metabolic conversion to the active metabolite, R-138727 (Wallentin 2009, Floyd, Passacquale et al. 2012). This is a process that occurs predominantly in the intestine as opposed to the liver.

The key cytochrome P450 isoenzymes responsible for this metabolic biotransformation are CYP3A4, CYP2B6 and CYP2C19 (Floyd, Passacquale et al. 2012). This is in contrast to clopidogrel, which relies predominantly on CYP2C19 in both the intestine and liver to facilitate conversion of the inactive parent compound into its active metabolite. As discussed, in chapter 5,

genetic polymorphisms within the 2C19 allele, render it inactive in up to 30% of patients leading to significant variability in response (Contractor and Ruparelia 2012).

Since the pathways involved in the generation of the prasugrel active metabolite (P-AM) are not solely reliant on the CYP2C19 allele, its pharmacokinetic and pharmacodynamic profile is more efficient, streamlined and predictable when compared with clopidogrel. The one step conversion process following oral administration results in greater and more consistent levels of P-AM generation and levels of platelet inhibition; prasugrel is also much faster in terms of its onset of action resulting in approximately 80% inhibition of platelet aggregation (IPA) within 2 hours of administration in healthy patients (Brandt, Payne et al. 2007, Payne, Li et al. 2007).

1.6.2.3 Ticagrelor

Ticagrelor, as described in chapter 1, is a novel first in class antiplatelet agent belonging to the cyclopentyltriazolopyrimidine (CPTP) family.
It has a number of distinguishing pharmacokinetic and pharmacodynamic characteristics when compared to the thienopyridines; it is a directly acting, reversible inhibitor of the P2Y12 receptor (Floyd, Passacquale et al. 2012).
As well as allowing for greater and more consistent levels of platelet inhibition, like prasugrel, ticagrelor is subject to rapid and complete gastrointestinal absorption following oral administration (Wallentin 2009, Wallentin, Becker et al. 2009).

Since ticagrelor does not require biotransformation to its active form and as such it is rapid in terms of its speed of onset and is able to achieve far greater and more consistent level of IPA compared to clopidogrel (Wallentin, Becker et al. 2009).

1.6.2.3.1 Ticagrelor – Clinical Efficacy

The findings of the PLATO study in which ticagrelor was compared to clopidogrel demonstrated improved clinical outcomes (reduction in MACE and stent thrombosis) across the spectrum of ACS patients; those who were medically managed and those undergoing PCI following an NSTEMI/STEMI. In addition, ticagrelor is the first antiplatelet since aspirin that has shown a mortality benefit in AMI/STEMI patients as demonstrated by a 22% relative risk reduction in all cause mortality (Wallentin, Becker et al. 2009).

During PLATO, a number of “off-target” side effects of ticagrelor were noted and thought to be attributable to ticagrelor-induced blockage of adenosine re-uptake into red blood cells (eMC 2016b). Dyspnoea, the first of these side effects, was found to be a troublesome, yet transient effect experienced on treatment initiation, in approximately 13.8% of patients (eMC, 2014b). As a consequence, ticagrelor should be prescribed with caution in patients with a history of asthma or chronic obstructive pulmonary disorder (COPD) (eMC, 2014b). The administration of ticagrelor is also associated with an increase in the incidence of asymptomatic ventricular pauses; however, these are self-limiting and do not require any intervention. Following initiation, ticagrelor can also cause an increase in creatinine and uric acid levels, however, the former is not associated with a decline in renal function (eMC, 2014b). Although, not

a strict contraindication, the use of ticagrelor in patients with a with a history of cerebrovascular accident (CVA)/ stroke, should be undertaken with caution, since its administration is associated with an increase in non- CABG-related and, in particular, intracranial bleeding (Wallentin, Becker et al. 2009, eMC 2016b).

The superiority of ticagrelor over clopidogrel has been verified by the outcomes of a number of phase II studies, the first of which, a dose finding study (DISPERSE) was able to demonstrate that ticagrelor administration is associated with less variable and more rapid inhibition of platelet aggregation compared with clopidogrel (Husted, Emanuelsson et al. 2006). As a follow on from this initial dose finding study, a subsequent study, DISPERSE II, was undertaken to establish the safety and efficacy of ticagrelor when compared with clopidogrel in patients with NSTEMI. In terms of efficacy, ticagrelor was able to provide levels of platelet inhibition that were far greater than those achieved with clopidogrel. From a safety and tolerability perspective, there was no statistically significant difference in the incidence of minor or major bleeding events between the two groups. Although, patients assigned to treatment with ticagrelor experienced a greater incidence of dyspnoea and asymptomatic ventricular pauses; neither of which was significant enough to warrant the discontinuation of treatment (Cannon, Husted et al. 2007).
The findings of DISPERSE and DISPERSE II acted as a platform for the pivotal phase III study, PLATO, which was a randomised double blind parallel group study in which the safety and efficacy of ticagrelor was compared with clopidogrel in ACS patients. As described in chapter 1, ticagrelor was found

to be superior to clopidogrel in reducing the incidence of the primary efficacy end point, which was a composite of myocardial infarction, stroke or death from a vascular cause (Wallentin, Becker et al. 2009). The benefits are largely driven by the improved pharmacokinetic and pharmacodynamic profile of ticagrelor compared to clopidogrel.

1.6.2.3.2 Ticagrelor - Mechanism of Action

The mechanism of action by which ticagrelor is able to exert its antiplatelet effect is not dependent on the cytochrome P450 pathway, as such the parent compound does not require metabolic bioactivation in order to exert its antiplatelet effect (figure 7). A major advantage of this being that ticagrelor is not subject to genetic polymorphisms, as observed with clopidogrel. Despite this advantage, ticagrelor-parent compound (T-PC) is subject to enzymatic degradation, which is driven predominantly by the cytochrome P450 3A4 isoenzyme, leading to the generation of T-AM, which is present at a third of the concentration of T-PC and is comparable in terms of its potency at the P2Y12 receptor (Husted, Emanuelsson et al. 2006, Wallentin 2009, Teng, Oliver et al. 2010).

Figure 7. Pathway leading to the onset of action of ticagrelor and formation of its active metabolite.

In terms of onset of action, the administration of a 180mg loading dose of ticagrelor is able to provide 40% IPA within 30 minutes of administration compared to 5% IPA following the administration of clopidogrel 600mg loading dose; these values increased to 80% and 20% respectively after one hour (Gurbel, Bliden et al. 2009). These data support previous findings which state that ticagrelor allows for greater, more rapid, consistent and predictable levels of IPA when compared to clopidogrel.

The non-competitive binding of ticagrelor to the P2Y12 receptor also allows for its reversibility of action; following discontinuation of the drug, ticagrelor displays faster offset and recovery of platelet function when compared with clopidogrel. Within 24 hours of cessation, a 50% recovery of platelet function is observed, and it is this reversibility and rapid offset of effect that warrants twice daily dosing of ticagrelor (Gurbel, Bliden et al. 2009).

1.6.3 Glycoprotein IIb/IIIa Inhibitors (GPIs)

As shown in figures 3 and 5, the final common pathway of platelet aggregation involves activation of the glycoprotein IIb/IIIa receptor, which is located on the platelet surface. Binding of fibrinogen to the GPIIb/IIIa receptor, allows for cross-linking between platelets, which subsequently leads to platelet aggregation and thrombus formation (Saucedo 2010).

The glycoprotein IIb/IIIa inhibitors (GPIs) are administered as an adjunctive intravenous antiplatelet agent, since they allow for greater levels of platelet inhibition to be achieved prior to and at the time of PCI. Failure to achieve optimal levels of IPA has been shown to be associated with procedural MI and

catheter related thrombosis albeit at an increased risk of bleeding (Quinn, Plow et al. 2002, Van de Werf, Bax et al. 2008, Alexander and Peterson 2010).

In recent years, particularly following the introduction into clinical practice of the more potent P2Y12 inhibitors, the use of GPIs has diminished since both prasugrel and ticagrelor allow for greater and more consistent levels of platelet inhibition. This reduction in usage has also been influenced by studies that question the additive value of these agents to DAPT, in view of the extra bleeding complications that may arise secondary to such potent antiplatelet combinations (De Luca, Suryapranata et al. 2005, Mehilli, Kastrati et al. 2009). Although a rare phenomenon, thrombocytopaenia has also been reported following the administration of GPIs, and is thought to be mediated by the formation of antibodies that are stimulated by conformational changes in the GP IIb/IIIa receptor induced by the medication. Although an immune-mediated response, the development of antibodies does not diminish the efficacy of subsequently administered doses (Stangl and Lewis 2010, Kristensen, Wurtz et al. 2012).

1.7 Role of antithrombotic therapy

The administration of anticoagulant therapy during PCI performs two functions; firstly it prevents further thrombotic complications secondary to plaque rupture following angioplasty and/or stenting e.g periprocedural MI, and secondly it prevents periprocedural thrombus formation on intravascular catheters and PCI equipment e.g. catheter related thrombosis (Rao and Ohman 2010). Therefore, anticoagulants are important adjuncts in the

management of patients who undergo emergent or elective PCI. Since the procedure itself can also cause endovascular damage, which as described above, increases the stimulation of the procoagulant tissue factor, leading to the activation of the coagulation cascade and activated factor Xa. Factor Xa leads to thrombin generation, the conversion of fibrinogen to fibrin and subsequent thrombus formation. This process occurs simultaneously to that of platelet adhesion, activation and aggregation as described in figure 5.

In view of the significant contribution of thrombin to thrombus formation, as a result of its pro-inflammatory effects and ability to stimulate platelet activation and enhance aggregation, it is also an important target for antithrombotic therapies, particularly for patients who present following an ACS.

1.7.1 Unfractionated heparin (UFH)

Anticoagulation with heparin has been the mainstay of treatment for decades in PCI. UFH has a very short, dose-dependent half-life such that heparin is administered as a continuous infusion and the dose administered is dictated by the activated partial thromboplastin time (APTT) or activated clotting time (ACT). In view of the short half-life, discontinuation of the infusion is sufficient to allow for reversal of its anticoagulant effect.

While UFH is an effective thrombin inhibitor, monotherapy is not sufficient to protect against further ischaemic complications, since heparin administration itself can cause induce platelet activation and contribute to the formation of further platelet aggregates (Xiao and Theroux 1998). However, in the era of

modern PCI, the administration of potent oral antiplatelet agents and concomitant administration of GPIs mitigate against this complication.

1.7.2 Low Molecular Weight Heparin (LMWH)

The LMWHs carry less risk of bleeding than UFH and do not require any monitoring since they can be administered as simple and convenient once/twice daily sub-cutaneous injections depending on the indication. The safety and efficacy of enoxaparin compared with UFH has been investigated as part of a meta-analysis. The authors found that in the context of ACS, there was a marginally significant reduction in the composite end point of death at 30 days or MI in those treated with enoxaparin, but no statistically significant difference in the incidence of major bleeding events (Murphy, Gibson et al. 2007). A more recent systematic review and meta-analysis reported the superiority of enoxaparin over UFH in reducing bleeding complications and mortality following PPCI (Silvain, Beygui et al. 2012). Despite these encouraging outcomes, the uptake of LMWH into clinical practice in the context of peri-procedural administration during PCI remains low.

1.7.3 Fondaparinux

Fondaparinux, a synthetic pentasacchiride, is an irreversible inhibitor of factor Xa (figure 8) with a half-life of 17 to 21 hours (Rao and Ohman 2010). Fondaparinux has been investigated in the context on NSTEMI/UA in the OASIS-5 study, in which it was compared against enoxaparin. The study demonstrated that in the short term, fondaparinux was comparable to enoxaparin in the prevention of death, MI or recurrent ischeamia in those

presenting within 24 hours with symptoms of UA/NSTEMI. Fondaparinux also demonstrated a reduced risk of bleeding, which was associated with lower, long-term mortality (Fifth Organization to Assess Strategies in Acute Ischemic Syndromes, Yusuf et al. 2006).

However, it should be noted that during the study there was an increase in the incidence of catheter related thrombus during PCI (Rao and Ohman 2010). In addition, fondaparinux is known to accumulate in patients with renal impairment, such that its use is contra-indicated in patients with a CrCl < 20ml/min (eMC 2014).

While OASIS-5 supports the use of fondaparinux in NSTEMI/UA, the OASIS-6 trial, which investigated fondaparinux administration and outcomes in STEMI patients reported a significantly higher rate of reinfarction and death at 30 days in those assigned to the fondaparinux arm. For this reason the administration of fondaparinux during primary PCI is not recommended (Yusuf, Mehta et al. 2006).

1.7.4 Bivalirudin

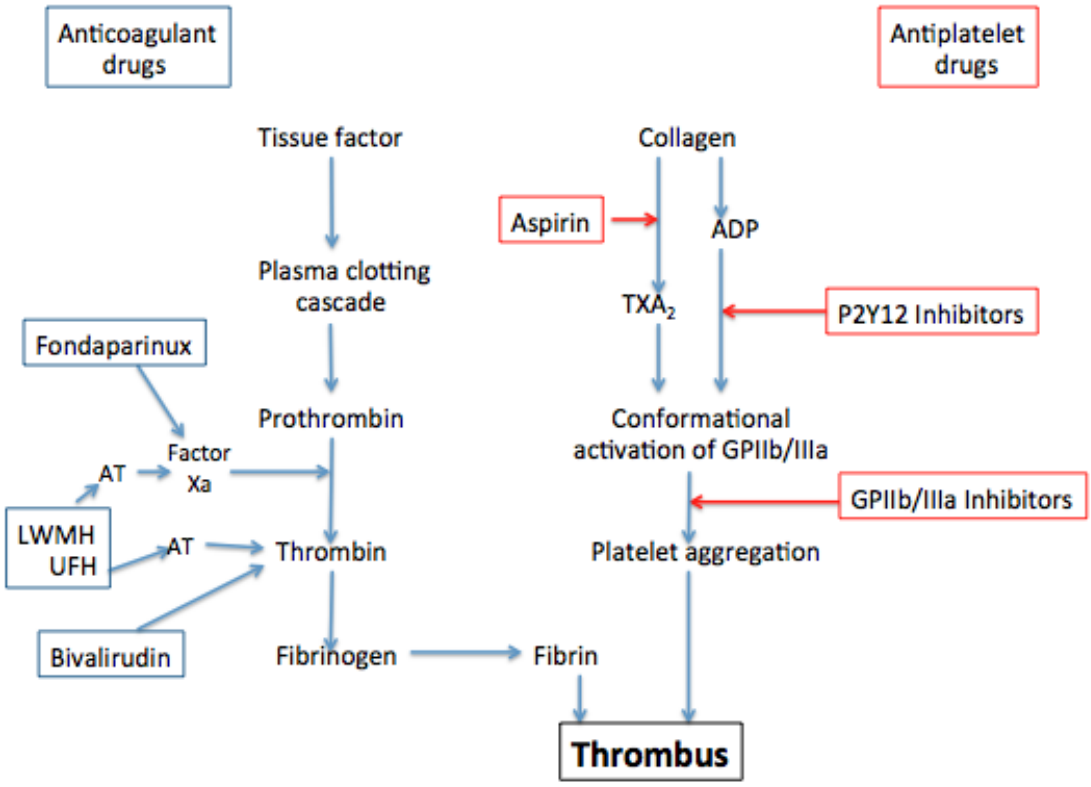
Bivalirudin, as described in figure 8, is a reversible direct thrombin inhibitor with additional antiplatelet activity, that is recommended for use in combination with aspirin and clopidogrel in patients who present following a STEMI and are to undergo primary PCI (NICE TAG 230 2011).
The main study on which the NICE recommendation is based, is HORIZONS-AMI, in which patients presenting with STEMI intended for PPCI were

assigned to bivalirudin or heparin plus a glycoprotein IIb/IIIa inhibitor. Both treatment arms received aspirin and clopidogrel (NICE TAG 230 2011).

The findings of HORIZONS-AMI demonstrated that bivalirudin alone, as compared with heparin plus glycoprotein IIb/IIIa inhibitors, led to a significant reduction in major bleeding and an overall reduction in net adverse clinical events at 30 days. There was however, an increase in the incidence of acute stent thrombosis (Stone, Witzenbichler et al. 2008). In clinical practice however, clopidogrel is rarely administered during PPCI and as such the use of more potent agents such as prasugrel and ticagrelor may well mitigate against the risk of acute stent thrombosis (Khan 2015).

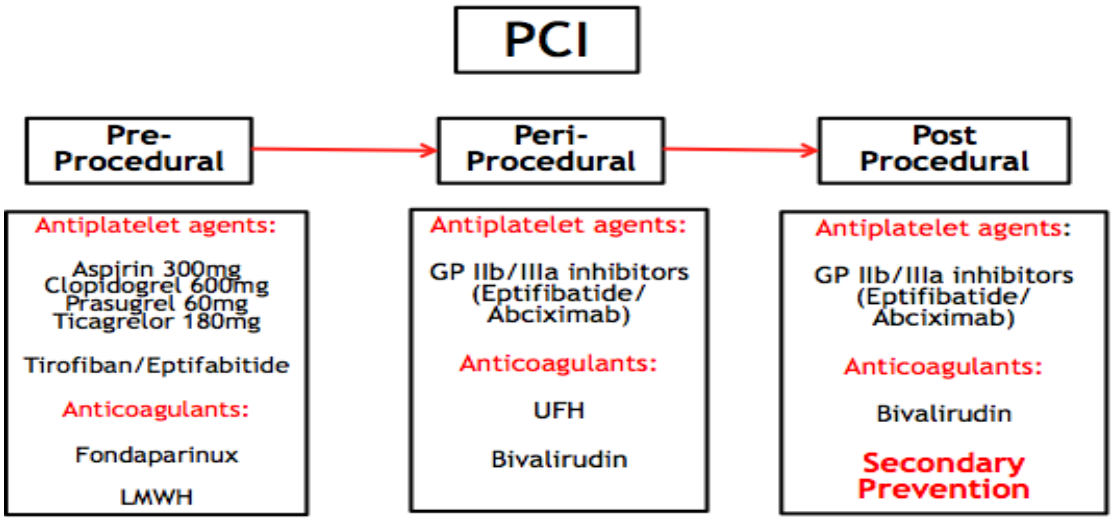
Following the publication of HEAT-PPCI, the clinical efficacy of bivalirudin in PPCI/STEMI patients has recently been called into question. Patients were recruited following an admission for PPCI and were assigned to either bivalirudin plus bail-out GP IIb/IIIa inhibitor (abxicimab) or heparin plus bail out GP IIb/IIIa inhibitor (abciximab). The investigators found that bivalirudin was inferior to UFH, and was associated with an increase in adverse cardiovascular events, due to increased incidence of myocardial infarction and stent thrombosis. In addition, the administration of bivalirudin did not lead to a reduction in major bleeding as claimed in HORIZONS-AMI (Shahzad, Kemp et al. 2014). As a consequence, the use of bivalirudin has very much fallen out of favour in clinical practice.

Figure 8. Antithrombotic drugs used in the treatment of ACS. This figure outlines the targets of antithrombotic drugs used to inhibit coagulation and platelet aggregation during and after thrombus formation.



Combinations of antithrombotic therapies are necessary to target all pathways that may lead to further ischaemic and thrombotic complications in patients who are treated with PCI following an ACS, figure 9 outlines the place in therapy of the many drugs available for use in this context.

Figure 9. The place in therapy of anticoagulant and antiplatelet agents utilised in the management of patients who undergo PCI following presentation.



The information in this chapter provides some background to the complexities of antithrombotic management of patients who present with cardiovascular disease; the remainder of my thesis will focus on the role of oral antiplatelet therapies and their clinical efficacy in the context of ACS.

Chapter 2 – Oral P2Y12 Inhibitors Administration and Outcomes in STEMI patients Undergoing PPCI - A Single Tertiary Centre Retrospective Observational Analysis

2.1 Introduction

The contemporary management of ST-elevation myocardial infarction (STEMI) patients has evolved considerably in the last decade with primary percutaneous coronary intervention (PPCI) being the default revascularisation strategy of choice in this setting (Keeley, Boura et al. 2003, Thomas and French 2014, De Luca, Danchin et al. 2015). In this time we have also seen an evolution in stent technologies with each generation of stents promising improved biocompatibility and reduced thrombogenicity, leading to a reduction in the incidence of stent thrombosis; a rare yet potentially fatal complication associated with coronary artery stent implantation (Curzen and Sambu 2011, Mehran, Giustino et al. 2015, Riegger, Byrne et al. 2015).

The success of mechanical reperfusion is also dependent on the administration of antithrombotic therapies, prior to, during and post stent implantation (as described in chapter 1, figure 9). To this end, early treatment with dual antiplatelet therapy consisting of aspirin plus an oral P2Y12 inhibitor forms the cornerstone of management for patients who not only present with STEMI but all forms of acute coronary syndromes (De Luca, Danchin et al. 2015). STEMI, the most malignant manifestation of this clinical syndrome requires the administration of oral P2Y12 inhibitors that are rapid in terms of their onset, as well as providing adequate and consistent levels of platelet inhibition at the time of angioplasty and stent deployment. Although, a robust clinical evidence base supports clopidogrel, its use in clinical practice has

been superseded by the introduction of newer more potent agents, prasugrel and ticagrelor. With the latter agents providing a superior pharmacological profile which translates into improved clinical outcomes such as reduction in cardiovascular death, recurrent MI and stroke (Wiviott, Braunwald et al. 2007, Wallentin, Becker et al. 2009) National and international guideline recommendations indicate the use of all three agents in the context of STEMI, however, the place in therapy of prasugrel and ticagrelor is assigned a higher class/level of recommendation (NICE TAG 236 2011, Steg, James et al. 2012, NICE CG 167 2013, NICE TAG 317 2014).

2.2 Rationale for Study

Cardiovascular medicine is a dynamic and rapidly evolving field in which the timescales from the publication of clinically sound and robust evidence to implementation into clinical practice are often short. Our tertiary referral centre has used all three agents in the last five years as part of the PPCI pathway. We aimed to determine the effect of clopidogrel, prasugrel and ticagrelor in a “real-world, all comers” STEMI population on the incidence of in-hospital and 30 day mortality in addition to their impact on the incidence of in-hospital major bleeding.

In addition, this data will form the basis from which our patient population in subsequent chapters in which the pharmacokinetic and pharmacodynamic activity of the oral P2Y12 inhibitors in the context of acute myocardial infarction will be investigated.

2.3 Methods

2.3.1 Ethical Conduct of the Study

Data were collated as part of a service evaluation and clinical audit; all patient identifiable information was anonymised prior to analysis. Since all analyses were undertaken on anonymised patient data, ethics approval and patient consent was not required as advised by our local ethics committee and Caldicott Guardian.

2.3.2 Study Population

The Heart and Lung Centre is the tertiary specialist cardiac centre for the Black Country and surrounding areas serving a population of approximately 1.2 million and carrying out approximately 500 PPCI cases per year. Data were collected for 2,200 patients who were treated at the The Royal Wolverhampton Hospitals NHS Trust (RWH) Heart and Lung Centre following activation of the primary percutaneous coronary intervention (PPCI) pathway (2010-2015). This time period was selected since it is reflective of the temporal changes in and uptake of guideline recommendations with regards to the use of oral P2Y12 inhibitors in the context of STEMI at our centre.

2.3.3 Study Design

This was a single centre retrospective observational analysis of specified outcomes following the administration of oral P2Y12 inhibitors prior to PPCI. Data relating to patient clinical characteristics/demographics, clinical presentation and procedural outcomes were collated from multiple data sources by a single individual (myself) to minimise bias and maintain the quality of data collected. Information sources utilised included, local coronary

artery disease databases (CARDAS), Office for National Statisitics (ONS) data, pharmacy systems and patient health records.

2.3.4 Outcome measures

The primary outcome measure for this study was to assess 30-day mortality (all cause and cardiovascular only) following the administration of an oral P2Y12 inhibitor (clopidogrel, prasugrel or ticagrelor) in patients presenting with ST-elevation myocardial infarction (STEMI) immediately prior to primary percutaneous coronary intervention (PPCI).

Secondary endpoints were, in-hospital mortality and in-hospital major bleeding. Major bleeding was assessed using the BARC score and was defined as a gastrointestinal, intracerebral or a bleed requiring a blood transfusion (Mehran, Rao et al. 2011).

Patients were categorised into three treatment groups based on the choice of oral P2Y12 inhibitor administered immediately prior to angioplasty/PPCI. These groups were: (1) clopidogrel group, (2) prasugrel group and (3) ticagrelor group. Background and peri-procedural antithrombotic therapy consisted of aspirin, unfractionated heparin +/- bivalirudin and/or a glycoprotein IIb/IIIa receptor antagonist (abciximab/eptifibatide/tirofiban).

Mortality data were retrieved from the UK Office for National Statistics (ONS) using the patient's NHS number to track status (alive/dead). This was further linked to the patient's unique hospital identifier to collate information relating

to pre-, peri-procedural and post-procedural drug administration and outcomes such as in-hospital complications e.g. bleeding. Local databases, CARDAS, pharmacy systems and electronic patients records were utilised to retrieve this data. All data were recorded in a case report form (CRF) which can be viewed in appendix 1.

2.4 Statistics and Data Analysis

2.4.1 Descriptive statistics

Continuous variables are described as a mean ± standard deviation (± SD). Categorical data are expressed as frequencies and percentages. Continuous variables were analysed using student's independent samples t-test or analysis of variance where appropriate. Categorical variables were assessed using chi-square (Pearson's chi square) test as appropriate.

The unadjusted effect of oral P2Y12 inhibitor administration on overall survival/mortality was determined through construction of Kaplan-Meier survival curves and compared using the log-rank test. In order to correct for known confounders, the effect of oral P2Y12 inhibitor administration on overall survival was also assessed using a Cox regression analysis, which was adjusted/standardised for age, gender, weight, diabetes, previous MI/PCI, call to balloon time and systolic blood pressure, heart rate and haemaglobin on admission. Other co-morbidities included in the standardised analysis were peripheral vascular disease, congestive heart failure, atrial fibrillation, chronic obstructive pulmonary disease, family history, hypercholesterolaemia and hypertension.

I undertook all statistical calculations and analyses using SPSS (SPSS Version 21; SPSS, Inc, Chicago, Il, USA). A p-value < 0.05 (2-sided) was considered statistically significant.

2.5 Results

The study population comprised 2,200 patients with a confirmed diagnosis of ST-elevation myocardial infarction who underwent PPCI at our centre.

2.5.1 Patient Demographics and Baseline Characteristics

Table 2. Baseline demographics and patient characteristics

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
Age (mean) years	70.51 ±13.36	59.36 ± 10.84	63.04 ± 12.72	< 0.0001
Sex Male	338 (68)	848 (80)	446 (75)	< 0.0001
Sex Female	182 (32)	210 (20)	146 (25)	< 0.0001
Ethnicity Caucasian	519 (91)	961 (91)	533 (90)	0.765
Ethnicity Asian	46 (8.1)	87 (8.2)	56 (9.5)	0.765
Ethnicity Black	5 (0.9)	10 (0.9)	3 (0.5)	0.765

(Continous variables are expressed as a mean ± SD. Categorical data are expressed as frequencies and percentages)

Table 3. Patient risk factors and co-morbidities

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
Previous MI	81 (14)	107 (10)	67 (11)	0.056
Previous CABG	14 (2.5)	17 (1.6)	8 (1.4)	0.314
Previous PCI	61 (10.7)	90 (8.5)	51 (8.6)	0.256
Diabetes Mellitus	88 (15)	129 (12.2)	73 (12.3)	0.298
Diabetes Insulin	22 (3.9)	41 (3.9)	29 (4.9)	0.298
BMI	26.62 ± 4.84	28.20 ± 5.51	27.92 ± 5.30	< 0.0001
PVD	38 (6.7)	48 (4.5)	20 (3.4)	0.028
CHD	161 (28)	190 (18)	141 (24)	< 0.0001
Previous Stroke/TIA/CVA	92 (16)	20 (1.8)	22 (3.7)	< 0.0001
CHF	15 (2.6)	8 (0.8)	9 (1.5)	0.010
Atrial Fibrillation	64 (11.2)	37 (3.5)	24 (4.0)	< 0.0001
COPD/Asthma	60 (10.5)	98 (9.3)	77 (13)	0.060
VHD	41 (7.2)	21 (2)	15 (2.5)	< 0.0001
Family History CAD	182 (32)	398 (37.6)	190 (32)	< 0.0001
Current Smoker	166 (29)	487 (46)	228 (38.5)	< 0.0001
Ex Smoker	158 (28)	221 (21)	121 (20)	< 0.0001
Hypercholesterolaemia	378 (66)	795 (75)	425 (72)	0.001
Hypertension	312 (55)	430 (41)	277 (47)	< 0.0001

Table 4. Clinical parameters on admission.

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
Total Cholesterol	4.80 ±1.27	5.21 ± 1.23	5.13 ±1.33	< 0.0001
SBP	121.64 ±23.48	123.51± 23.38	125.3 ±23.74	0.034
HR	77.28 ±15.55	77.44 ±15.63	78.74 ± 18.40	0.229
Hb	134.5 ±19.25	142 ±16.61	139.4 ±20.13	< 0.0001
Glucose	8.39 ± 3.66	8.34 ± 3.30	8.98 ± 4.43	0.005
Creatinine	102.1 ± 64.3	89.41 ±48.76	89.13 ±33.02	< 0.0001

Table 5. Procedural complications.

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
CVA Embolic	3 (0.5)	4 (0.4)	1 (0.2)	0.591
CVA Bleed	1 (0.2)	0 (0)	0 (0)	0.235
Blood Transfusion	30 (5.3)	19 (1.8)	26 (4.4)	< 0.0001
GI Bleed	8 (1.4)	4 (0.4)	7 (1.2)	0.061

Table 6. In-patient length of stay.

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
Days	5.34 ± 7.15	3.62 ± 5.46	4.50 ± 6.93	<0.0001

The average length of stay (LOS) following a STEMI in our centre is 3 days, our results indicate a significant difference in LOS between the three groups with clopidogrel treated patients having the highest and prasugrel the lowest LOS (5.34 ±7.15 vs 3.62 ± 5.46, p = 0.000).

Table 7. Discharge medications.

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
Aspirin	551 (97)	1034 (90)	576 (97)	0.443
Clopidogrel	454	95	41	< 0.0001
Prasugrel	77	927	0	< 0.0001
Ticagrelor	23	15	535	< 0.0001
Beta blocker/RLCCB	452 (79)	920 (87)	535 (90)	< 0.0001
ACEI/ARB	446 (78)	950 (90)	522 (88)	< 0.0001
Statin	524 (92)	1014 (96)	570 (96)	0.001
Aldosterone antagonist	25 (4.4)	59 (5.6)	48 (8.1)	0.021

The data in table 7 indicates that all patients following PPCI were discharged on the recommended combination of secondary prevention medications.

2.5.2 In-hospital Bleeding

Table 8. Bleeding complications.

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
CVA Embolic	3 (0.5)	4 (0.4)	1 (0.2)	0.591
CVA Bleed	1 (0.2)	0 (0)	0 (0)	0.235
Blood Transfusion	30 (5.3)	19 (1.8)	26 (4.4)	< 0.0001
GI Bleed	8 (1.4)	4 (0.4)	7 (1.2)	0.061

Our data indicates that across the three treatment groups there is no significant difference in bleeding complications relating to CVA events and GI bleeds as demonstrated in table 8. However, there is a highly statistically significant difference (p = 0.000) in the number of patients who required a blood transfusion in the clopidogrel treatment arm. This again is most likely attributable to the age of patients who were assigned to treatment with clopidogrel (as described in table 2).

Table 9. Mortality outcomes (unadjusted).

	Clopidogrel (n = 570)	Prasugrel (n = 1058)	Ticagrelor (n = 592)	p-value
All cause in-hospital mortality	52 (9.1)	42 (4.0)	30 (5.1)	< 0.0001
All cause 30 day mortality	59 (10.4)	46 (4.3)	34 (5.7)	0.032

The numbers for cardiovascular only and all-cause mortality for both in-hospital and 30 day mortality are comparable, as such only all-cause will be considered in the final analysis.

2.5.3 In-hospital Mortality

Our data indicates that the unadjusted in-hospital mortality is statistically significantly different between the three treatment groups (p = 0.000). This significance however, is driven by the increased mortality observed in the clopidogrel group. When comparing clopiogrel to prasugrel and ticagrelor in terms of in-hospital mortality, the latter agents demonstrate superiority over clopidogrel with p values of p = 0.000 (clopidogrel vs prasugrel) and p = 0.002 (clopidogrel vs ticagrelor) which is indicative of higher in-hospital mortality following treatment with clopidogrel. However, when comparing the incidence of in-hospital mortality between prasugrel and ticagrelor, there is no significant difference in outcome as demonstrated by p = 0.438.

The patients assigned to clopidogrel according to our patient demographic are older and have increased number of co-morbidities which may well account for the increased mortality observed in our clopidogrel treatment arm.

2.5.4 Unadjusted 30 day Mortality

Unadjusted mortality data for the three treatment groups are presented as Kaplan-Meier survival curves, which demonstrate the number of indviduals who are still alive over a 30-day period and includes data regarding those patients in whom the survival outcome may be unknown i.e is censored (figures 8 - 11). This is a crude analysis however, since the variables included are not adjusted for any confounders. Unadjusted thirty-day all-cause and cardiovascular only mortality is lower for both prasugrel and ticagrelor when compared to clopidogrel. Log-rank testing confirmed significantly lower mortality following the administration of prasugrel and ticagrelor ($p < 0.001$ for prasugrel vs clopidogrel and ticagrelor vs clopidogrel). There was a non-significant difference in mortality when comparing prasugrel with ticagrelor (log rank test, $p = 0.785$)

Figure 8. All cause mortality at 30 days - Kaplan-Meier Survival Curves - clopidogrel, prasugrel and ticagrelor.

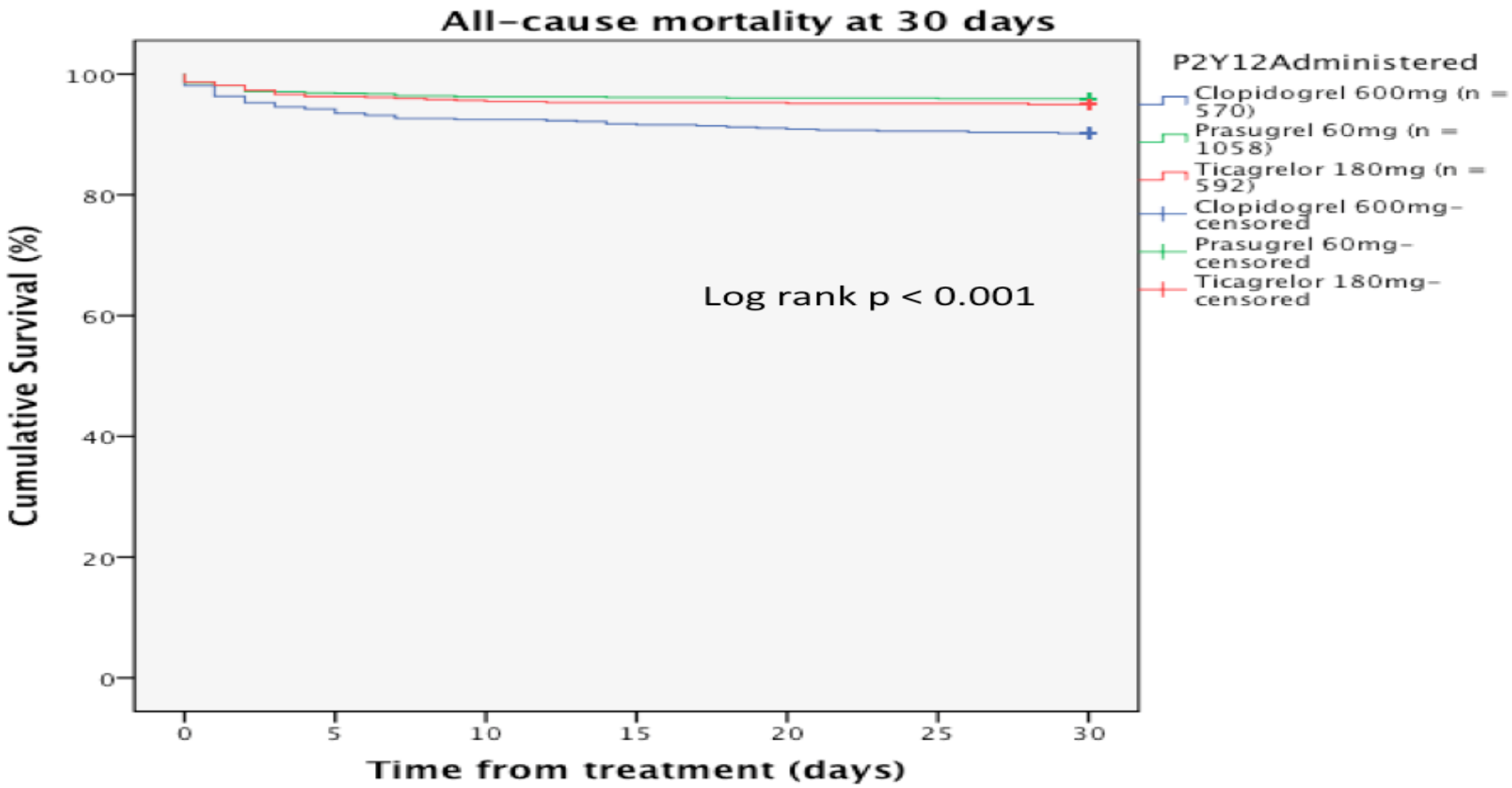


Figure 9. All cause mortality at 30 days - Kaplan-Meier Survival Curves – clopidogrel vs prasugrel

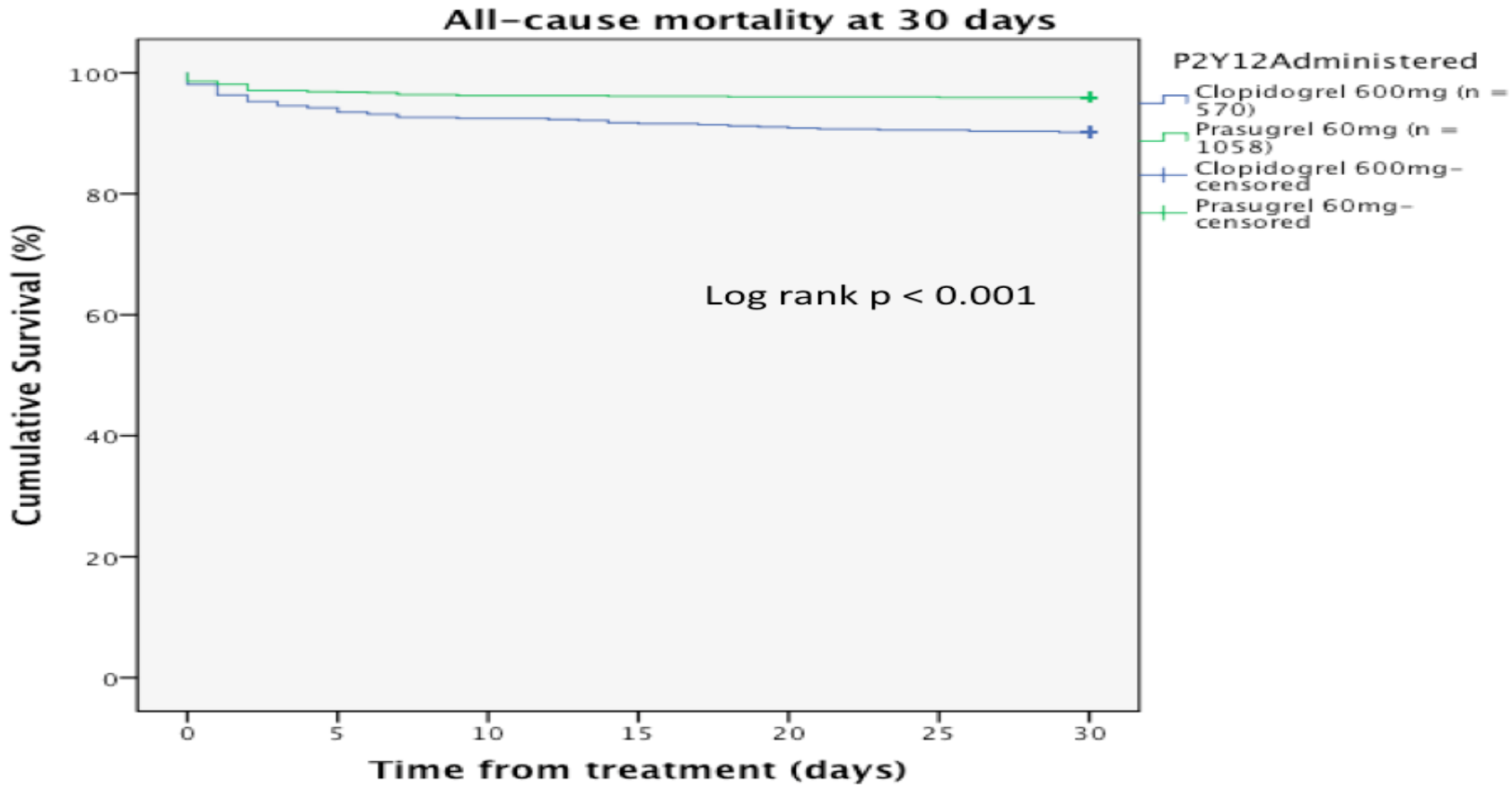


Figure 10. All cause mortality at 30 days - Kaplan-Meier Survival Curves – clopidogrel vs ticagrelor

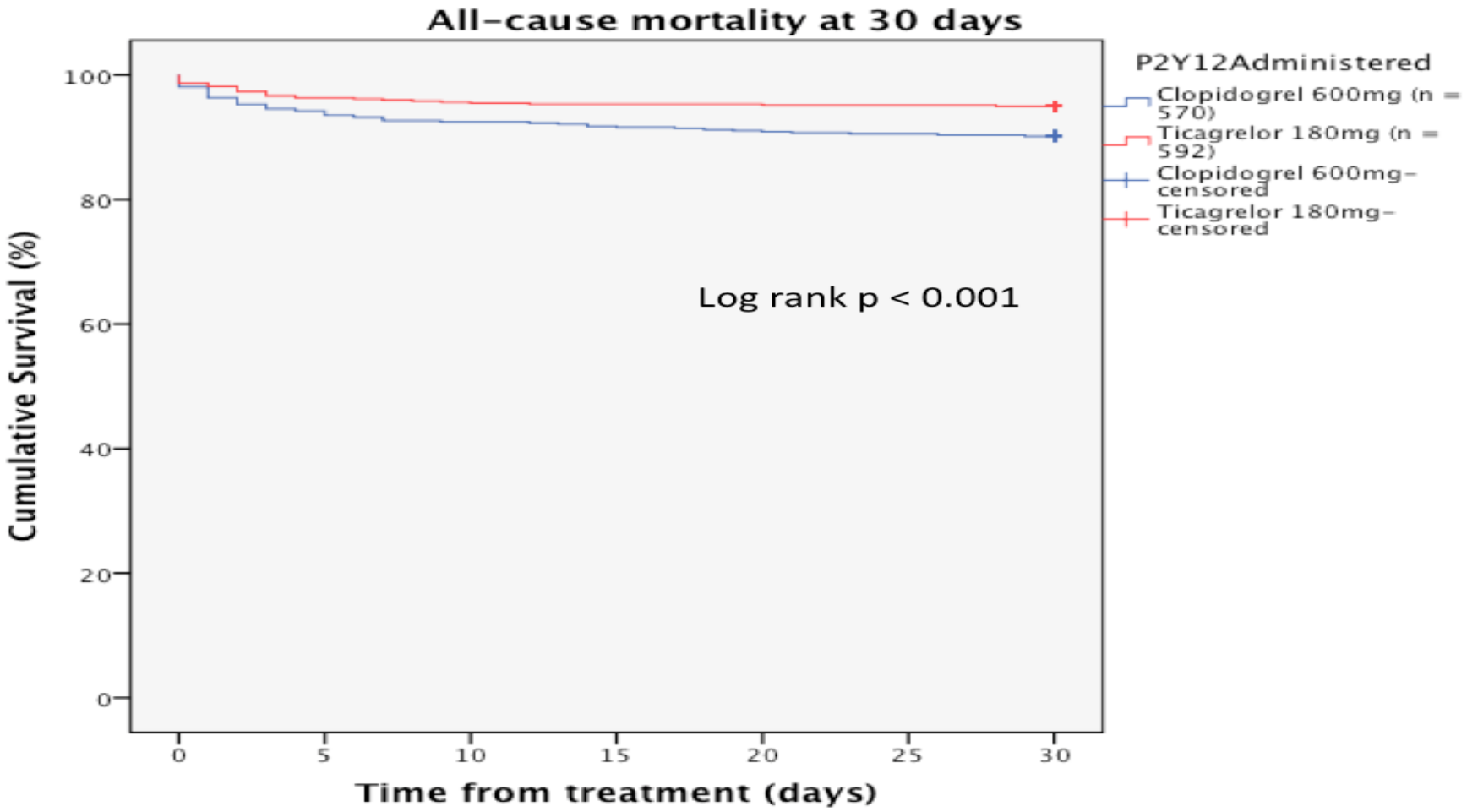
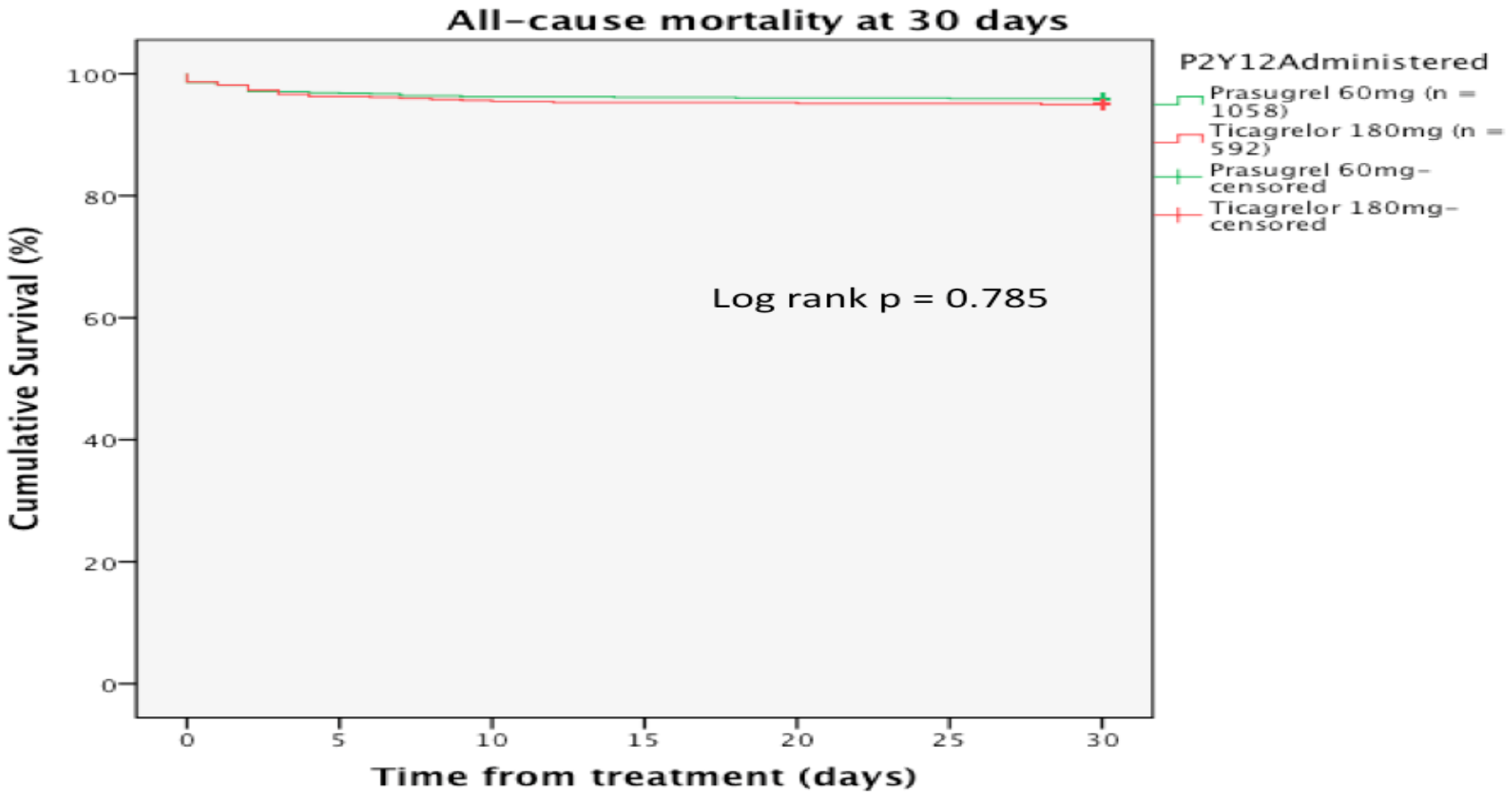


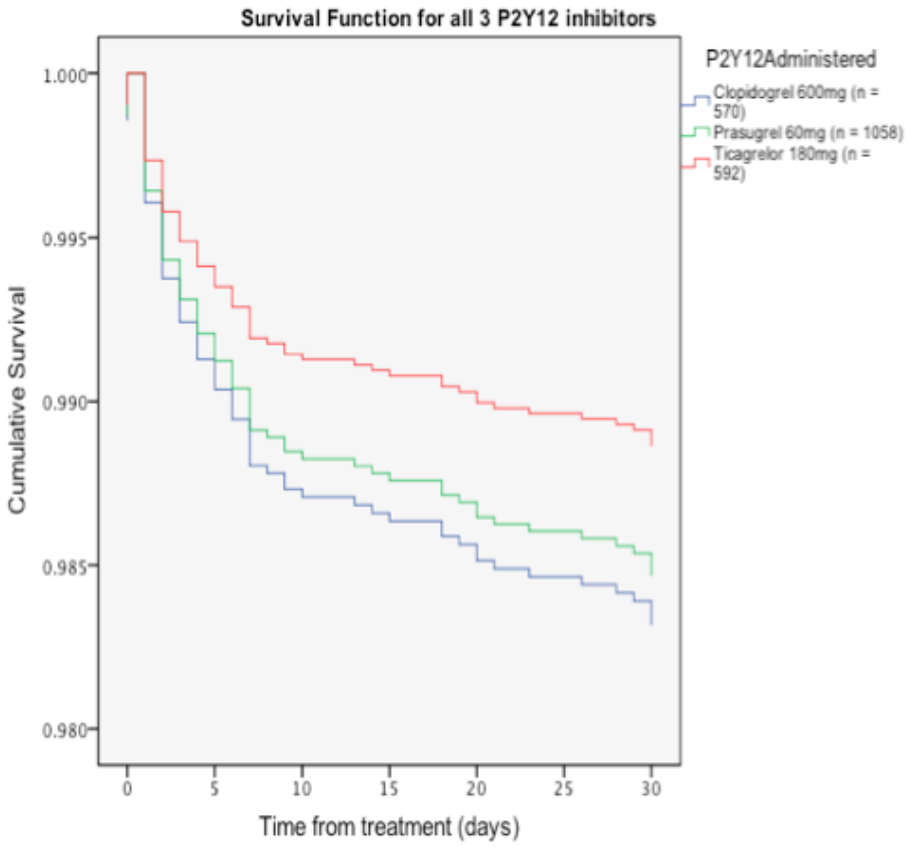
Figure 11. All cause mortality at 30 days - Kaplan-Meier Survival Curves – prasugrel vs ticagrelor



2.5.5 Adjusted 30 day Mortality

A cox-regression analysis was also undertaken and mortality outcomes were standardised/adjusted for the following variables; age, sex, ethnicity, weight, previous MI, diabetes, PVD, CHD, CHF, atrial fibrillation, COPD, VHD, family history, hypercholesterolaemia, hypertension, systolic blood pressure, heart rate and call to balloon time (CBT). The nationally mandated CBT is 150 minutes and relates to the time from the patients call for help to reperfusion and is the benchmark against which all PPCI centres are measured (MINAP 2014). The survival curves (figures 12 - 15) differ once adjusted for confounders; following adjustment ticagrelor appears to be numerically superior to prasugrel in terms of surivival, however, this difference is not significant ($p = 0.438$). Both prasugrel and ticagrelor are numerically superior to clopidogrel, however, again this difference is not of significance with p values of $p = 0.746$ and $p = 0.169$ respectively

Figure 12. Cox regression survival and hazard curves – clopidogrel vs prasugrel vs ticagrelor.



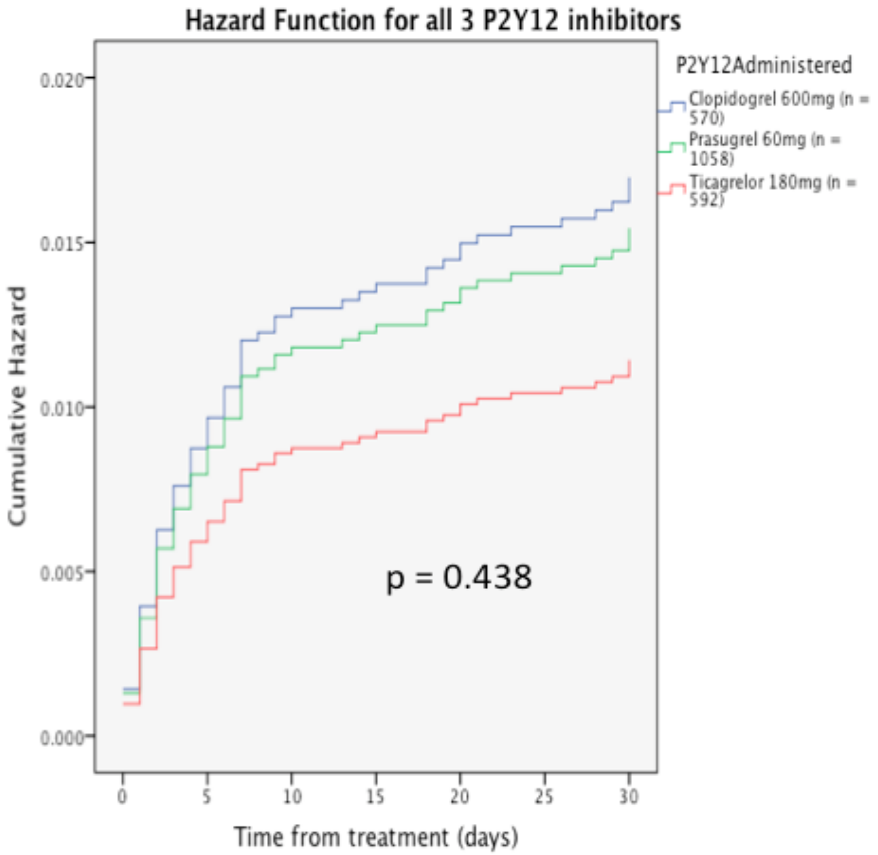


Figure legends - the y-scale does not begin at zero

Figure 13. Cox regression survival and hazard curves – clopidogrel vs prasugrel

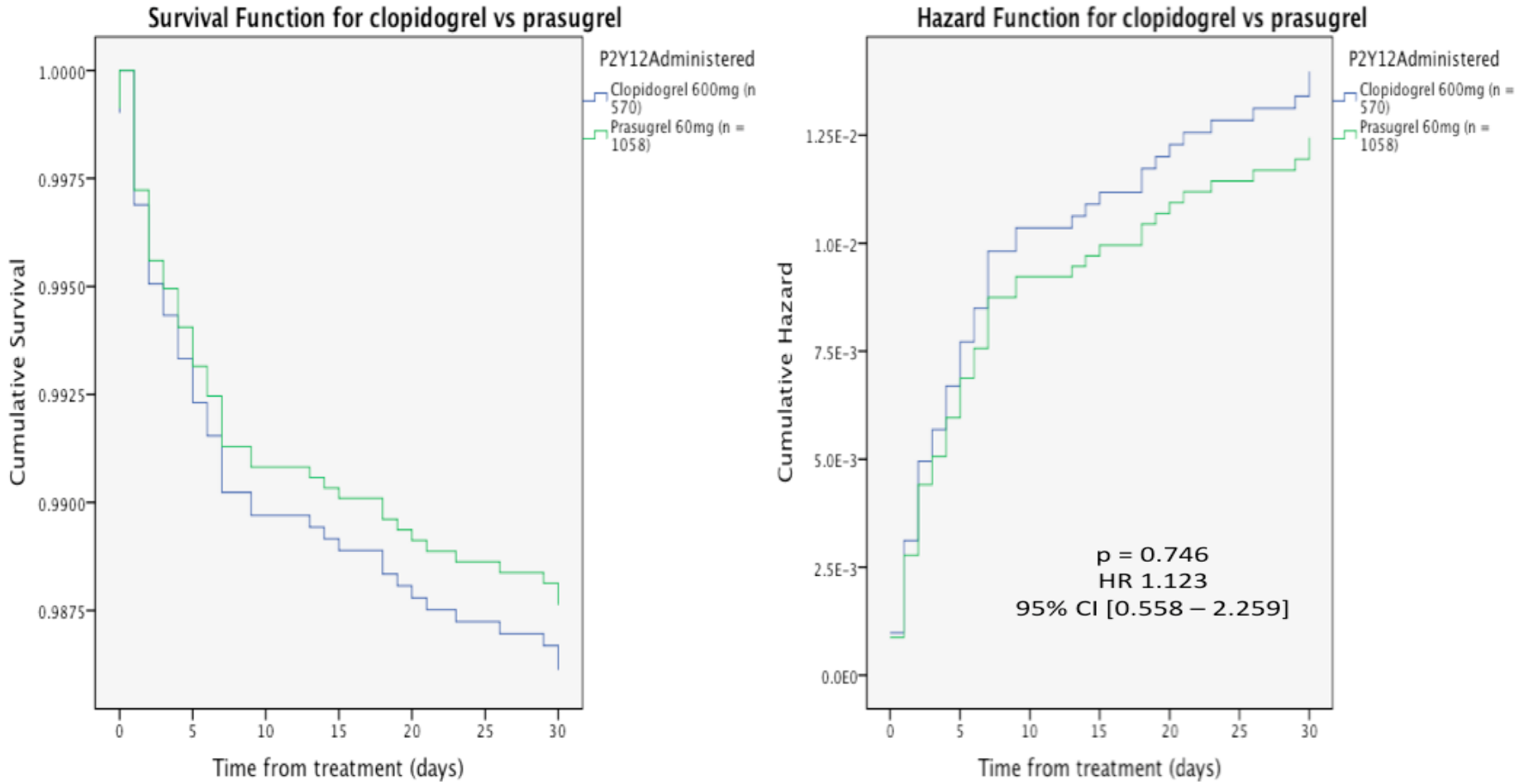


Figure legends - the y-scale does not begin at zero

Figure 14. Cox regression survival and hazard curves – clopidogrel vs ticagrelor

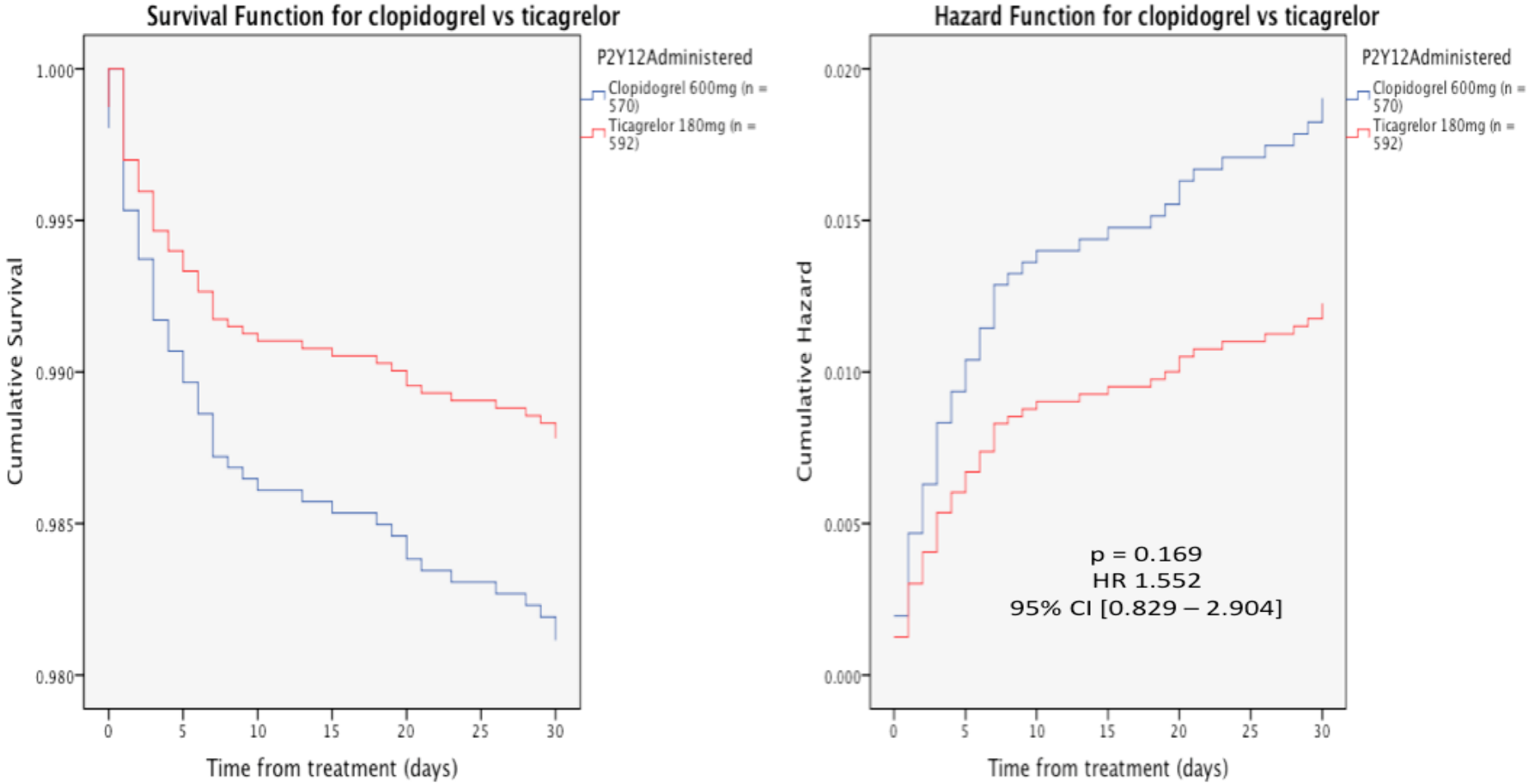


Figure legends - the y-scale does not begin at zero

Figure 15. Cox regression survival and hazard curves – prasugrel vs ticagrelor

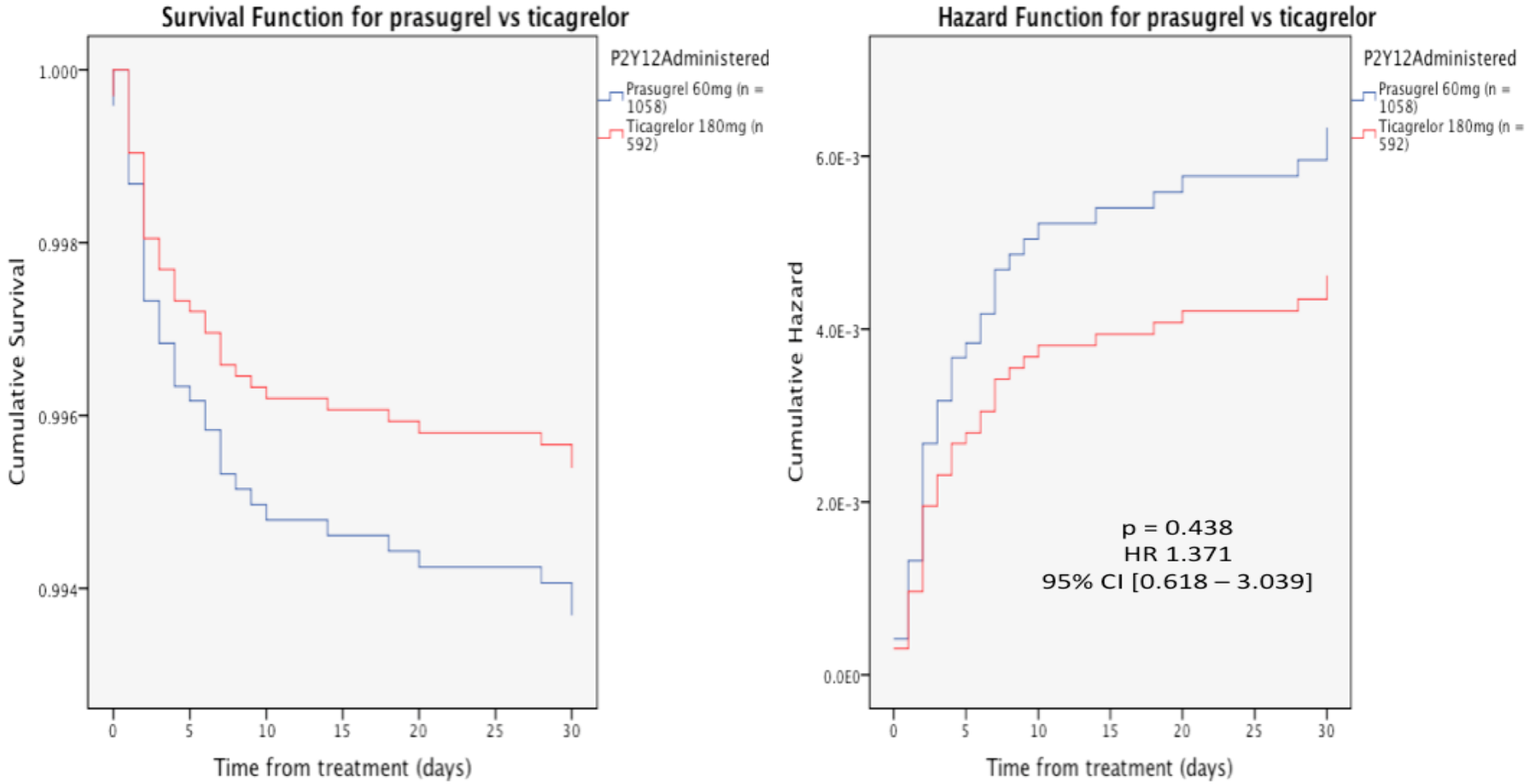


Figure legends - the y-scale does not begin at zero

2.6 Discussion

Observations from the antiplatelet registry data indicate that the patient demographic presenting with STEMI to be treated with PPCI at our centre consists mainly of Caucasian people with an average BMI ranging from 26 to 28 indicating that our population is predominantly overweight. A statistically significant difference in BMI is seen between our three treatment groups ($p = 0.000$) with patients with the highest BMI receiving treatment with prasugrel. This is due to prasugrels licensing restrictions, which state that it should not be administered to patients under 60kg in view of the increased incidence of bleeding complications that can occur in patients with low body weight. There are statistically significant differences in the age at presentation between the three groups ($p = 0.000$), with younger patients receiving treatment with prasugrel and older patients clopidogrel. Which is again reflective of the licensed indications for prasugrel, administration in patients > 75 years is not recommended due to the increased bleeding risk in older patients.

Baseline characteristics in terms of risk factors and co-mordibities differ between the three treatment groups; there are statistically significantly greater incidences of CHD, atrial fibrillation, VHD and hypertension in our clopidogrel treated group. However, this seems reasonable in view of the older age of patients in this particular group; advancing age is known to be associated with increased number of co-morbidities.

The patient demographic described in this chapter provides some insights into the population from which patients for the pharmacokinetic/pharmacodynamic study have been selected (chapters 5-8).

2.6.1 In-hospital bleeding

The increased bleeding complications in older patients in TRITON-TIMI 38 resulted in the recommendation to avoid the use of prasugrel in patients over the age of 75 years (Wiviott, Braunwald et al. 2007). Ticagrelor is also associated with higher rates of non-CABG related major bleeding (Wallentin, Becker et al. 2009). Such adverse effects are expected since prasugrel and ticagrelor provide greater levels of platelet inhibition when compared to clopidogrel resulting in a reduction in both ischaemic and thrombotic complications following an ACS event.

Data regarding the optimal use of P2Y12 inhibitors in older patients are limited. Pharmacokinetic and pharmacodynamic drug handling in older patients is markedly different to that seen in younger patients with often greater exposure to the antiplatelet drug which can contribute to the increased risk of bleeding complications that are seen in this patient group (Wiviott and Mega 2010).

The prevalence of co-morbidities such as atrial fibrillation requiring concomitant anticoagulation and the incidence of renal impairment and anaemia in combination with the age-related changes in drug handing are known to contribute to the increased bleeding risk seen in older patients treated with P2Y12 inhibitors (Kinnaird, Stabile et al. 2003, Moscucci, Fox et al. 2003).

This antiplatelet registry data demonstrates increased bleeding complications (GI bleeds) and need for the blood transfusions in our clopidogrel treated

patients. This patient group is older (mean age 70.51 ± 13.36) and presented with a statistically significantly higher incidence of renal dysfunction (p = 0.000) and anaemia (p = 0.000) when compared to the prasugrel and ticagrelor treated groups (refer to tables 4 and 8).

2.6.2 Mortality

The publication of large clinical trials have demonstrated that the administration of both prasugrel and ticagrelor versus clopidogrel leads to a reduction in the composite of cardiovascular death, myocardial infarction and stroke (Wiviott, Braunwald et al. 2007, Wallentin, Becker et al. 2009)

Although ticagrelor is associated with a mortality benefit in patients who present with an acute MI, this benefit is not seen in our patient population. With regards to survival outcomes, this data indicates that all three oral P2Y12 inhibitors are associated with comparable levels of in-hospital and 30 day mortality. Suggesting that survival outcomes are not solely determined by the antiplatelet drug administered, but are also related to the condition of STEMI itself which has an impact on drug handling and subsequent survival outcomes. There may be additional/unknown factors that prevent the drugs from exerting their effect and benefit.

The physiological changes that occur secondary to the disease state of STEMI, such as reduced gastric absorption and increased platelet reactivity may attenuate the benefit of all orally administered antiplatelet agents (Alexopoulos, Xanthopoulou et al. 2013).

2.6.3 Limitations

The registry represents the daily practice of our PPCI centre and incorporates a “real world all-comers” population, which may introduce recruitment, selection or screening biases.

This is a single centre study in which a relatively small sample size has been scrutinised. In addition, due to time constraints, it was not possible to compare the same outcomes in NSTEMI/UA patients who also received oral antiplatelets and were treated with PCI.

Statistical analyses are conducted on unmatched/balanced populations since propensity matched analyses has not been undertaken at this stage.

2.7 Conclusions

This local registry data indicates that despite the rapid uptake into practice of newer more potent antiplatelet agents, in-hospital and 30 day mortality does not differ between the clopidogrel, prasugrel and ticagrelor groups. Despite the use of newer agents, that offer enhanced pharmacological profiles, which should translate into improved therapeutic outcomes in clinical practice, this is not evident in our local patient population.

We therefore postulate that all oral P2Y12 inhibitors have reduced clinical efficacy in the context of STEMI possibly secondary to gastric malabsorption and reduced hepatic perfusion. This leads us to question whether the disease state rather than drug administration in the acute phase has more of an impact on clinical outcomes.

Although not an outcome driven trial, our pharmacokinetic/pharmacodynamic pilot study data, which will be discussed in the remainder of my thesis, will help to explore this question.

Chapter 3 - Pharmacokinetics and Pharmacodynamics of oral P2Y12 inhibitors during the acute phase of a myocardial infarction: A Systematic Review.

3.1 Introduction

The immediate administration of oral antiplatelet therapy in the form of aspirin plus a P2Y12 inhibitor is the mandated and universally recognised standard of care for patients who present with an acute myocardial infarction (Steg, James et al. 2012). Despite such strong recommendations for their use, there are a paucity of data regarding their onset of action and clinical efficacy during the short time frames from confirmation of diagnosis to reperfusion with primary percutaneous coronary intervention. In order to establish the current level of evidence regarding drug handling of oral P2Y12 inhibitors during the acute phase of a myocardial infarction I conducted a systematic review of the available literature.

3.2 Study Objective

To undertake a systematic review of the available evidence regarding the pharmacokinetic and pharmacodynamic drug handling of orally administered P2Y12 inhibitors (clopidogrel, prasugrel and ticagrelor) during the acute phase of a ST-elevation myocardial infarction (STEMI) (that is from symptom onset/diagnosis to the time of angioplasty) to determine whether their administration allows for and/or achieves adequate levels of platelet inhibition in patients undergoing primary percutaneous coronary intervention.

3.3 Methods

This review has been conducted in line with the recommendations made within the PRISMA-P guideline and has been registered with the International Prospective Register of Systematic Reviews (PROSPERO - CRD 42015023393) on June 19th 2015 (Khan 2015, Moher, Shamseer et al. 2015). Of note, the systematic review process was completed 36 months after the conception of the research project, formulation of the research question and initiation of the study as outlined in chapter 4 onwards.

3.3.1 Search Strategy

An initial literature search was undertaken to determine whether this research question had already been addressed. At the time of review, there were no indications that a review of this nature had already been completed. The main reviewer (NK) and secondary reviewer (AC) agreed the systematic review question, search terms, search strategy and inclusion/exclusion criteria for the final studies to be included. A structured and comprehensive literature search was performed in January 2014 using Pubmed (from inception to January 2014) and EMBASE (Ovid) (from 1974 to December week 3 2013). In addition, a search of the Cochrane database of systematic reviews was also undertaken, however, this did not reveal any relevant or related review topics. Secondary references found during the initial literature search were deemed to fall under the category of “grey” data/literature.

3.3.2 Study Selection

The key medical subheading search terms used during the literature search included, clopidogrel (Plavix), prasugrel (Effient), ticagrelor (Brilique), P2Y12

inhibitors, myocardial infarction, STEMI, inhibition of platelet activity (IPA), gastrointestinal absorption, cardiogenic shock, pharmacokinetics and pharmacodynamics.

As shown in figure 16, a total of 4,005 papers were retrieved following the literature search using PubMed and further 3,242 from EMBASE. A search of the Cochrane database did not yield any results. All search results were exported to a reference manager programme (EndNote Version X7.3 2015) where duplicate searches were excluded. Of those searches that remained (n = 4,532), a title and abstract review was undertaken to determine whether the contents of the selection were in line with the inclusion and exclusion criteria stipulated at the outset (Appendix 2). For the final title/abstracts selected, the full papers were retrieved and the contents scrutinised in more detail to determine their relevance in relation to the research question and inclusion criteria. Any discrepancies in the search results identified were discussed by NK and AC, compared against the inclusion/exclusion criteria and screening questions and a decision made as to whether the paper should be included in the final review. Where a decision regarding inclusion could not be made, the opinion of a third reviewer (JC) was sought. In order to ensure the appropriateness of the final selections, a number of screening questions, based on the CASP and SURE checklists were devised and utilised (Appendix 3). A modified tool, incorporating only the key screening questions relevant to nature of the papers collated for my systematic review was developed to remove duplication and provide a more focused assessment of

the quality and robustness of the final randomised controlled trials and qualitative reviews included in the list of papers retrieved for scrutiny.

3.3.3 Data Synthesis

This review was able to provide insights in to whether sufficient levels of platelet inhibition are achieved at the time of primary percutaneous coronary intervention in patients who are administered loading doses of oral P2Y12 inhibitors following a diagnosis of STEMI. The degree of platelet inhibition (pharmacodynamics) will be expressed as either percentage platelet reactivity index (%PRI), P2Y12 reaction units (PRU), ADP-induced aggregation and whole blood aggregation. Pharmacokinetic assessment will be determined through the amount of active metabolite generated. A narrative overview of the data extracted from studies included in the final review will be given.

3.4 Results

3.4.1 Study Selection and Data Extraction

The process of screening and selection of the final citations included in the systematic review are outlined in figure 16. We reviewed the full text of 101 of the 4,532 records identified through the initial database search. Of these, a final eight papers were selected for inclusion in our analysis; six relate to pharmacodynamic studies and one relates to both pharmacodynamic and pharmacokinetic and the remainder is a pharmacokinetic study only.

A summary of patient and study characteristics along with key study findings is included in table 10.

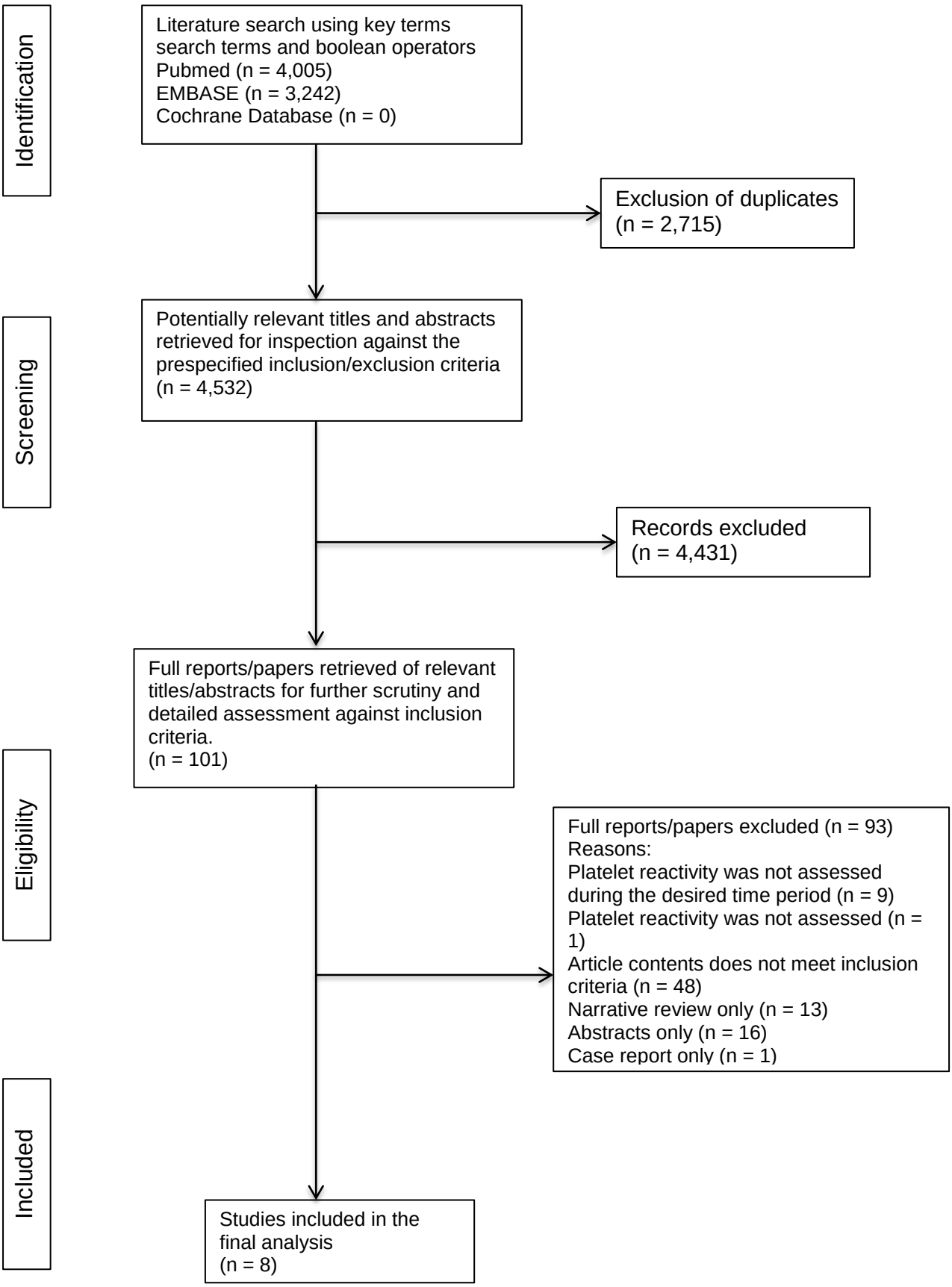
Table 10. Study and patient characteristics with key findings.

Reference	Study type	Population	Intervention	Comparators	Platelet Function Test/Analysis	Findings
Alexopoulos (Alexopoulos, Xanthopoulou et al. 2012)	PD	STEMI (n = 55)	PPCI	Prasugrel 60mg (n = 28) Ticagrelor 180mg (n = 27)	VerifyNow and Multiplate Analyzer at baseline, 1,2,6, 24 hrs and 5 days post loading	VerifyNow PRU at 1hr prasugrel 257 and ticagrelor 231. (PRU ≥ 230 indicates HTPR). There is an initial delay in antiplatelet effect; taking approx. 2hrs to see sufficient levels of IPA (PRU <208)
			Background antithrombotic therapy: unspecified			
Beigel (Beigel, Fefer et al. 2013)	PD	STEMI (n = 79)	PPCI	Clopidogrel 600mg (n = 49) Prasugrel 60mg (n = 30)	LTA at baseline, at PPCI and after 72hrs	Mean DTB – 48 +/-20 mins At baseline, ADP induced aggregation – comparable between prasugrel and clopidogrel. At PPCI - ADP induced platelet aggregation significantly less in the prasugrel group compared with clopidogrel group; but less than 50% of prasugrel treated patients achieve IPA < 70%.
			Background antithrombotic therapy: aspirin 100mg (PO) +/- GPI (tirofiban)			
Heestermans (Heestermans, van Werkum et al. 2008)	PK	STEMI vs healthy controls (n = 21)	PPCI	STEMI clopidogrel 600mg (n = 11) Healthy controls clopidogrel 600mg (n = 10)	LC-MS/MS pre-dose, 0.5, 1, 1.5,2,3,4,6 and 24 hrs post-loading	Plasma concentration of the active thiol metabolite of clopidogrel is significantly lower in STEMI patients compared to the healthy controls. Impaired bioavailabilty of clopidogrel in STEMI patients leads to suboptimal levels of IPA.
			Background antithrombotic therapy: aspirin 900mg (IV) + UFH 70IU/kg			
Hobl (Hobl, Stimpfl et al. 2014)	PK/ PD	Healthy subjects (n = 24)	None	Clopidogrel 600mg + morphine 5mg Clopidogrel 600mg + placebo	PD assessment: VASP phosphorylation assay PK assessment: LC-MS/MS	PD: morphine administration is associated with a 2hr delay in achieving maximal IPA PK: morphine administration significantly reduces the maximal concentration on the active thiol metabolite (Cmax) and prolongs the time taken to reach maximal concentration (Tmax) A clinically significant drug/drug interaction
			Background antithrombotic therapy: none administered			

						is apparent following the co-administration of morphine and clopidogrel.
Orban (Orban, Mayer et al. 2014)	PD	STEMI (complicated by cardiogenic shock) (n = 145)	PPCI	Clopidogrel 600mg (n = 95) Prasugrel 60mg (n = 50)	Multiplate Analyser	42% of patients showed HTPR following loading doses of either clopidogrel or prasugrel. All-cause mortality lower at 30 days in patients treated with prasugrel without any increase in bleeding risk.
			Background antithrombotic therapy: aspirin 500mg (IV) + UFH 5,000IU			
Osmancik (Osmancik, Jirmar et al. 2010)	PD	STEMI (critically ill) (n = 40)	PPCI	Clopidogrel 600mg unstable STEMI (n=20) Clopidogrel 600mg stable STEMI (n=20)	VASP phosphorylation assay at baseline, 4, 24 and 48 hrs post clopidogrel loading	PRI >53% is indicative of clopidogrel unresponsiveness. A greater reduction in %PRI was observed in stable compared to unstable STEMI patients.
			Background antithrombotic therapy: aspirin 500mg (IV) + UFH 150IU/kg +/- GPI			
Parodi (Parodi, Valenti et al. 2013)	PD	STEMI (n = 50)	PPCI	Prasugrel 60mg (n = 25) Ticagrelor 180mg (n = 25)	VerifyNow at baseline, 2,4,8 and 12hrs post loading	Only 50% of patients demonstrate effective levels of IPA at 2hrs and at least 4 hrs is required to see sufficient IPA in the majority of patients. The administration of morphine is an independent predictor of HRPR at 2 hrs
			Background antithrombotic therapy: aspirin 500mg (IV) + bivalirudin only			
			Background antithrombotic therapy: aspirin 300-500mg + bivalirudin only			

AMI – acute myocardial infarction, DTB –door to balloon time, PPCI - primary percutaneous coronary intervention, PD - pharmacodynamics PK – pharmacokinetic UFH – unfractionated heparin GPI – Glycoprotein IIb/IIIa inhibitor PRU – P2Y12 reactivity units PRI – platelet reactivity index IPA – inhibition of platelet activity HTPR – high on treatment platelet reactivity LTA – light transmission aggregometry LC-MS/MS – liquid chromatography tandem mass spectrometry

Figure 16. PRISMA-P Flow chart/Study Selection Process



3.5 Discussion

Although the place in therapy and longer-term benefits of DAPT are well established, there are very little data regarding the clinical utility of these agents during the acute phase of a STEMI. This systematic review has highlighted a potential gap in the evidence base regarding the use of antiplatelet agents, since it is apparent that the speed of onset, degree of platelet inhibition and clinical efficacy of the currently available oral P2Y12 inhibitors has not been fully assessed during the narrow door to balloon times that are necessary to allow for successful PPCI following a STEMI.

3.5.1 Phamacodynamic Studies

The pharmacodynamic studies included compared the administration of clopidogrel, prasugrel or ticagrelor in various patient populations; healthy volunteers, STEMI patients who are haemodynamically stable and unstable, or STEMI complicated by cardiogenic shock. A number of platelet function assays were utilised and the time points at which samples were collected in relation to the administration of the loading dose were variable. Irrespective of the differences in study designs, drugs administered and platelet function assays used, a number of key themes are apparent.

Firstly, despite the administration of prasugrel or ticagrelor loading doses, there is an initial delay in their onset of action, with an increase intrinsic platelet reactivity/HRPR is present at 2 hours indicated by PRU ≥ 230, indicating that neither agent has a particularly potent antiplatelet effect at the

time of PPCI (Alexopoulos, Xanthopoulou et al. 2012, Parodi, Valenti et al. 2013)

Secondly, STEMI is a clinical state which is often accompanied by haemodynamic instability and complications such as cardiogenic shock, the administration of catecholamines, systemic vasoconstriction, adrenergic activation and shunting of blood flow away from non-essential organs leads to impaired perfusion of the gut and liver with subsequent impairment of gastrointestinal absorption and metabolic biotransformation of orally administered drugs into their pharmacologically active forms. The impact of such physiological changes on the pharmacological effect of the oral P2Y12 inhibitors has also been investigated and quantified for clopidogrel and prasugrel, with both agents being subject to HRPR as demonstrated by %PRI > 50% (Osmancik, Jirmar et al. 2010, Parodi, Valenti et al. 2013, Orban, Mayer et al. 2014).

Thirdly, the co-administration of morphine introduces a potentially clinically significant drug-drug interaction, which leads to a delay in the onset of action of all three oral antiplatelet agents (clopidogrel, prasugrel and ticagrelor), with a consequent reduction in IPA and corresponding HRPR (Parodi, Bellandi et al. 2015). The nature of the interaction will be described in more detail in chapter 8, but it is has been proposed that the administration of morphine reduces gastric emptying, which in turns leads to a delay in the absorption of orally administered antiplatelets and a consequent reduction in their antiplatelet effects (Parodi, Bellandi et al. 2015).

3.5.2 Pharmacokinetic Studies

Only one study reports on pharmacokinetic data regarding clopidogrel and its active metabolite in the context of STEMI; there are no data for prasugrel or ticagrelor active metabolite generation in this setting.

Heestermans et al, work from 2008, which focuses on clopidgrel pharmacokinetics, provides some insights into the altered drug handling that occurs secondary to a STEMI. Generation of clopidogrel active metabolite was shown to be significantly reduced in STEMI patients when compared to healthy controls. The consequent reduction in bioavailability and platelet inhibition is thought to be secondary to impaired GI absorption (Heestermans, van Werkum et al. 2008).

Although not undertaken STEMI patients, the study by Hobl et al has been included in this systematic review, since it investigates the extent of the morphine-antiplatelet drug-drug interaction in healthy patients and demonstrates that the co-administration of morphine leads to a reduction in active metabolite generation, demonstrated by a delay in the time take to achieve maximum concentration (Tmax), decrease in maximum concentration (Cmax) and a 34% reduction in area under the curve (AUC). Consequently, a decrease in antiplatelet effect is seen as demonstrated by a PRI >50% (Hobl, Stimpfl et al. 2014).

3.5.3 Limitations

There are several limitations to the systematic review undertaken, mainly attributable to the heterogeneity of final studies included. Patient characteristics and the type of platelet function assays utilised were variable between the studies reviewed. In addition the timing of maximal IPA in relation to administration of the loading dose is not always clear and the use of background antithrombotic therapy between the different studies was markedly different.

The reporting of clinical outcomes, pharmacodynamic and pharmacokinetic data is variable and the impact this may have on patient outcomes is not clear. Lastly, the studies included are not adequately powered to make inferences with regards to clinical outcomes, but they do provide further insights into and support emerging evidence indicating that even the newer generation oral P2Y12 inhibitors are not effective in the setting of STEMI.

3.6 Conclusion

The pharmacokinetic and pharmacodynamic data collated and scrunitised during the review demonstrate inadequate levels of platelet inhibition in the first few hours after presentation in STEMI patients. The results of this systematic review indicate that despite the administration of oral P2Y12 inhibitors such as prasugrel and ticagrelor that should allow for greater and more consistent levels of platelet inhibition, the physiological state of STEMI and the co-administration of opioid based analgesia (e.g. morphine) are

associated with a reduction in the degree of platelet inhibition achieved following their administration.

The results from chapter 2 and the findings of this systematic review, (although the latter was completed following the commencement of my research project), provide further justification and rationale for undertaking and completing the pharmacokinetic and pharmacodynamic assessment of oral P2Y12 inhibitor activity during the acute phase of a myocardial infarction (Chapter 4 onwards).

Chapter 4 – Materials and Methods describing the Pharmacokinetic and Pharmacodynamic assessment of Oral P2Y12 Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction

4.1 Introduction

PPCI is an emergency procedure, which should be performed within 60 minutes of a patient's arrival at hospital (or within 120 minutes of symptom onset). The currently available oral agents within the armamentarium of antiplatelet therapies have all demonstrated clinical benefits in patients who present following a myocardial infarction. Much of the available pharmacodynamic and pharmacokinetic data are largely derived from healthy volunteers (Brandt, Payne et al. 2007). In addition to the short time scales involved for PPCI, it is possible that the condition of STEMI itself, with the effects of concomitant treatments (e.g. opioid based analgesia) might limit the effectiveness of oral antiplatelet agents (Lincoff, Steinhubl et al. 2008)

It is unlikely that gastro-intestinal absorption during a STEMI is equivalent to that of a healthy resting patient. The physiological effects of severe pain and neurohormonal activation will redirect blood flow away from the gut, this coupled with the administration of opioid analgesia will lead to delayed absorption of oral antiplatelet agents from the gut (Parodi, Bellandi et al. 2015). The increase in intrinsic platelet reactivity seen during the acute phase of a MI will further impair the effectiveness of the antiplatelet agents (Kumana, Rambihar et al. 1982, Mathur, Robinson et al. 2001, Frelinger, Michelson et al. 2011).

As demonstrated by the findings of the systematic review undertaken and reported in chapter 3, there are little data concerning the comparative speed of onset in terms of IPA/clinical efficacy of the oral antiplatelet agents in the context of an acute STEMI when compared to other more stable presentations of ACS e.g. NSTEMI/UA. Much of the data reported are derived from STEMI patients only or healthy patients or a comparison between the two. The findings of the current study demonstrate the extent to which clopidogrel, prasugrel or ticagrelor are having a significant clinical effect during the primary angioplasty (STEMI patients) and provide insights into the pharmacodynamic and pharmacokinetic properties of these agents in the setting of STEMI. By also studying patients with UA/NSTEMI, we were able to determine whether the condition of STEMI per se affects the pharmacokinetics and pharmacodynamics of these orally administered P2Y12 inhibitors.

4.2 Study Objectives

1. To determine the degree and time course of platelet inhibition by clopidogrel, prasugrel and ticagrelor administered acutely before emergency primary percutaneous coronary intervention (PPCI) for STEMI, during the procedure and in the following four hours.
2. To determine whether the state of acute STEMI reduces the absorption and/or subsequent efficacy of the oral $P2Y_{12}$ inhibitors when compared to patients with other acute coronary syndromes e.g. unstable angina/NSTEMI.

4.3 Study Design

This was a single-centre, non-randomised, prospective observational study, in which the efficacy of oral antiplatelet agents, clopidogrel, prasugrel and ticagrelor were investigated during the acute phase of a myocardial infarction. A total of 87 patients were recruited to the study following a diagnosis of STEMI or UA/NSTEMI; since this was a pilot study, power calculations were not undertaken to determine sample size. The study was non-randomised to allow for adherence to local prescribing protocols as well as compliance with national and international guideline recommendations regarding the use of antiplatelet therapies in the setting of ACS. In terms of allocation to a treatment arm, the initial phase of patient recruitment involved the use of clopidogrel and prasugrel only, as per local treatment guidelines. Following successful and complete recruitment to the prasugrel arm of the study, the use of prasugrel was discontinued in our centre and ticagrelor was introduced in its place. Our local antiplatelet prescribing guidelines were updated to reflect the change in practice.

4.4 Study Outcome Measures

In order to determine the pharmacokinetic and pharmacodynamic profile of oral P2Y12 inhibitors, in addition to the degree and time course of platelet inhibition following the administration of clopidogrel, prasugrel and ticagrelor in patients presenting with a STEMI and UA/NSTEMI, the following parameters were recorded for all three oral agents:

1. The degree of platelet inhibition (pharmacodynamic effect) as measured by VerifyNow.

2. The degree of platelet inhibition (pharmacodynamic effect) as measured with VASP flow cytometry.
3. The concentration of active metabolite or parent compound) present in plasma (pharmacokinetic effect) as measured by liquid chromatography with tandem mass spectrometry.

4.5 Materials and Methods

4.5.1 Subject Selection

The study recruited those patients who presented following an acute coronary syndrome; either a STEMI or UA/NSTEMI.

STEMI patients were identified following activation of the STEMI/PPCI pathway and admission to the Heart and Lung Centre for PPCI. UA/NSTEMI patients were identified with the assistance of the Cardiac Assessment Team nurses following their admission to either the Accident and Emergency department or the Emergency Admissions Unit at RWH.

Patient groups were carefully selected such that all patients who were to undergo PCI received anti-platelet medications as per current national and local guidelines and manufacturers datasheet recommendations.

Table 11. Patient allocation to treatment groups.

Group 1 (n=15)	Patients admitted with STEMI who were under the age of 75 years and greater than 60kg were administered prasugrel 60mg as a single loading dose followed by 10mg daily as a maintenance dose.
Group 2 (n=15)	Patients admitted with NSTEMI who were under the age of 75 years and greater than 60kg were administered prasugrel 60mg as a single loading dose, however - After sample collection, **diabetic** patients who were stented received maintenance treatment with prasugrel 10mg daily as per the licensing agreement and NICE recommendations. - After sample collection, **non-diabetic** patients and those who were not stented received maintenance treatment with clopidogrel 75mg daily.
Group 3 (n=13)	Patients admitted with STEMI who were over the age of 75 years or under 60kg were administered clopidogrel 600mg as a single loading dose followed by 75mg daily as a maintenance dose.
Group 4 (n=14)	Patients admitted with UA/NSTEMI who were over the age of 75 years and under 60kg were administered clopidogrel 600mg as a single loading doses followed by 75mg daily as a maintenance dose.
Group 5 (n=15)	Patients admitted with STEMI were administered ticagrelor 180mg as a single loading dose, followed by 90mg twice daily as a maintenance dose.
Group 6 (n=15)	Patients admitted with NSTEMI were administered ticagrelor 180mg as a single loading dose, followed by 90mg twice daily as a maintenance dose.

NB – patient recruitment to the ticagrelor STEMI or NSTEMI groups did not commence until all prasugrel STEMI and NSTEMI patients were recruited.

4.5.2 Inclusion Criteria

1. STEMI patients who presented for PPCI (characterised by chest discomfort and prominent ST-segment elevation).
2. NSTEMI patients as characterised by their clinical presentation; chest discomfort, raised levels of myocardial enzymes and/or ST-segment depression or prominent T-wave inversion.
3. Provision of verbal assent (STEMI patients pre-procedure) and/or written consent (STEMI patients post-procedure and NSTEMI patients prior to enrolment).
4. Age > 18 years.
5. Able to take aspirin and either clopidogrel, prasugrel or ticagrelor.

6. Do not have a concurrent septic or inflammatory illness.
7. Are P2Y12 inhibitor naïve (are not currently taking clopidogrel or prasugrel or ticagrelor).

4.5.3 Exclusion Criteria

1. Are unable to provide verbal assent and written consent.
2. Have a documented allergy to aspirin or clopidogrel or prasugrel or ticagrelor.
3. Are diagnosed with pre-existing cardiogenic shock.
4. Have concurrent septic or inflammatory disease e.g. rheumatoid arthritis, lupus or pneumonia.
5. Are already taking a P2Y12 inhibitor.
6. Have known bleeding diathesis.
7. Patients over the age of 75 years or under 60kg or those who have had a previous stroke/transient ischaemic attack would not receive treatment with prasugrel.
8. Patients with a history of intracranial haemorrhage would not receive treatment with prasugrel or ticagrelor but would receive clopidogrel.
9. Peri-procedural administration of glycoprotein IIb/IIIa inhibitor (abciximab or eptifibatide) due to their adverse effects on platelet reactivity and ability to generate meaningful results using VerifyNow.

4.5.4 Withdrawal Criteria

For those patients who chose to withdraw consent to participate in the study, all collected samples were to be discarded. All patient specific information collected for the purpose of the study would be erased. Patient withdrawal would be documented in the clinical notes and in the study patient

identification log. The patients treatment would however, continue in accordance with clinical guidelines should the participant choose to withdraw or not.

4.5.6 Assessment and Follow-up

4.5.6.1 Subjects

A total of 87 patients were recruited to the study of which 43 were recruited to the STEMI arm following activation of the PPCI pathway and 44 to the NSTEMI/UA arm following identification and assessment against the inclusion criteria.

4.5.6.2 Sampling

4.5.6.2.1 STEMI Patients

Following verbal assent, an oral $P2Y_{12}$ inhibitor was administered (as per current local and national guidelines). The time at which the loading dose was administered was recorded on the case report form (CRF – refer to appendices 4 and 5) and documented within the medical notes; the patient was then transferred to the cardiac catherisation suite for PPCI.

After insertion of the radial or femoral sheath, 15ml of whole blood was drawn from this sheath at 20 minutes post-loading (or as close to this time as practicable) and also at the time of first balloon inflation. A further 15ml of blood was collected at 60 minutes post-loading. A final 15ml sample was collected at 4 hours post-loading; at this point the patient will have returned to a ward environment and as such the 15ml blood sample was collected from an anticubital vein using a 21 gauge needle.

4.5.6.2.2. NSTEMI Patients:

Following written consent, a $P2Y_{12}$ inhibitor was administered (as per current local and national guidelines). The time at which the loading dose was administered was recorded, and 15ml of whole blood was taken at 20 minutes, 60 minutes and 4 hours post loading from an anticubital vein using a 21 gauge needle.

Aspirin was administered by the ambulance staff or medical staff in the Accident & Emergency/Emergency Admissions Unit and samples for assessment were collected at 20 minutes, the time of balloon inflation (for PPCI patients) and 60 minutes following the administration of the $P2Y_{12}$ inhibitor loading dose. The process of patient recruitment and sample collection are described in appendices 6 - 9. The corresponding patient information sheet given to patients and consent forms can be viewed in appendices 10 - 13.

Each sample was collected for:

1. Estimation of P2Y12 inhibition using the near patient VerifyNow test.
2. Estimation of P2Y12 inhibition by flow cytometry using a VASP-phosphorylation test.
3. Estimating the effect of aspirin on platelet inhibition using the near patient VerifyNow test
4. Estimation of the active metabolites generated for clopidogrel and prasugrel in addition to the parent compound of ticagrelor and its active metabolite which is generated following enzymatic degradation of

ticagrelor using liquid chromatography in tandem with mass spectrometry.

Table 12. Sample collection and sampling times.

Time	Pre-dosing	20 minutes post dosing	Balloon time (STEMI only)	60 minutes post dosing	240 minutes post dosing
Procedure	Consent	15ml blood	15ml blood	15ml blood	15ml blood
VN Aspirin		X		X	X
VN $P2Y_{12}$		X	X	X	X
VASP Phosphorylation		X	X	X	X
Estimation of plasma concentration		X	X	X	X

4.6 Pharmacodynamic Analysis

4.6.1 VerifyNow for aspirin and $P2Y_{12}$ analysis

VerifyNow™ (Accumetrics, San Diego, California, USA) is a near patient test comprising a turbidimetric based optical detection system that measures platelet aggregation as an increase in light transmittance (Bouman, Parlak et al. 2010).

The VerifyNow point of care assay has an established role in facilitating the pharmacodynamic assessment of platelet reactivity following the administration of either aspirin a P2Y12 inhibitor or an GP IIb/IIIa inhibitor and is endorsed by the FDA in this capacity (Michelson, Frelinger et al. 2006). The utility of using VerifyNow to assess the effectiveness of clopidogrel, prasugrel and ticagrelor is also well established in clinical practice (Varenhorst, James et al. 2009, Jeong, Bliden et al. 2012).

While undertaking the VerifyNow assay, 2ml of whole blood was transferred into a Greiner Bio-one Vacuette containing 3.2% sodium citrate and inverted carefully, after which the tube was left at room temperature for a minimum of 15 and 30 minutes for both the $P2Y_{12}$ and aspirin assays respectively. After the appropriate rest period, the vacuette was loaded into the assay device and analysed as per the manufacturers instructions (Accumetrics). The VerifyNow device was calibrated on a daily basis and full quality control was carried out for each batch of new assays to ensure the integrity of the products being used. For this component of the analysis, I assisted with sample collection and the preparation of samples for analysis using the VerifyNow point of care device.

In terms of assessing platelet function, the VerifyNow assay employs the same principles as those used in the current gold standard for assessing platelet function; light transmission aggregometry (LTA) (Michelson, Frelinger et al. 2006).

The aspirin specific assay utilises arachadonic acid as the principal agonist to stimulate platelet aggregation; aspirin 300mg is often administered at the point of presentation and initial assessment by the ambulance staff. In terms of assessing the anti-platelet effects of aspirin, samples were collected at 20 minutes, balloon inflation (for STEMIs only), 60 and 240 minutes after administration of the oral $P2Y_{12}$ inhibitor.

The $P2Y_{12}$ assay contains 20micromol/L of adenosine diphosphate (ADP) as the principle agonist of platelet aggregation. ADP however, can activate platelet aggregation via both the P2Y1 and P2Y12 cell surface receptors. In order to reduce platelet activation via the $P2Y_1$ receptor, a second agonist prostaglandin E_1 (PGE_1) was also added to the vacuette (Bouman, Parlak et al. 2010).

The magnitude of arachadonic acid induced platelet activation is expressed as aspirin response units (ARU) and the magnitude of ADP-induced platelet activation is expressed as P2Y12 reaction units (PRU).

4.6.1.1 Interpretation of VerifyNow pharmacodynamic data

4.6.1.1.1 The P2Y12 reaction unit (PRU)

The measure of P2Y12 reaction unit (PRU) provides an indication of the degree of ADP-mediated aggregation specific to the P2Y12 receptor:

- PRU ≥ 230 is indicative of high on treatment platelet reactivity and patients who display such high values are termed "clopidogrel non-responders".
- PRU ≥ 208 indicates patients are at increased risk of experiencing an adverse cardiac event.
- PRU ≤ 95 indicates an increased risk of experiencing bleeding complications.

These values suggest that there is a "therapeutic PRU window" which provides insights into the degree optimal inhibition of platelet aggregation that can be achieved with an antiplatelet agent, whilst minimising bleeding complications, and can be found at PRU values between 95-208.

4.6.2 VASP Flow Cytometry

VASP is another assay commonly used to determine the degree of P2Y12 inhibition; a flow cytometric analysis of the vasodilator-stimulated phosphoprotein phosphorylation provides an indication of the platelet reactivity index (% PRI). A high PRI value (>50%) is indicative of poor levels of platelet inhibition (Sinhal and Aylward 2013).

VASP, an intracellular actin regulatory protein, is a substrate of both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) dependent protein kinases (Waldmann, Nieberding et al. 1987, Aleil, Ravanat et al. 2005). The final platelet reactivity index (PRI) generated following a VASP analysis provides an indication of the degree of P2Y12 inhibition/activation seen in the presence of a P2Y12 inhibitor. P2Y12 receptor stimulation leads to the dephosphorylation of VASP and inhibition of the P2Y12 receptor, for example, by clopidogrel and stimulation of the PGE_1-activated adenylcyclase induces phosphorylation of VASP by cAMP dependent protein kinase. Consequently, the resulting levels of VASP phosphorylation/dephosphorylation provide an indication of $P2Y_{12}$ receptor activation/inhibition (Aleil, Ravanat et al. 2005).

Although VASP flow cytometry is commercially available as an assay (Biocytex, Asnieres, France), it is a labour intensive, complex and highly specialised procedure that requires significant technical expertise in order to conduct the test accurately. As such it is not freely available in cardiac treatment centres and is primarily housed in research /higher education

institutes. For the purposes of my study, I assisted in the preparation of samples for analysis within the cardiac department at RWH. Prepared samples were stored for no more than 48 hours within our Clinical Chemistry department, prior to transfer to The University of Wolverhampton.

The method for carrying out VASP flow cytometry has been previously described by Bonello et al (Bonello, Camoin-Jau et al. 2008), but in summary, whole blood is transferred to tubes containing 3.2% sodium citrate and incubated for 10 minutes with PGE_1 or with PGE_1 and ADP and then fixed with paraformaldehyde. The platelets are then permeablised with a non-ionic detergent prior to being "labeled with a primary monoclonal antibody against serine 239-phosphorylated VASP (16C2), followed by a secondary fluorescein isothiocyanate-conjugated polyclonal goat antimouse antibody"(Aleil, Ravanat et al. 2005)

The VASP flow cytometry analysis was performed on a Becton Dickinson (Plymouth, UK) FACS Calibur flow cytometer, and 10,000 platelets were gated per assessment as per the manufacturers instructions. VASP flow cytometry was performed within 48 hours of sampling and the subsequent results were expressed as the percentage platelet reactivity (%PRI) (Aleil, Ravanat et al. 2005, Cotton, Worrall et al. 2010). The VASP-phosphorylation analysis was undertaken within the School of Applied Science at the University of Wolverhampton.

4.6.2.1 Interpretation of VASP-P pharmacodynamic data

VASP-PRI (%) provides an indication of platelet reactivity following the administration of a P2Y12 inhibitor:

- A PRI (%) value =100% is indicative of a complete lack of antiplatelet effect
- PRI (%) value >50% is indicative of poor levels of platelet inhibition (Sinhal and Aylward 2013).
- A PRI (%) value < 50% is indicative of adequate levels of platelet inhibition
- A PRI (%) value at or near 0% is indicative of excessive levels of platelet inhibition.

4.7 Pharmacokinetic Analysis

Liquid chromatography in tandem with mass spectrometry (LC-MS/MS) was used to determine the plasma concentrations of clopidogrel and prasugrel active metabolites, as well as the presence of ticagrelor parent compound and active metabolite, using already established and validated methods (Farid, McIntosh et al. 2007, Takahashi, Pang et al. 2008, Sillen, Cook et al. 2010, Peer, Spencer et al. 2012). The active metabolites of clopidogrel and prasugrel are not stable in blood and therefore to ensure metabolite stability during sample handling and storage, 2-bromo-3'methoxy acetophenone (MPB) was added within 30 seconds of sample collection to the blood collected in the EDTA tubes. MPB, an alkylating agent, was therefore, used to derivatise and stabilise the active metabolites (Payne, Li et al. 2007). The

derivatised samples were centrifuged at 1500g for 10 minutes, the plasma was then extracted and frozen at -80C until analysis.

Blood samples for ticagrelor parent compound and active metabolite quantification were collected in lithium tubes; the ticagrelor parent compound and active metabolite are stable in blood and therefore did not require the addition of a stabilising agent

The derivatised clopidogrel and prasugrel samples and ticagrelor samples underwent solid phase extraction before being separated using liquid chromatography and quantified using mass spectrometry. A Principal Clinical Scientist carried out this activity in the Clinical Chemistry department at RWH.

The primary pharmacokinetic parameters (i.e. the active metabolites of clopidogrel, prasugrel and ticagrelor) are expressed as plasma concentration (ng/ml) (Farid, McIntosh et al. 2007).

4.7.1 Interpretation of LC-MS/MS pharmacokinetic data

Previously reported pharmacokinetic studies, indicate that maximal antiplatelet effects of clopidogrel, prasugrel and ticagrelor are observed when the following peak plasma concentrations are observed following LC-MS/MS:

- In healthy patients a clopidogrel active metabiolite (C-AM) concentration of 43ng/ml is associated with a maximal antiplatelet effect (Taubert, Kastrati et al. 2004).

- Prasugrel active metabolite (P-AM) concentration between the range of 87 ng/ml to 512 ng/ml have been documented in healthy patients (Brandt, Payne et al. 2007, Payne, Li et al. 2007, Cattaneo 2010).
- Ticagrelor parent compound (T-PC) and ticagrelor active metabolite (T-AM) concentrations of 549 ng/,ml and 135ng/ml, respectively, were associated peak antiplatelet effects in patients with stable coronary artery disease (SCAD) (Wallentin 2009).

4.8 Statistics and Data Analysis

All statistical analyses were undertaken using SPSS (IBM SPSS Statistics for Mac, Version 21.0. Armonk, NY: IBM Corp). Continuous variables were expressed as mean ± SD and categorical variables as frequencies (%). Continuous variables were analysed individually using student's independent sample t-tests. Categorical variables were assessed using separate Fisher's exact (Chi-square) test. A p value < 0.05 was considered to be statistically significant.

Comparison of means between groups was assessed using analysis of variance (ANOVA) technique. ANOVA allowed for a comparison of more than two means and enabled an assessment to be made of the relationship between, different drugs (clopidogrel vs prasugrel vs ticagrelor), different clinical states (STEMI vs NSTEMI/UA) and different time points.

In order to determine the relationship between the different platelet function assays utilised and the plasma concentrations observed following LC-MS/MS, a Pearson Correlation Co-efficient test was used.

4.8.1 Interim Analyses

Due to the small study population, an interim analysis was not be performed.

4.8.2 Number of subjects to be enrolled

Since this was a pilot study, power calculations were not undertaken to determine sample size; fifteen patients were recruited into each group. This study was non-randomised such that current local and international guidelines could be fully adhered to.

4.8.3 Definition of the end of the trial

The trial aimed to recruit 15 patients to each arm; on collection of the four-hour sample in patient 90, all data collection was discontinued.

4.9 Ethical Considerations

The study was carried out in line with the spirit and the letter of the Declaration of Helsinki and in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice Guidelines.

4.9.1 Consent

All patients freely gave their informed assent and/or consent to participate in the study in accordance with the process outlined below. Patients were able to withdraw from the study at any time without any compromise to their future care.

STEMI patients were likely to be acutely unwell and therefore, it would be inappropriate to request written consent the time of admission. In order to avoid unnecessary delays in treatment initiation, a two-staged consent process was implemented. STEMI patients were read a shortened patient information sheet (appendix 10) enabling them to provide verbal assent.

Because the study medications were still to be administered as per local protocol, this approach would not delay urgent medical intervention and initiation of PPCI, while allowing collection of the first 3 blood samples. This practice was in line with National Research Ethics Service (NRES) guidelines, which suggest that patients admitted with a diagnosis of STEMI are considered as unconscious patients initially. If verbal consent was gained post-procedure, it was recorded in the patient's clinical notes and the site file consent record.

The second stage of consent was initiated once the patient was pain free and following a period of rest and stabilisation on return to the ward. At this time, a full patient information leaflet was given to the patient and written consent sought and documented in the medical notes. If the patient did not wish to participate in the study, their collected samples would be discarded and their data not used for analysis. However, this did not occur with the patients we recruited since all gave their full consent for the collection and subsequent analysis of samples obtained throughout the study period.

For UA/NSTEMI patients, full written consent was requested and recorded after the patient was able to read the full patient information sheet.

The patient's participation in the trial ceased with the collection of the four-hour sample. Patient data was fully anonymised and stored on RWH fully encrypted computers.

4.9.2 Ethics Approval

An initial study protocol was seen, reviewed and approved by RWH R&D peer review and ethics committee. The initial study protocol consisted of four groups only, as outlined in table 13.

Table 13. Patient allocations to treatment groups (prior to substantial amendment and REC re-submission).

Group 1 (n=15)	Patients admitted with STEMI who were under the age of 75 years and greater than 60kg were administered prasugrel 60mg as a single loading dose followed by 10mg daily as a maintenance dose.
Group 2 (n=15)	Diabetic patients admitted with NSTEMI who were under the age of 75 years and greater than 60kg were administered prasugrel 60mg as a single loading dose, followed by a 10mg maintenance dose
Group 3 (n=13)	Patients admitted with STEMI who were over the age of 75 years or under 60kg were administered clopidogrel 600mg as a single loading dose followed by 75mg daily as a maintenance dose.
Group 4 (n=14)	Patients admitted with UA/NSTEMI who were over the age of 75 years under 60kg were administered clopidogrel 600mg as a single loading doses followed by 75mg daily as a maintenance dose.

Ethical approval for the above study protocol and patient groups was granted by the South Birmingham Research and Ethics Committee (REC reference number: 13/WM/0025 – appendix 13) in January 2013 and the pilot study was designated a non-CTIMP.

At the time at which the initial protocol was presented to the South Birmingham REC, ticagrelor was a non-formulary medication at RWH. In January 2013 the South Birmingham REC were informed that once patient recruitment to the clopidogrel and prasugrel groups was near to completion, a formulary application would be submitted for the inclusion of ticagrelor to our hospital formulary. In parallel to this process we would request a substantial amendment to allow for its inclusion in the study protocol.

Due to difficulties in recruiting to the diabetic NSTEMI prasugrel group (group 2), it was decided that as part of the substantial amendment, in addition to the

inclusion of ticagrelor, we would also request extension of the eligibility criteria for group 2, to include non-diabetic NSTEMI patients also.

In June 2014, a substantial amendment was submitted to IRAS, in view of the amendment to group 2, the study was deemed to be a CTIMP and therefore required MHRA approval also. All patient recruitment was subsequently halted until the necessary approvals were granted.

The amended protocol was presented to the Coventry and Warwick REC in November 2014, following which a favourable opinion was granted (REC reference number 14/WM/1236 – appendix 14). MHRA approval was granted on February 13th 2015 an active patient recruitment recommenced on February 16th 2015 and the final patient was recruited to the study on December 14th 2015.

The final study protocol consisted of six groups that are outlined in Table 11. Recruitment to groups 5 and 6 (ticagrelor groups) commenced only after all patients were recruited to groups 1 and 2 (prasugrel groups). The switch over from prasugrel to ticagrelor also coincided with the adoption of a new antiplatelet prescribing protocol within the Black Country (Wolverhampton, Walsall and Dudley) in which ticagrelor became our first line antiplatelet for STEMI patients.

Chapter 5 - A Pharmacokinetic and Pharmacodynamic Assessment of Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction

5.1 Introduction

A detailed overview of clopidogrel, its mechanism of action, clinical efficacy and place in therapy has been covered extensivley in chapter 1.

5.2 Objectives

This chapter aims to compare the degree and time course of platelet inhibition observed following the administration of a clopidogrel 600mg loading dose in STEMI and NSTEMI patients. In addition, we will also determine whether the state of STEMI per se adversely affects the clinical efficacy of clopidogrel.

5.3 Ethical Considerations and Consent

All ethical considerations and procedures for patient consent and recruitment are described in Chapter 4.

5.4 Materials and Methods

All materials and methods are described in Chapter 4.

5.5 Statistics and Data Analysis

The methods for data collection and statistical analyses are outlined in chapter 4.

5.6 Results

Table 14. Baseline patient characteristics - clopidogrel treatment group

Characteristic	STEMI (n = 13)	NSTEMI (n = 14)	*P-value*
Age (yrs)	78 ± 9.7	62.4 ± 15.4	0.005
Sex			
Female	6 (46)	2 (14)	0.103
Risk Factors			
Diabetes Mellitus	0 (0)	2 (14)	0.481
Hypertension	6 (46)	7(50)	1.000
Current Smoker	2 (15)	3 (21)	1.000
Ex Smoker	4 (31)	7(50)	0.440
Hyperlipidaemia	4 (31)	8 (57)	0.252
Familial History of CAD	6 (46)	6 (43)	1.000
Previous MI	2 (15)	6 (43)	0.209
Previous PCI	2 (15)	3 (21)	1.000
Previous CABG	0 (0)	0 (0)	1.000
Previous stroke/TIA	1 (8)	0 (0)	0.481
Patient therapy on admission			
Analgesia	10 (77)	7 (57)	0.236
Comprising of:-			
Morphine	10	2	0.002
GTN	0	5	0.041

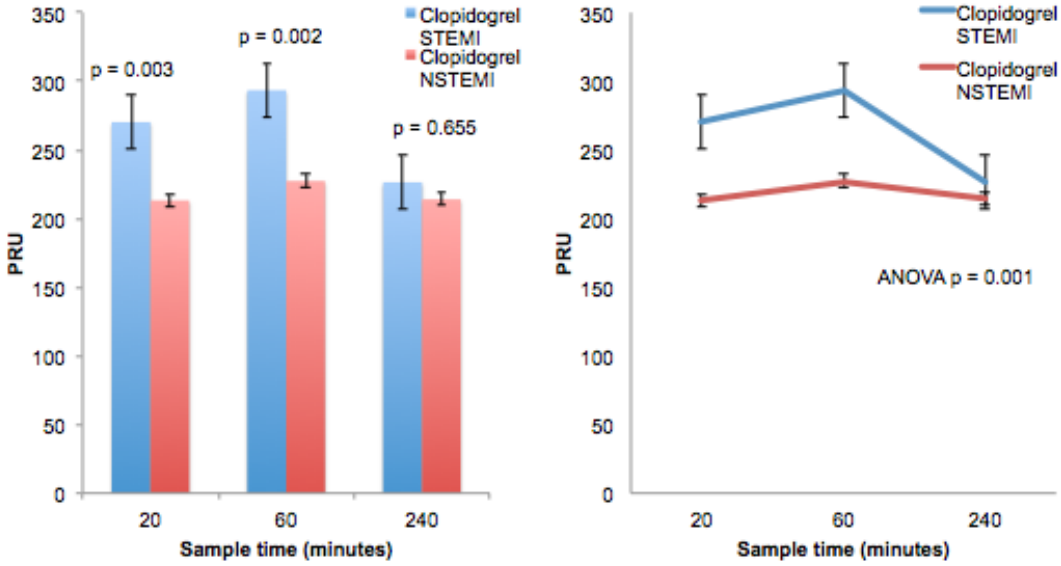
5.6.1 Pharmacodynamic analysis of the degree of platelet inhibition following clopidogrel loading (600mg) STEMI vs NSTEMI

5.6.1.1 VerifyNow Results

Table 15. Clopidogrel - VerifyNow (PRU)

Verify Now result expressed as PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	13	270.23	57.02 [20.62,93.42]	38.56	10.693	0.003
	NSTEMI	14	213.21		51.74	13.828	
60 minutes	STEMI	13	293.46	66.10 [27.01, 105.20]	31.68	8.787	0.002
	NSTEMI	14	227.36		61.19	16.355	
240 minutes	STEMI	12	226.42	12.42 [-44.20,69.03]	69.44	20.046	0.655
	NSTEMI	14	214.00		69.98	18.702	

Figure 17. VerifyNow mean PRUs (and standard error) after the administration of clopidogrel in STEMI vs NSTEMI patients.



5.6.1.1.2 Summary

Following administration of a 600mg loading dose of clopidogrel, this data indicates a statistically significant difference in mean PRU values between STEMI and NSTEMI samples taken at 20 minutes (270.2 ± 10.69 vs 213.12 ± 13.83 p<0.003) and 60 minutes (293.5 ± 8.79 vs 227.4 ±16.36 p < 0.002).

At 240 minutes following the administration of a 600mg loading dose of clopidogrel, there is no statistically significant difference in the mean PRU values observed between the STEMI and NSTEMI groups (226.4 ± 20.05 vs 214 ±18.70 p = 0.655).

The data in table 15 were analysed using a two-way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (PRU) over time was examined. There was no significant difference between the effect of disease state on the degree of platelet inhibition, F(2,23) = 3.094, p = 0.065. However, there was a highly significant

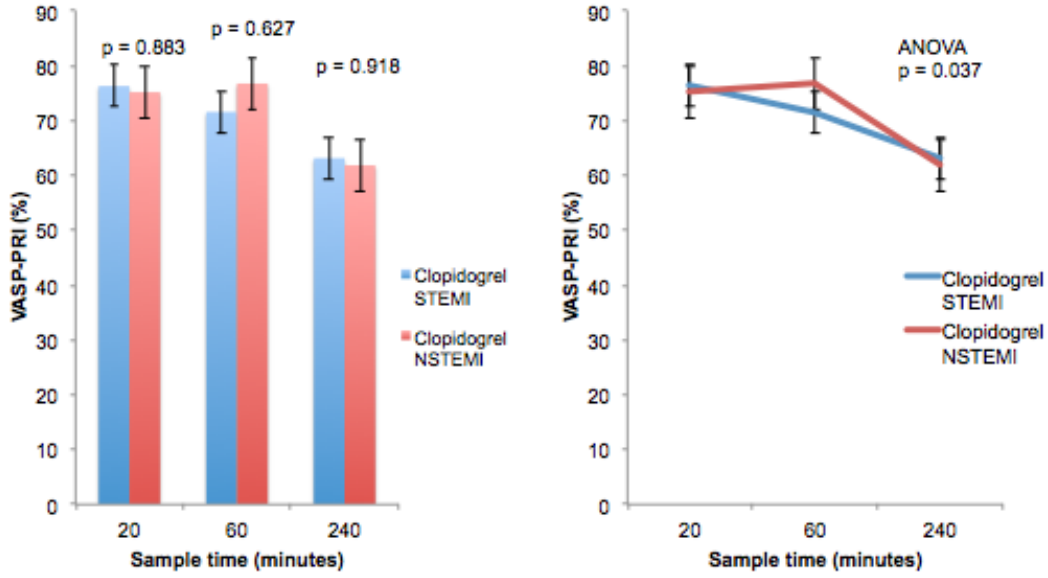
increase in the degree of platelet inhibition observed over time (that is at 240 minutes compared to 20 minutes), F(2,23) = 8.890, p = 0.001.

5.6.1.2 VASP Results

Table 16. Clopidogrel - VASP-PRI (%)

VASP-PRI (%) result post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	7	76.29	1.12 [-14.69,16.93]	23.214	8.774	0.883
	NSTEMI	12	75.17		9.476	2.735	
60 minutes	STEMI	7	71.57	5.18 [-27.26,16.90]	30.908	11.682	0.627
	NSTEMI	12	76.75		15.076	4.352	
240 minutes	STEMI	7	63.14	1.31 [-25.19, 27.81]	35.08	13.26	0.918
	NSTEMI	12	61.83		20.16	5.82	

Figure 18 Mean VASP-PRI (%) (and standard error) after the administration of clopidogrel 600mg in STEMI vs NSTEMI patients.



5.6.1.2.1 Summary
Following administration of a 600mg loading dose of clopidogrel, this data does not indicate a statistically significant difference in mean VASP-PRI (%)

at 20 minutes (76.29 ± 8.77 vs 75.17 ± 2.74 p<0.883) at 60 minutes (71.57 ± 11.68 vs 76.75 4. ± 35 p < 0.002) and at four hours (63.14 ±13.26 vs 61.83 ± 5.82 p = 0.510) following the administration of a 600mg loading dose of clopidogrel, there are no statistically significant difference in the mean VASP-PRI (%) values observed between the STEMI and NSTEMI groups.

The data in table 16 were analysed using a two-way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (expressed as mean %PRI) over time was examined. No significant difference between the effect of disease state on the degree of platelet inhibition was observed, $F(2, 16) = 0.702$, $p = 0.510$. However, a significant increase in the degree of platelet inhibition over time was observed in both groups, $F(2,26) = 4.060$, $p = 0.037$.

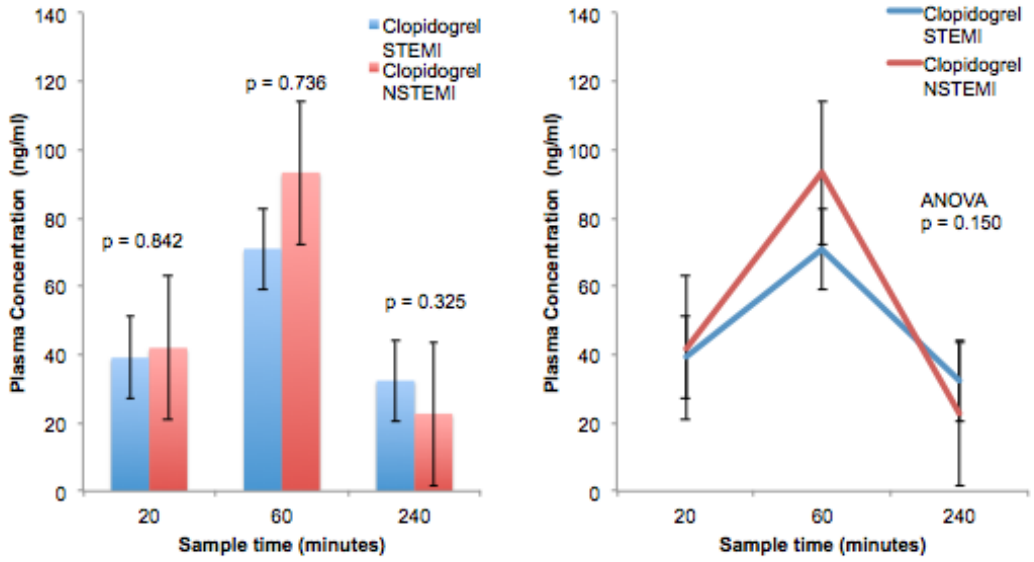
5.6.2 Pharmacokinetic analysis of active metabolite generation following clopidogrel loading (600mg) STEMI vs NSTEMI

Liquid Chromatography/Mass Spectrometry Results

Table 17. Clopidogrel active metabolite plasma concentration (ng/ml)

Active metabolite generation (ng/ml) result post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	12	39.12	-6.37 [-72.01, 59.26]	56.00	16.17	0.842
	NSTEMI	12	45.49		94.25	27.21	
60 minutes	STEMI	12	71.02	-19.10 [-134.42, 96.23]	94.24	27.21	0.736
	NSTEMI	14	90.11		172.43	46.09	
240 minutes	STEMI	12	32.41	9.70 [-10.26, 29.65]	23.23	6.71	0.325
	NSTEMI	12	22.71		23.90	6.90	

Figure 19. Mean active metabolite generation (ng/ml) (and standard error) after administration of Clopidogrel in STEMI vs NSTEMI patients.



5.6.2.1 Summary

Following administration of a 600mg clopidogrel loading dose, the degree of clopidogrel active metabolite generation as assessed by liquid chromatography in tandem with mass spectrometry indicates that there is no difference in the generation of clopidogrel active metabolite at any of the time points following loading between the two groups (table 17).

The data in table 17 were analysed using a two-way analysis of variance test in which the extent of clopidogrel active metabolite generation/plasma concentration over time in STEMI vs NSTEMI patients was examined. There was no significant difference between the effect of disease state on the generation of clopidogrel active metabolite, $F(2, 19) = 0.133$, $p = 0.876$. There was no significant difference in the degree of active metabolite generation observed over time, $F(2,19) = 2.104$, $p = 0.150$.

5.6.3 Correlation between platelet function assays (VerifyNow and VASP-PRI) and active metabolite generation.

A Pearson's Correlation Co-efficient test was undertaken to determine the strength of the relationship between the two platelet function assays utilised during the study. Since all VerifyNow samples were collected and assessed at the base hospital and the results generated are more reliable, this test has been used as the baseline against which to compare the VASP-PRI (%) and active metabolite plasma concentration (ng/ml) results.

Table 18. Correlation the degree of platelet inhibition observed using VerifyNow compared with VASP-PRI following the administration of a clopidogrel 600mg loading dose

	VASP-PRI			
	STEMI		**NSTEMI**	
VerifyNow	**r**	**p-value**	**r**	**p-value**
20 minutes post loading	0.528	0.223	-0.178	0.581
At Balloon Inflation	0.657	0.109	x	x
60 minutes post loading	-0.059	0.900	0.063	0.845
240 minutes post loading	0.636	0.125	0.523	0.081

Both VerifyNow (expressed as PRU) and VASP-PRI (%) results provide an indication as to the degree of platelet inhibition achieved following the oral administration of a clopidogrel loading dose to both STEMI and NSTEMI patients.

Table 19. Correlation between the degree of platelet inhibition observed using VerifyNow compared with clopidogrel active metabolite plasma concentration following the administration of a 600mg loading dose.

	Clopidogrel active metabolite plasma concentration			
	STEMI		NSTEMI	
VerifyNow	r	p-value	r	p-value
20 minutes post loading	-0.005	0.989	-0.273	0.390
At Balloon Inflation	-0.096	0.767	x	x
60 minutes post loading	-0.124	0.700	-0.324	0.280
240 minutes post loading	0.412	0.183	0.481	0.113

Based on the mechanism of action of clopidogrel and the nature of the platelet function assays, one would expect a negative correlation between the degree of platelet inhibition (PRU) and the amount of active metabolite generated in both the STEMI and NSTEMI groups.

5.7 Discussion

Baseline patient characteristics are described in table 14. There are no significant differences with regards to risk factors/co-morbidites between the STEMI and NSTEMI treated clopidogrel groups. There are distinct and clinically significant differences between the two groups with regards to the administration of opioid-based analagesia. These differences are based on guideline recommendations that do not advocate the administration of opioids in NSTEMI patients in view of the increase in mortality associated with this practice (Meine, Roe et al. 2005). As a result there is a statistically significant difference in the number of patients administered morphine between the STEMI and NSTEMI groups ($p = 0.002$).

In terms of the patient population recruited to the clopidogrel arm of the study, a statistically significant difference in the mean age of patients recruited to the STEMI group in comparison to the NSTEMI group (78 ± 9.7 vs 62.4 ± 15.4 p = 0.005) is apparent. At the time at which the study was commenced, our hospital prescribed both prasugrel and clopidogrel for STEMI patients; the licensing of prasugrel is such that its use in older patients (i.e over the age of 75 years) and lighter patients (under 60kg body weight) is not recommended due to an increased associated risk of bleeding complications in these groups. As a consequence, the default P2Y12 inhibitor in patients aged over 75 years was clopidogrel in this study.

5.7.1 Pharmacodynamic analysis - VerifyNow

This pharmacodynamic VerifyNow data reported indicates that at the time of PPCI and for at least four hours after the administration of a 600mg loading dose in STEMI patients, clopidogrel does not afford sufficient IPA, since all values are >208 PRU (figure 17). Furthermore, there is no significant difference in the observed degree of platelet inhibition over time in the NSTEMI group. This is in contrast to the pharmacodynamic data derived from healthy volunteers in whom the administration of a 600mg loading dose leads to 40 – 45% reduction in inhibition of platelet reactivity after 2-3 hours (Wallentin, Becker et al. 2009, Floyd, Passacquale et al. 2012).

This VerifyNow data further indicates that in the context of NSTEMI, although a significant difference in terms of the PRU at 20 and 60 minutes is calculated when compared to the STEMI group ($p = 0.003$ and $p = 0.002$ respectively), the administration of a clopidogrel 600mg loading dose does not lead to

sufficient levels of platelet inhibition as demonstrated by PRU values > 208 at all data collection time points (figure 17). Therefore, this VerifyNow pharmacodynamic data indicates a lack of platelet inhibition over time, in addition, to no difference between the STEMI and NSTEMI groups. This is the first study to demonstrate reduced antiplatelet effectiveness of clopidogrel in STEMI and NSTEMI patients using VerifyNow, as such there are no baseline data against which we can compare our NSTEMI results.

5.7.2 Pharmacodynamic analsysis – VASP-PRI(%)

VASP phosphorylation provides an indication of ADP induced platelet reactivity and subsequent clopidogrel induced platelet inhibition, the final value is expressed a percentage of the platelet reactivity index (% PRI). VASP-PRI (%) values can also be used to indicate the pharmacodynamics effects of clopidogrel following the administration of a loading dose. A VASP-PRI (%) > 50% provides an indication of an inadequate response to ADP-induced platelet aggregation and in the context of clopidogrel can be viewed as “non-response” (Cuisset, Grosdidier et al. 2013). A PRI% >50% is associated with an increased risk of recurrent ischaemic and thrombotic events e.g. MACE and stent thrombosis. A PRI% <50% is indicative of an adequate antiplatelet response with consequent inhibition of platelet activity. A PRI% value of 100% would indicate a complete absence of clopidogrel effect and a lack of any antiplatelet cover.

This data demonstrates that in STEMI patients administered a 600mg clopidogrel loading dose, there is insufficient clopidogrel induced inhibition of

platelet of activity, since all % VASP-PRI values are ＞50%. Indicating inadequate levels of platelet inhibition over time, this trend is observed in our NSTEMI patient group also (figure 18). When comparing the VASP-PRI (%) results between the STEMI and NSTEMI groups, there is no observed difference in platelet inhibition at all time points, indicating a lack of pharmacodynamics effect in both disease states.

On examining the relationship the VerifyNow and VASP phosphorylation assays (table 18), there is a reasonable to high correlation between the results generated using both assay. Both VerifyNow and VASP phosphorylations assays confirm that the pharmacodynamic effect of clopidogrel is suboptimal following the administration of a 600mg loading dose in both STEMI and NSTEMI patients, since neither assay demonstrates adequate levels of platelet inhibition.

5.7.3 Pharmacokinetic analysis – LC-MS/MS

The pharmacokinetic analysis of clopidogrel active metabolite generation was assessed as the maximum plasma concentration determined using liquid chromatography in tandem with mass spectrometry. A number of analyses have been undertaken to determine the pharmacokinetic profile of clopidogrel and its active metabolites following the administration of a 600mg loading dose. The works of Taubert et al have postulated that a peak plasma concentration of clopidogrel active metabolite of 43 ng/ml leads to a maximal antiplatelet effect (Taubert, Kastrati et al. 2004). This is in contrast to the data

reported in this thesis, which shows widely variable plasma concentrations of active metabolite (figure 19).

The observed LC-MS/MS data demonstrates a non-linear relationship between the time from administration and plasma concentration observed. The plasma concentration in both STEMI and NSTEMI groups at 20 minutes and 60 minutes indicates a positive trend; increasing plasma concentrations with time, however at 240 minutes, the plasma concentration in both groups is less than that seen at 20 minutes.
Furthermore, these results indicate that the disease state of STEMI does not have any effect on active metabolite generation when compared with the NSTEMI patient group, as demonstrated by non-significant differences in mean plasma concentration values obtained at each time point (figure 19).

On examination of the relationship between pharmacodynamic and pharmacokinetic profiles captured following the administration of a clopidogrel 600mg loading dose (table 19), no significant correlation was observed between the results of the degree of platelet inhibition assessed using the VerifyNow platelet function assay and plasma concentration of the clopidogrel active metabolite in the STEMI and NSTEMI groups at 20 and 60 minutes. However, at 240 minutes, in both groups, a reasonable positive correlation between the degree of platelet inhibition and plasma concentration was calculated. In practice, this final result seems somewhat spurious since the plasma concentration at 240 minutes is less than that observed at 20 minutes. There is no reasonable explanation for this trend.

5.7.4 General overview

Platelet adherence, activation and subsequent aggregation is a core component in the initiation and propagation of atherothrombosis and consequent ischaemic and thrombotic complications in patients who present with ACS and are managed with PCI. Despite the administration of aspirin and clopidogrel, up to 25% of patients who undergo PCI with subsequent coronary artery stent implantation are clopidogrel non-responders and are therefore at increased risk of recurrent ischaemic events and thrombotic complications such as stent thrombosis, further myocardial infarction or death (Matetzky, Shenkman et al. 2004, Wallentin 2009).

Different clinical manifestations of ACS will have varying effects on platelet behaviours (Mathur, Robinson et al. 2001). As described by Alexopolous et al the condition of STEMI is a highly pro-thrombotic state in which platelet reactivity is greatly enhanced in comparison to patients with stable coronary artery disease (Alexopoulos 2013). Platelet reactivity becomes heightened during the acute phase of a myocardial infarction, this in combination with a revascularization strategy of percutaneous coronary intervention, justifies the need for potent platelet inhibition, since PCI itself can cause further endovascular injury, inflammation and additional activation of platelets (Sibbing, Kastrati et al. 2016)

Platelet response to endothelial dysfunction/injury in STEMI is markedly different to that seen in UA/NSTEMI; the former is associated with a high thrombotic burden with significantly enhanced levels of platelet activation

(intrinsic platelet reactivity) even prior to the administration of clopidogrel (Alexopoulos, Xanthopoulou et al. 2013). Intrinsic platelet reactivity can contribute high levels of platelet reactivity even after the administration of high-dose clopidogrel (P2Y12 inhibition), this phenomenon is known as high residual platelet reactivity (HRPR) and is associated with poor clinical outcomes (Frelinger, Michelson et al. 2011, Garabedian and Alam 2013).

The high pre-treatment platelet reactivity observed in STEMI has a significantly detrimental effect on the time dependent inhibition of platelets after clopidogrel loading. Hence the statistically significant increase in mean PRU at 20 minutes and 60 minutes in the STEMI group compared with the NSTEMI group ($p = 0.003$).

Following oral administration, the need for clopidogrel to undergo gastrointestinal absorption and metabolic biotransformation into its active metabolite, creates a time lag between oral administration and clinical effect in terms of inhibition of platelet activity and hence the delayed antiplatelet effect seen in both STEMI and NSTEMI patients (Cattaneo 2008). As a consequence, clopidogrel is subject to both increased intrinsic (elevated baseline) platelet reactivity and high residual platelet reactivity. This may well account for the increased PRU (>230 and 208) and %PRI (>50%) values observed in the STEMI patients.

Clopidogrel treatment failure or lack of clinical efficacy is attributable to unpredictable pharmacodynamics and variable pharmacokinetics observed

following the administration of either loading and/or maintenance doses (Floyd, Passacquale et al. 2012). Clinical outcomes in terms of reduction in further MACCE and complications such as stent thrombosis will vary depending on whether the patient is a clopidogrel non-responder or not. The variability in pharmacokinetic and pharmacodynamics profiles and subsequent response to clopidogrel therapy can occur secondary to a number of factors, as described below.

The physiological changes that occur during a STEMI and in particular the heightened platelet reactivity that manifests secondary to endovascular dysfunction and/or stent implantation are known to reduce the effectiveness of clopidogrel (Aradi, Vorobcsuk et al. 2010, Alexopoulos, Xanthopoulou et al. 2013).

The diversion of blood flow away from the gut during a myocardial infarction leads to reduced/delayed gastrointestinal absorption (Heestermans, van Werkum et al. 2008). In addition, variations in P-gp efflux co-transporter may affect clopidogrel absorption via the intestine (Floyd, Passacquale et al. 2012).

Genetic polymorphisms in the cytochrome P450 2C19 allele (and to a lesser extent the CYP450 3A4 allele), can lead to marked differences in the activity of these enzymes; these differences often correlate with the variability in the degree of inhibition of platelet activity observed in patients (Wallentin 2009). The mean prevalence of clopidogrel non-response is 21%, polymorphisms

within the 2C19 allele is known to account for only 12% of the variability observed in clopidogrel response (Perry and Shuldiner 2013). Thereby indicating that the variability in response seen is multifactorial and is attributable to both genetic (C19 loss of function allele, P-gp which is encoded by ABCB1) and non-genetic (smoking, diabetes, BMI, concomitant drug therapy) factors.

Although weight was not always documented in the case report forms, the data collated in chapter 2 indicates that patients treated with clopidogrel in our centre have an average BMI of 26.6 ± 4.8, and are therefore considered to be overweight. Patient demographics from table 14 indicate that there were no statistically significant differences between the STEMI and NSTEMI groups with regards to smoking status, incidence of diabetes or concomitant drug therapies (with the exception of morphine). Smoking is known to alter CYP450 levels and can adversely affect drug metabolism and biotransformation. In comparison to those with a normal weight, patients who are overweight, demonstrate higher levels of platelet reactivity and may display a sub-optimal response to clopidogrel therapy (Wallentin 2009). The presence of diabetes is associated with high levels of platelet reactivity and an impaired response to clopidogrel (Wallentin 2009).

Advancing age, is also an important independent predictor of an individual's response to clopidogrel; the ability of clopidogrel to lead to sufficient levels of platelet inhibition during the acute phase of a MI is dependent on the patients age. Advancing age not only leads to a reduction in gastric motility and

absorption, but is also associated with a reduction in the activity of CYP450 substrates in the liver which can compromise the ability of an older patient to convert the clopidogrel prodrug into its active metabolite (Gurbel, Bliden et al. 2003, Maree and Fitzgerald 2007). The observed data demonstrates that there is a significant difference in age between our STEMI and NSTEMI groups ($p = 0.005$). This difference is largely driven by the licensing of prasugrel which does not recommend its use in those over the age of 75 years.

A number of drug-drug interactions relating to the P2Y12 inhibitors and medications such as opioids, statins and proton pump inhibitors have also been proposed. However, with the exception of the opioids, co-administration of statins/proton pump inhibitors is not considered to be clinically significant and would not warrant any intervention.

The COGENT study in which the co-administration of omeprazole with clopidogrel was thought to lead to an increase in CV events was actually underpowered to make such an inference. In addition, the period of co-administration was not long enough to draw any firm conclusions regarding the validity of the interaction (Bhatt, Cryer et al. 2010). A number of clinical trials in both healthy patients and in those who present with ACS have quantified the adverse impact of opioid based administration on the clinical efficacy of clopidogrel and indeed all P2Y12 inhibitors (Heestermans, van Werkum et al. 2008, Hobl, Reiter et al. 2015, Parodi, Bellandi et al. 2015).

Clopidogrel biovailability has been shown to be significantly impaired in STEMI patients compared with healthy volunteers and this has been attributed to impaired intestinal absorption of clopidogrel during STEMI. (Heestermans, van Werkum et al. 2008). This reduction in intestinal absorption occurs secondary to a number of physiological changes that occur during a STEMI; reduction in cardiac output, increased sympathetic drive, vasoconstriction of peripheral arteries, reduced gut perfusion, permeability and motility are the main contributors to impaired drug absorption. In addition, the administration of opioid-based analgesia has also been shown to reduce clopidogrel absorption with a consequent reduction in active metabolite generation and possible treatment failure (Hobl, Stimpfl et al. 2014).

Patients who present with high baseline/intrinsic platelet reactivity will as a consequence be predisposed to high residual platelet reactivity and have less antithrombotic protection (Gurbel, Bliden et al. 2003). This in part can be mitigated by the co-administration of intravenous antithrombotic agents (e.g. UFH or GPIs), which have the potential to achieve adequate levels of platelet inhibition during the acute phase for those patients who are scheduled for PCI, however, may predispose patients to bleeding complications.

5.8 Conclusion

In terms of the degree and time course of platelet inhibition following the administration of a 600mg loading dose of clopidogrel, there is no significant reduction in IPA observed over time. Clopidogrel is ineffective for the first four hours after administration in both STEMI and NSTEMI patients.

As a consequence of the physiological state of STEMI, platelet activation secondary to endothelial dysfunction/vascular injury has already occurred; the platelets are activated to such a degree that they have gone past the point at which the P2Y12 inhibitor can exert its antiplatelet effect. The administration of clopidogrel will prevent further platelet aggregation, but won't be able to inhibit the already activated platelets. The results of this study in which the pharmacokinetics and pharmacodynamics of clopidogrel in STEMI vs NSTEMI have been investigated support the already proven concept as to why clopidogrel does not provide maximum efficacy in terms of IPA during an acute STEMI. High residual platelet reactivity secondary to both genetic and non-genetic factors can compromise the clinical efficacy of clopidogrel during the acute phase of an MI.

The inherent limitations of clopidogrel in terms of its sub-optimal characteristics coupled with the physiological changes that occur in a STEMI in addition to opioid administration and age related dysfunction with regards to drug metabolism, may account for the lack of antiplatelet effectiveness observed in our STEMI group. It is apparent that interindividual variability in the degree of intestinal absorption may be an important determinant of the response in variability seen with clopidogrel.

In conclusion, the state of STEMI itself leads to platelet hyperreactivity. The heightened/elevated levels of PRU in the context of STEMI indicate an increase in the degree of intrinsic platelet reactivity even before the administration of the P2Y12 inhibitor. Pre-treatment/high intrinsic platelet

reactivity can lead to high residual platelet reactivity, which in turn can lead to further MACCE and ischaemic complications, particularly in patients treated with clopidogrel.

Subsequent prasugrel and ticagrelor data (chapters 6 and 7) will provide further insights into whether those drugs which provide faster, greater and more consistent levels of platelet inhibition can overcome this increase in intrinsic and high residual platelet reactivity seen during a STEMI.

Chapter 6 - A Pharmacokinetic and Pharmacodynamic Assessment of Prasugrel in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction

6.1 Introduction

A detailed overview of prasugrel, its mechanism of action, clinical efficacy and place in therapy has been covered extensivley in chapter 1.

6.2 Objectives

This chapter aims to compare the degree and time course of platelet inhibition observed following the administration of a prasugrel 60mg loading doses in STEMI versus NSTEMI patients. In addition, we will also determine whether the state of STEMI per se adversely affects the clinical efficacy of prasugrel, during the first few hours after a myocardial infarction.

6.3 Ethical Considerations and Consent

All ethical considerations and procedures for obtaining patient consent and patient recruitment are described in Chapter 4.

6.4 Materials and Methods

All materials and methods are described in Chapter 4.

6.5 Statistics and Data Analysis

The methods for data collection and statistical analyses are outlined in Chapter 4.

6.6 Results

Table 20. Baseline patient characteristics – prasugrel treatment group.

Characteristic	STEMI (n =15)	NSTEMI (n =15)	*P-value*
Age (yrs)	56 ± 12.9	61 ± 7.6	0.214
Sex			
Female	3 (20)	2 (13)	1.000
Risk Factors			
Diabetes Mellitus	4 (27)	7 (47)	0.450
Hypertension	7 (47)	6 (40)	1.000
Current Smoker	3 (20)	1 (7)	0.598
Ex Smoker	8 (53)	7 (47)	1.000
Hyperlipidaemia	5 (33)	5 (33)	1.000
Familial History of CAD	9 (60)	8 (53)	1.000
Previous MI	2 (13)	3 (20)	1.000
Previous PCI	1 (7)	4 (27)	0.330
Previous CABG	1 (7)	0 (0)	1.000
Previous stroke/TIA	0 (0)	0 (0)	-
Patient therapy on admission			
Analgesia	13 (87)	7 (47)	0.050
Comprising of:-			
Morphine	13	12	< 0.001
GTN	0	5	0.017

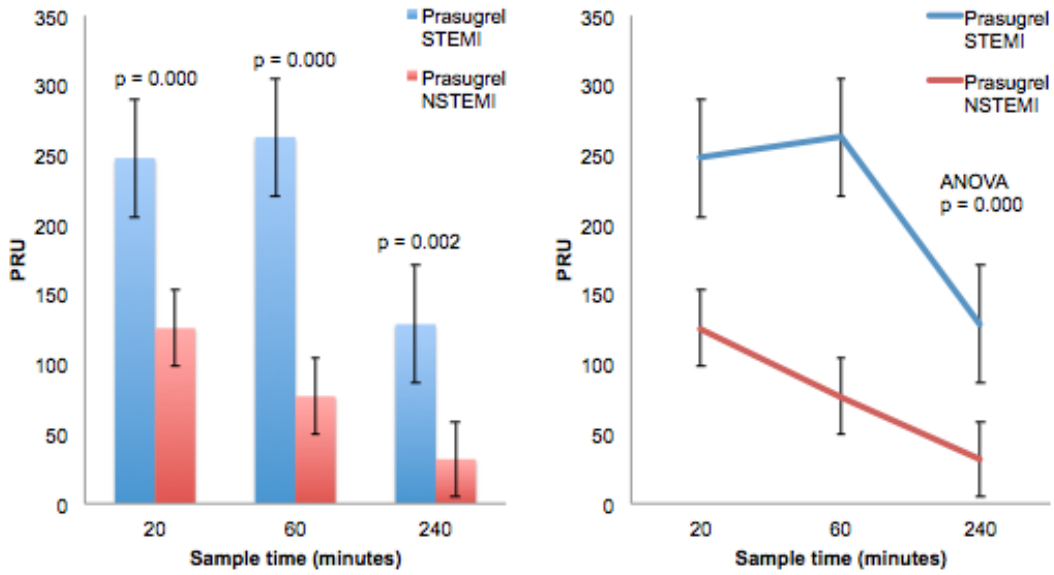
6.6.1 Pharmacodynamic analysis of the degree of platelet inhibition following prasugrel loading (60mg) STEMI vs NSTEMI

6.6.1.1 VerifyNow Results

Table 21. Prasugrel - VerifyNow (PRU)

Verify Now result expressed as PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	15	247.73	121.93 [68.10, 175.77]	48.78	12.60	0.000
	NSTEMI	15	125.80		89.34	23.07	
60 minutes	STEMI	15	262.87	185.93 [128.64, 243.23]	43.43	11.21	0.000
	NSTEMI	15	76.93		99.24	25.62	
240 minutes	STEMI	14	128.64	96.78 [43.49, 150.16]	89.16	23.83	0.002
	NSTEMI	15	31.87		45.52	11.75	

Figure 20. Mean VerifyNow PRUs (and standard error) following the administration of Prasugrel in STEMI vs NSTEMI patients



6.6.1.1.1 Summary

Following administration of a 60mg loading dose of prasugrel, this data indicates a statistically significant difference in mean PRU values between STEMI and NSTEMI samples taken at 20 minutes (247.73 ± 12.60 vs 125.80 ± 83.07 p = 0.000) and 60 minutes (262.87 ± 11.21 vs 79.93 ±25.62 p = 0.000). At four hours following the administration of a 60mg loading dose of prasugrel, there is a highly statistically significant difference in the mean PRU values observed between the STEMI and NSTEMI groups (128.64 ± 23.83 vs 31.87 ± 11.75 p = 0.002).

The data in table 21 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (PRU) over time was examined. There was a highly

significant increase in the degree of platelet inhibition observed over time, for both the STEMI and NSTEMI groups, F(2,26) = 17.897 p = 0.000

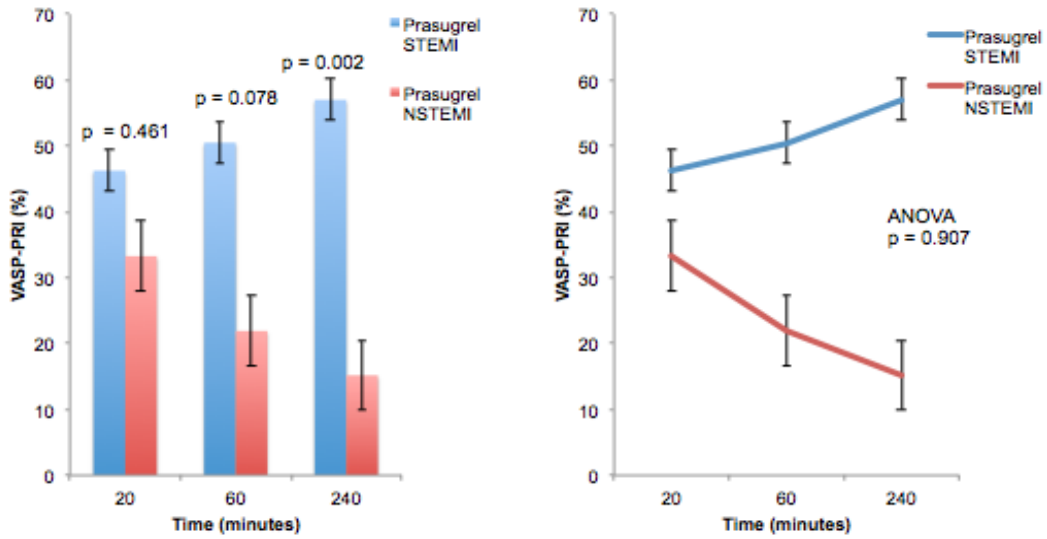
There was highly significant difference between the effect of disease state on the degree of platelet inhibition over time, F(2,26) = 7.114, p = 0.003.

6.6.1.2 VASP-PRI (%) Results

Table 22. Prasugrel – VASP-PRI (%)

VASP-PRI (%) result post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	8	46.25	12.98 [-23.34, 49.30]	33.34	13.91	0.461
	NSTEMI	11	33.27		35.36	10.66	
60 minutes	STEMI	8	50.50	28.59 [-3.57, 60.75]	33.62	11.89	0.078
	NSTEMI	11	21.91		32.23	9.72	
240 minutes	STEMI	8	57.00	41.82 [18.27, 65.36]	29.38	10.39	0.002
	NSTEMI	11	15.18		19.40	5.85	

Figure 21. Mean VASP-PRI% (and standard error) following the administration of Prasugrel in STEMI vs NSTEMI patients.



6.6.1.2.1 Summary

Following administration of a 60mg loading dose of prasugrel, this data indicates a non-significant difference in mean %PRI values between STEMI and NSTEMI samples taken at 20 minutes (46.25 ± 13.91 vs 33.27 ± 10.66 p = 0.461). At 60 minutes there was a significant difference in mean %PRI (50.50 ± 11.86 vs 21.91 ± 9.72 p = 0.078). At four hours following the administration of a 60mg loading dose of prasugrel, there is a highly statistically significant difference in the mean %PRI **v**alues observed between the STEMI and NSTEMI groups (57.00 ± 29.38 vs 15.18 ± 5.85 p = 0.002). The data in table 22 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (PRU) over time was examined. There was a significant difference between the effect of disease state on the degree of platelet inhibition $F(2,16) = 1.004$, $p = 0.389$. The observed reduction in the degree of platelet inhibition over time for both disease states was not significant, $F(2,16) = 0.098$, $p = 0.907$.

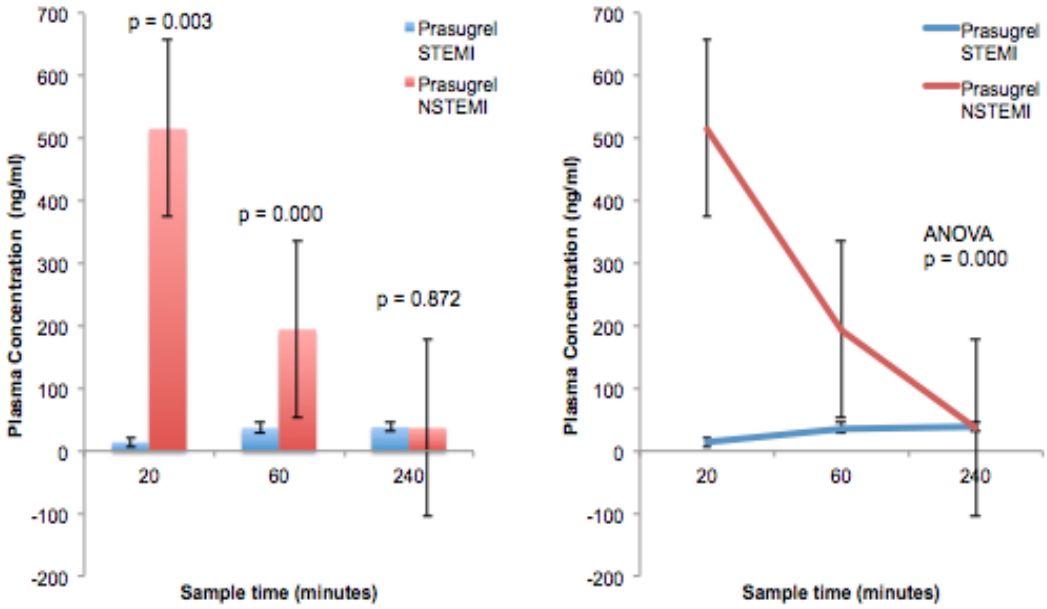
6.6.2 Liquid Chromatography/Mass Spectrometry Results

6.6.2.1 Pharmacokinetic analysis of active metabolite generation following prasugrel loading (60mg) STEMI vs NSTEMI

Table 23. Prasugrel active metabolite plasma concentration (ng/ml)

Plasma concentration of active metabolite (ng/ml) post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	15	14.27	-501.32 [-714.61, -288.44]	34.10	8.80	0.003
	NSTEMI	10	515.80		401.10	126.84	
60 minutes	STEMI	14	36.86	-157.73 [-228.82, -86.65]	49.66	13.27	0.000
	NSTEMI	10	194.59		94.79	29.97	
240 minutes	STEMI	15	38.44	-1.63 [-19.20, 22.47]	29.40	7.59	0.872
	NSTEMI	10	36.80		14.53	4.60	

Figure 22: Mean prasugrel active metabolite plasma concentration (ng/ml) (and standard error) following the administration of a prasugrel 60mg loading dose in STEMI vs NSTEMI patients.



6.6.2.1.2 Summary

Following administration of a 60mg loading dose of prasugrel, this data indicates a highly significant difference in mean plasma concentration of P-AM (ng/ml), between STEMI and NSTEMI samples taken at 20 minutes (14.27 ± 8.80 vs 515.80 ± 126.38 p = 0.003). At 60 minutes there was a highly significant difference in mean plasma concentration (ng/ml) (36.86 ± 13.27 vs 194.59 ± 29.97 p = 0.000). At four hours following the administration of a 60mg loading dose of prasugrel, there no statistical difference in the mean plasma concentration (ng/ml) values observed between the STEMI and NSTEMI groups (38.44 ± 7.59 vs 36.80 ± 4.60 p = 0.872).

The data in table 23 were analysed using a two-way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of P-AM generation expressed as the plasma concentration (ng/ml) over time was examined. There was a highly significant difference between the effect of disease state on the degree of platelet inhibition, $F(2,21) = 13.475$, $p = 0.000$. The observed reduction in the degree of platelet inhibition over time for both disease states was highly significant, $F(2,21) = 11.779$ $p = 0.000$.

6.6.3 Correlation between platelet function assays, VerifyNow and VASP-PRI (%) and active metabolite generation/plasma concentration.

As explained in chapter 5, since all VerifyNow samples were collected and assessed at the base hospital and I was able to undertake a majority of these tests myself, the results generated are more reliable. Therefore, our

VerifyNow results have been used as a baseline against which to compare the VASP-PRI (%) and active metabolite plasma concentration (ng/ml) results.

Table 24 Correlation the degree of platelet inhibition observed using VerifyNow compared with VASP-PRI following the administration of a prasugrel 60mg loading dose

	VASP-PRI			
	STEMI		NSTEMI	
VerifyNow	r	p-value	r	p-value
20 minutes post loading	0.202	0.631	0.912	0.002
At Balloon Inflation	-0.614	0.105	x	x
60 minutes post loading	0.357	0.385	0.532	0.113
240 minutes post loading	0.667	0.071	0.158	0.663

VerifyNow (expressed as PRU) and VASP-PRI (%) results provide an indication as to the degree of platelet inhibition achieved following the oral administration of a prasugrel loading dose to both STEMI and NSTEMI patients.

Table 25. Correlation between the degree of platelet inhibition observed using VerifyNow compared with prasugrel active metabolite plasma concentration following the administration of a 60mg loading dose.

	Prasugrel active metabolite plasma concentration			
	STEMI		NSTEMI	
VerifyNow	r	p-value	r	p-value
20 minutes post loading	0.055	0.846	0.284	0.426
At Balloon Inflation	0.096	0.733	x	x
60 minutes post loading	-0.169	0.564	0.532	0.113
240 minutes post loading	-0.512	0.061	0.158	0.663

Based on the mechanism of action of prasugrel and the nature of the platelet function assays, one would expect a negative correlation between the degree

of platelet inhibition (PRU) and the amount of active metabolite generated in both the STEMI and NSTEMI groups.

6.7 Discussion

In terms of the patient population recruited to the prasugrel arm of the study (table 20), there are no statistically significant differences between the STEMI and NSTEMI groups; both groups are well matched in terms of their baseline characteristics. There is a numerical but not statistical difference in the number of diabetic patients in the NSTEMI group compared with the STEMI group. This difference is attributable to national guideline recommendations stipulating that diabetic NSTEMI patients under the age of 75 years and over 60kg in body weight would be assigned the prasugrel arm of the study (NICE TAG 317 2014). This inclusion criterion was amended part way through the study to extend the eligibility to non-diabetic patients also (as per the manufacturers recommendations), to facilitate recruitment to the NSTEMI arm (eMC 2016a).

There is a highly statistically significant difference in the administration of morphine ($p < 0.001$) and statistically significant difference in the administration of sub-lingual glyceryl trinitrate ($p = 0.017$) between the STEMI and NSTEMI groups. Again, this difference is attributable to evidence base and guideline recommendations which advise against the administration of either drug to NSTEMI patients in view of the increased mortality associated with their use in this particular patient group (Meine, Roe et al. 2005).

6.7.1 Pharmacodynamic analysis - VerifyNow

A detailed explanation of the interpretation of VerifyNow data is given in chapter 4.

The observed VerifyNow pharmacodynamic data demonstrates a statistically significant difference in PRU values between our STEMI and NSTEMI groups at all data collection time points (figure 21). Thereby indicating the disease state of STEMI significantly impairs the onset of platelet inhibition when compared to NSTEMI.

The pharmacodynamic superiority of prasugrel over clopidogrel results from its complete and rapid absorption following oral administration; 100% of the loading dose is absorbed via the gastrointestinal tract. However, in the condition of STEMI, delays in gastric motility adversely affect the amount of prasugrel that undergoes gastrointestinal absorption, leading to a decrease in the amount of active metabolite generated because less parent compound is absorbed and available for conversion by intestinal esterases to the intermediate metabolite, which is then converted to the active moiety.

The time delay between oral administration and subsequent gastric absorption and metabolic activation is evident by the increased PRU values observed at 20 minutes and 60 minutes (242 and 262 respectively) and adequate PRU value (128) at 240 minutes. So despite, an enhanced and more efficient pathway for P-AM generation, prasugrel is still subject to and

limited by HRPR and provides sub-optimal levels of platelet inhibition at the time of angioplasty, as demonstrated by the reported data.

HRPR observed during the immediate period following prasugrel administration is most likely attributable to heightened platelet reactivity that occurs secondary to acute endovascular injury following vulnerable atherosclerotic plaque rupture and the acute inflammatory response that occurs subsequent coronary artery stent implantation (Angiolillo, Fernandez-Ortiz et al. 2007). These manifestations in addition to the reduction in gastrointestinal absorption and hepatic metabolism due to the diversion of blood flow away from the gut and liver plus the administration of opioid-based analgesia can also contribute to reduced prasugrel effectiveness and the occurrence of HRPR.

In contrast, the NSTEMI patients demonstrate adequate levels of platelet inhibition at 20 minutes (PRU < 208), and excessive levels of platelet inhibition at 60 and 240 minutes (PRU < 95). This data indicates, that in comparison to our STEMI patients, the NSTEMI cohort are at increased risk of experiencing bleeding complications due to excessive inhibition of platelet aggregation.

6.7.2 Pharmacodynamic analysis - VASP-PRI (%)

The interpretation of VASP-PRI (%) results is explained extensively in chapter 5. In contrast to the VerifyNow results, the pharmacodynamic VASP data indicates that there is no statistically significant difference in the degree of platelet inhibition observed over time following the administration of a

prasugrel 60mg loading dose (p = 0.389). In addition, the disease state of STEMI does not affect the degree of platelet inhibition achieved when compared to our NSTEMI patient group as shown in figure 22 and documented in table 22.

On further analysis of the VASP-PRI% data, an explanation for the increasing %PRI trend observed in our STEMI group cannot be given; the data appears to indicate a reduction in antiplatelet efficacy over time (figure 22).

An explanation for this may be due to the lag time between oral administration to onset of action allows for even more platelets to become activated thereby reducing the clinical efficacy of prasugrel, such that it is only active against new platelets. However, were this the case, then we would see a similar trend in our VerifyNow data.

The NSTEMI data in comparison, demonstrates increasing antiplatelet effectiveness of prasugrel over time; the %PRI at each time point is below 50% indicating an adequate level of platelet inhibition. At 240 minutes, the %PRI observed, indicates that degree of platelet inhibition could predispose patients to increased bleeding risks. These results are in line with previously reported data in which pre-treatment with prasugrel in the context of NSTEMI increases the risk of bleeding complications (Montalescot, Collet et al. 2014).

On examining the relationship between our VerifyNow and VASP phosphorylation assays (table 24), there is no significant correlation

between the two assays in neither the STEMI or NSTEMI groups. The numerical trend in our NSTEMI group for both assays indicates increasing antiplatelet effectiveness over time as demonstrated by the decreasing PRU and %PRI values recorded. However, the data for our STEMI group is inconsistent between the two assays.

As a result of the inconsistencies observed in the VASP data, which is most likely to due to inexperience of conducting the analytical techniques required to run the analysis and operate the flow cytometer, the VASP results reported in this work will be used and applied with caution.

6.7.3 Pharmacokinetic analysis – LC-MS/MS

The pharmacokinetic analysis of prasugrel active metabolite generation was assessed as the maximum plasma concentration determined using LC-MS/MS. The pharmacokinetic profile of prasugrel and its active metabolite has been investigated extensively in healthy patients. The maximum plasma concentration of P-AM reported in these studies ranged from 87 ng/ml to 512 ng/ml and usually occurred within 30 minutes of administration of a 60mg loading dose (Brandt, Payne et al. 2007, Payne, Li et al. 2007, Cattaneo 2010) .

Following oral administration, hydrolysis by intestinal esterases leads to the generation of an intermediate metabolite (R-95913), the speed of conversion of R-95913 to the P-AM (R-138727) via CYP3A4 and CYP2B6 is so rapid and efficient that the intermediate metabolite is not detectable (Floyd,

Passacquale et al. 2012). For this reason, our pharmacokinetic analysis focused on detection of the final P-AM (R-138727) only.

The observed pharmacokinetic data demonstrates a marked and statistically significant difference in the pharmacokinetic profile and P-AM generation between the STEMI and NSTEMI groups over time ($p = 0.000$) as shown in figure 23. However, this should be interpreted with caution in view of the erroneous NSTEMI results recorded.

P-AM in the STEMI patients' increases over time, although, the P-AM plasma concentrations are numerically quite low compared to the values previously reported data from healthy subjects. Since this data are derived from STEMI patients during the acute phase of their presentation, the reduction in gastric absorption is likely to result in less prasugrel being available for conversion by intestinal esterases to the intermediate and subsequent active metabolite.

To date there are no studies, which provide pharmacokinetic insights into the plasma concentrations of P-AM during a STEMI, as such there are no data against which to benchmark the results of this current study. However, when comparing the outcomes to the average reported values in healthy patients (87ng/ml), it can be concluded that the physiological state of STEMI does adversely affect the pharmacokinetic profile of prasugrel.

Interestingly, P-AM generation in the NSTEMI group decreases over time, with the highest value observed at 20 minutes and lowest at 240 minutes

(figure 23). This finding is unusual and does not correlate with the pharmacodynamic data collated during the VerifyNow and VASP-PRI assays; both of which demonstrate a reduction in PRU and %PRI indicating an increase in antiplatelet effectiveness through a reduction in platelet reactivity over time.

It was initially thought that our erroneous NSTEMI P-AM results were due to a labelling error. However, all blood samples for the three analytical tests were collected and transferred to the appropriate sampling tubes and labelled at the same time for each patient. If these results were due to a labelling error, then the same trend/irregularities would be expected with the VerifyNow and VASP-PRI samples also.

Following discussion with our Clinical Chemist, the established and validated methods for sample collection, preparation and subsequent LC-MS/MS analysis as previously described in chapter 4 were utilised without modification (Farid, McIntosh et al. 2007, Payne, Li et al. 2007). In order to ensure that the results were not due to incorrect coding of the sampling tubes at the time of the LC/MS/MS analysis, all sampling tubes were cross-referenced against the original case report form documentation and the LC-MS/MS analyses was undertaken again using the same column and a new column. In addition, new internal standards for P-AM were also sourced and were again prepared using the validated method described by Farid et al (Farid, McIntosh et al. 2007). Unfortunately, the results of the second and third attempts also resembled those initially recorded. In view of this, we

decided to use the original results generated, since the clopidogrel and ticagrelor LC-MS/MS analyses were also undertaken using the original column on which the prasugrel anaylsis was carried out.

On examining the relationship between the pharmacodynamic and pharmacokinetic profiles generated following the administration of a prasugrel 60mg loading dose (table 25), there are no significant correlation between the two sets of data.

The pharmacokinetic and pharmacodynamic profile of prasugrel demonstrates homogeneity in terms of its clinical efficacy, speed of onset and resulting consistent and high levels of platelet inhibition when reviewing existing clinical trial outcomes and data (Brandt, Payne et al. 2007, Wiviott, Braunwald et al. 2007). However, platelet function assays and assessment of plasma active metabolite concentrations were not determined during the immediate period following diagnosis or at the time of angioplasty in patients scheduled to undergo PCI.

For the STEMI patients, the mean dose to balloon time was 26.8 ± 12.7 minutes; within this time frame, our prasugrel STEMI pharmacodynamic data indicates a PRU value of 240 and a %PRI of 46.25, and our pharmacokinetic data indicates a P-AM plasma concentration of 14.27ng/ml. As such the pharmacokinetic/pharmacodynamic data collated in our study very clearly demonstrates a lack of antiplatelet effect following the administration of prasugrel 60mg at the time of balloon inflation and subsequent coronary artery stent implantation and for at least 60 minutes following administration.

This data is supported by and is in line with the findings of other recently reported pharmacodynamic studies in which prasugrel administration in STEMI patients is associated with a delay in the onset of antiplatelet activity, this delay persists for at least two to four hours (Alexopoulos, Xanthopoulou et al. 2012, Parodi, Valenti et al. 2013). This delay in onset of action is most likely also influenced by the co-administration of morphine, which is associated with a reduction in the degree of platelet inhibition following the administration of prasugrel in both STEMI patients and healthy subjects (Hobl, Reiter et al. 2015, Parodi, Bellandi et al. 2015). Although the outcomes of these studies are informative they investigate only the pharmacodynamic effect of the newer antiplatelet agents in STEMI patients; the results from this study build on the works of Parodi at al (2015) since these results also provide insights into the effect of STEMI on plasma P-AM concentrations and make direct comparisons against NSTEMI patients to determine the effect of disease state on drug handling.

Based on its pathway of activation, a major determinant of the clinical efficacy of prasugrel is the degree of gastrointestinal absorption that will take place following oral ingestion. Emerging evidence suggests that gastric motility is reduced in the setting of STEMI, either as a consequence of the shunting of blood flow away from the gut and/or the co-administration of morphine. The delay in the onset of action of prasugrel and the inadequate antiplatelet effect observed is likely to be secondary to delayed gastrointestinal absorption.

While pretreatment/upstream administration of loading doses is necessary to partially overcome the lag time between administration and subsequent absorption and metabolic activation of antiplatelet pro-drugs in STEMI patients. Observed data from this study indicates that pre-treatment with prasugrel 60mg in the NSTEMI group leads to levels of platelet inhibition that can predispose patients to increased bleeding risk; PRU values of < 208 and %PRI < 50% were recorded at 20 minutes in comparison to the STEMI group. These values continued to decrease over time to less than PRU < 95 and %PRI < 25% at 60 and 240 minutes; indicating effective, if not possibly excessive levels of platelet inhibition in our NSTEMI prasugrel treated group. The NSTEMI prasugrel data is therefore in line with the outcomes of the recently reported ACCOAST-PCI study in which pre-treatment with prasugrel in the context of NSTEMI was not associated with a reduction in ischaemic events but rather a three fold increase in non-CABG related TIMI major bleeding (Montalescot, Collet et al. 2014). These findings have subsequently resulted in a change in the manufacturers recommendations and UK guideline recommendations, which no longer mandate upstream treatment with prasugrel in NSTEMI patients, but rather advise withholding administration of the loading dose until the time of PCI, particularly if coronary angiography is to be performed within 48 hours of the initial diagnosis (NICE TAG 317 2014). The relationship between pre-treatment with more potent P2Y12 inhibitors and bleeding risk in NSTEMI patients will be discussed further in chapter 8.

Increased levels of ADP-induced platelet reactivity have been observed in older patients, this in part has been used to explain apparent treatment failure

with clopidogrel in patients over the age of 75 years (Gremmel, Steiner et al. 2010). This concept is in contrast to prasugrel data, which indicates that older age predisposes patients to increased bleeding as a consequence of increased exposure to P-AM; in patients over the age of 75 years, exposure to P-AM was 19% greater when compared to those under the age of 75 years (Wiviott, Antman et al. 2010).

As described in earlier chapters, ACS and in particular the acute phase of a STEMI is associated with heightened platelet reactivity, despite the administration of potent antiplatelet agents (HRPR). This occurrence is further perpetuated by the process of angioplasty and coronary artery stent implantation which is necessary to allow for timely reperfusion and adequate restoration of myocardial blood flow. However, these mechanical interventions cause further platelet activation and contribute to the highly prothrombotic milieu that manifests during a STEMI.

Prasugrel still needs to undergo gastrointestinal absorption and subsequent metabolic biotransformation to an intermediate and then active metabolite. This process is not reliant on the CYP2C19 allele and is not subject to genetic polymorphisms which are known to affect the onset of action and effectiveness of clopidogrel. In contrast to clopidogrel, CYP3A4 is the key isoenzyme responsible for the biotransformation of prasugrel. Animal studies have shown that a significant amount of R-138727 is formed during first-pass metabolism in the intestine; thereby supporting the notion that the intestine is an important contributor to the formation of P-AM and is fundamental to the

pharmacodynamic activity of prasugrel and its subsequent clinical effect (Hagihara, Kazui et al. 2011).

Work undertaken by Small et al (2010) provides further insights into the mechanisms that contribute to the clinical effects of prasugrel. Moderate hepatic dysfunction was found to have a negligible effect on P-AM exposure or generation. When compared to healthy controls, in those with hepatic dysfunction the degree of platelet aggregation achieved following prasugrel administration remains unchanged (Small, Farid et al. 2009). This is of importance, since it further supports the concept that hepatic biotransformation is not the most important contributor to the in-vivo formation of P-AM (R-138727). Impaired gastric absorption, leads to a delay in the onset of action of antiplatelet effect as demonstrated by our VerifyNow and VASP-PRI data.

Increased or intrinsic platelet reactivity even prior to the administration of a P2Y12 inhibitor contributes to the phenomenon of HRPR, to such an extent that even the administration of a more potent agent such as prasugrel is unable to result in sufficient levels of platelet inhibition at the time of PPCI (Frelinger, Michelson et al. 2011).

6.8 Conclusion

The reported prasugrel pharmacodynamic data in this thesis is in line with previously reported data; we conclude that the delay in onset of action and impaired antiplatelet effect observed following the administration of prasugrel is attributable to the variable and impaired gastrointestinal absorption that

occurs in the STEMI group compared to the NSTEMI group. There is now increasing awareness of the inherent characteristics associated with the condition of STEMI (heightened platelet reactivity, diversion of blood flow away from the gut/liver, reduced peristalsis of the gut), which limit the clinical utility, and effectiveness of orally administered prasugrel during the acute phase of a myocardial infarction.

In summary, the prasugrel data demonstrates that the degree and time course of platelet inhibition observed reduces over time in both the STEMI and NSTEMI groups (based on VerifyNow pharmacodynamic results). However, prasugrel does not provide adequate levels of platelet inhibition at the time of angioplasty and coronary artery stent implantation. When compared to NSTEMI, the disease state of STEMI does adversely affect the clinical efficacy of prasugrel.

Chapter 7 - A Pharmacokinetic and Pharmacodynamic Assessment of Ticagrelor in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction

7.1 Introduction

A detailed overview of ticagrelor, its mechanism of action, clinical efficacy and place in therapy has been covered extensivley in chapter 1.

7.2 Objectives

This chapter aims to compare the degree and time course of platelet inhibition observed following the administration of a ticagrelor 180mg loading doses in STEMI and NSTEMI patients. In addition, we will also determine whether the state of STEMI per se adversely affects the clinical efficacy of ticagrelor.

7.3 Ethical Considerations and Consent

All ethical considerations and procedures for patient consent and recruitment are described in Chapter 4.

7.4 Materials and Methods

All materials and methods are described in Chapter 4.

7.5 Statistics and Data Analysis

The methods for data collection and statistical analyses are outlined in chapter 4.

7.6 Results

Table 26. Baseline patient characteristics

Characteristic	STEMI (n =15)	NSTEMI (n =15)	*P-value*
Age (yrs)	63.7 ± 11.6	62 ±13.9	0.714
Sex			
Female	4 (27)	2 (13)	0.651
Risk Factors			
Diabetes Mellitus	2 (13)	4 (27)	0.651
Hypertension	7 (47)	7 (47)	1.000
Current Smoker	4 (27)	3 (20)	1.000
Ex Smoker	4 (27)	6 (40)	0.700
Hyperlipidaemia	3 (20)	12 (80)	0.003
Familial History of CAD	8 (53)	8 (53)	1.000
Previous MI	0 (0)	1 (7)	1.000
Previous PCI	0 (0)	1 (7)	1.000
Previous CABG	0 (0)	0 (0)	-
Previous stroke/TIA	1 (7)	0 (0)	1.000
Patient therapy on admission			
Analgesia	13 (87)	3 (20)	0.001
Comprising of:-			
Morphine	13	0	<0.001
GTN	0	3	0.224

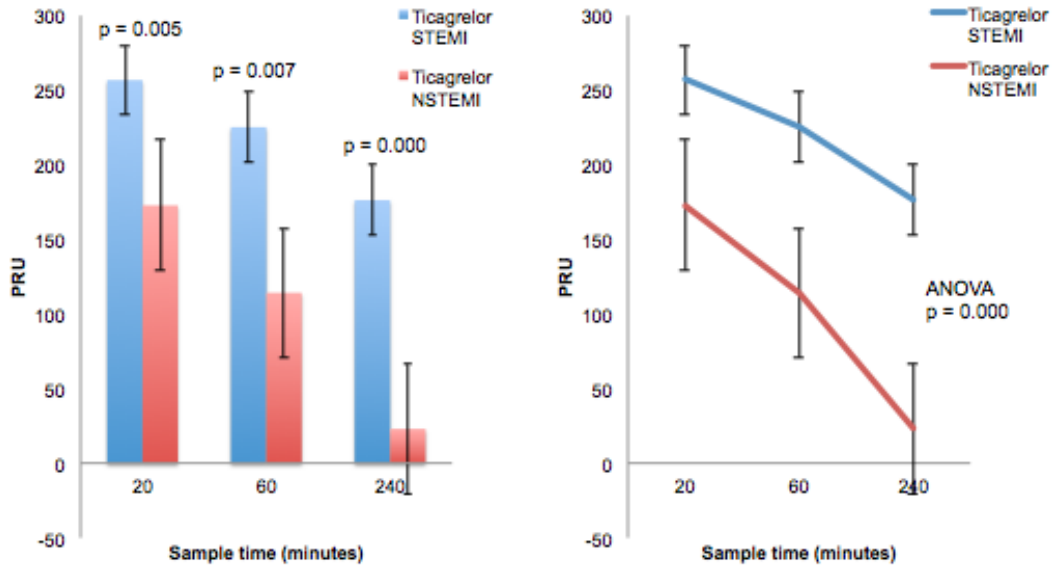
7.6.1 Pharmacodynamic analysis of the degree of platelet inhibition following ticagrelor loading (180mg) in STEMI vs NSTEMI patients.

7.6.1.1 VerifyNow Results

Table 27. Ticagrelor VerifyNow (PRU)

Verify Now result expressed as PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	15	256.73	83.93 [29.00, 139.77]	50.81	13.12	0.005
	NSTEMI	15	172.80		92.54	23.90	
60 minutes	STEMI	15	225.20	111.00 [32.95, 189.05]	82.70	21.35	0.007
	NSTEMI	15	114.20		122.22	31.56	
240 minutes	STEMI	15	176.27	153.27 [107.25,199.28]	84.92	21.93	0.000
	NSTEMI	15	23.00		18.94	4.89	

Figure 23. Mean VerifyNow PRUs (and standard error) after administration of a 180mg ticagrelor loading dose in STEMI vs NSTEMI patients.



7.6.1.1.1 Summary

Following administration of a 180mg loading dose of ticagrelor, the observed data indicates a statistically significant difference in mean PRU values between STEMI and NSTEMI samples taken at 20 minutes (256.73 ± 13.12 vs 172.80 ± 23.90 p = 0.005), 60 minutes (225.20 ± 21.35 vs 114.20 ± 31.56 p = 0.007). At four hours following the administration of a 180mg loading dose of ticagrelor, there is a highly statistically significant difference in the mean PRU values observed between the STEMI and NSTEMI groups (176.27 ± 21.93 vs 23.00 ± 4.89 p = 0.000). The data in table 27 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (PRU) over time was examined.

There was a highly significant decrease in the degree of platelet inhibition

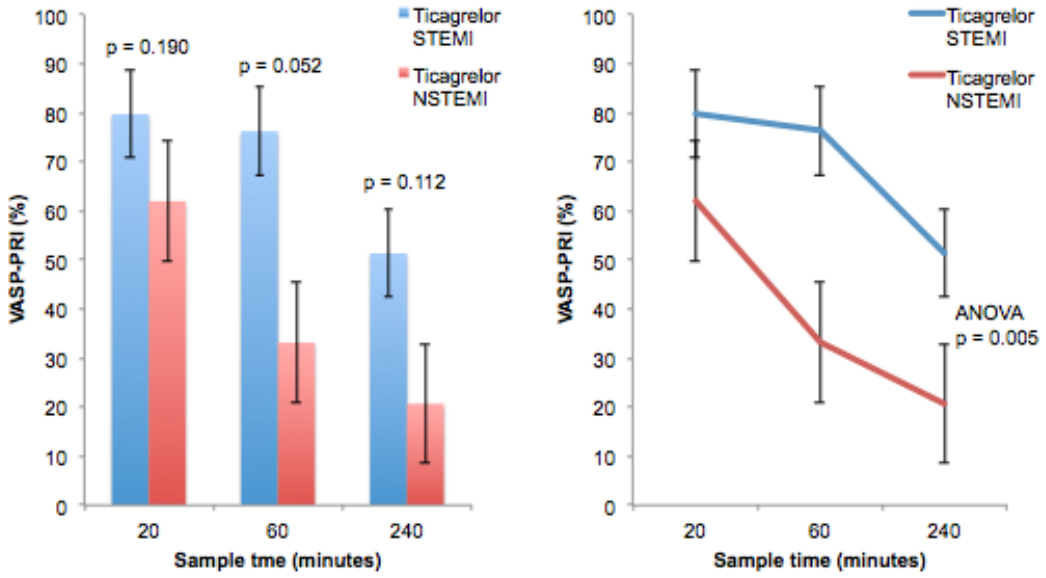
observed over time, for both the STEMI and NSTEMI groups, F(2,27) = 31.27 p = 0.000. There was no significant difference between the effect of disease state on the degree of platelet inhibition over time, F(2,27) = 2.834, p = 0.076.

7.6.1.2 VASP-PRI Results

Table 28. Ticagrelor - VASP-PRI(%)

VASP-PRI (%) result post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	8	79.75	17.75 [-9.98, 45.45]	26.01	9.20	0.190
	NSTEMI	7	62.00		23.25	8.79	
60 minutes	STEMI	8	76.25	43.08 [-0.37, 86.53]	31.46	11.12	0.052
	NSTEMI	6	33.17		43.44	17.73	
240 minutes	STEMI	8	51.38	30.66 [-8.19, 69.51]	39.78	14.07	0.112
	NSTEMI	7	20.71		27.74	10.48	

Figure 24. Mean VASP-PRI (%) (and standard error) following the administration of a 180mg ticagrelor loading dose in STEMI vs NSTEMI patients.



7.6.1.2.1 Summary

Following administration of a 180mg loading dose of ticagrelor, this data indicates a non-significant difference in mean %PRI values between STEMI and NSTEMI samples taken at 20 minutes (79.75 ± 9.20 vs 62.00 ± 8.79 p = 0.190). At 60 minutes following administration of a 180mg loading dose a significant difference is observed (76.25 ± 11.12 vs 33.17 ± 17.73 p = 0.052). At four hours following the administration of a 180mg loading dose of ticagrelor, a non-significant difference in the mean %PRI values observed between the STEMI and NSTEMI groups (51.38 ± 14.07 vs 20.71 ± 10.48 p = 0.112).

The data in table 28 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (%PRI) over time was examined.

Interaction over time: there was a highly significant increase in the degree of platelet inhibition observed over time, for both the STEMI and NSTEMI groups, $F(2,11) = 9.135$ $p = 0.005$.

Interaction between disease state by time: there was not a significant difference between the effect of disease state on the degree of platelet inhibition over time, $F(2,11) = 2.329$, $p = 0.143$.

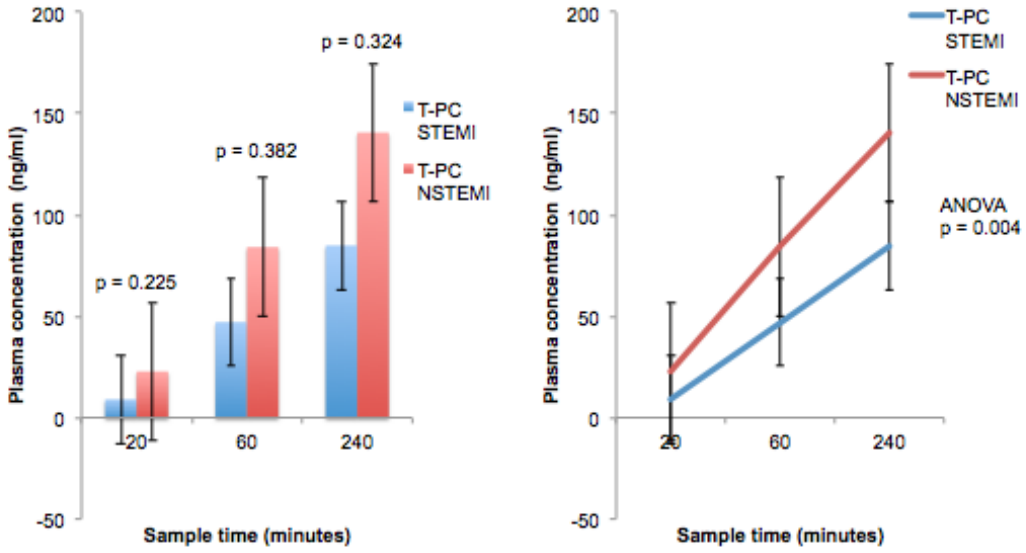
7.6.2 Pharmacokinetic analysis of ticagrelor and ticagrelor active metabolite (AR-C124910X) generation following ticagrelor loading (180mg) in STEMI vs NSTEMI patients.

7.6.2.1 Liquid Chromatography/Mass Spectrometry Results – Ticagrelor Parent Compound (T-PC)

Table 29. Ticagrelor parent compound plasma concentration (ng/ml)

Parent compound plasma concentration (ng/ml) post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	15	9.04	17.75 [-9.98, 45.45]	15.95	4.12	0.225
	NSTEMI	15	22.78		39.78	10.27	
60 minutes	STEMI	15	47.13	-37.00 [-122.39, 48.40]	93.28	24.09	0.382
	NSTEMI	15	84.14		131.78	34.03	
240 minutes	STEMI	15	84.92	-55.75 [-169.47, 57.97]	162.66	42.00	0.324
	NSTEMI	15	140.67		140.61	36.31	

Figure 25 Mean plasma concentration of ticagrelor parent compound (ng/ml) (and standard error) following administration of a loading dose in STEMI vs NSTEMI patients.



7.6.2.1.1 Summary

Following administration of a 180mg loading dose of ticagrelor, this data indicates a non-significant difference in mean plasma concentration values between STEMI and NSTEMI samples taken at 20 minutes (9.04 ± 4.12 vs 22.78 ± 10.23 p = 0.225), at 60 minutes (47.13 ± 24.09 vs 84.13 ± 34.03 p = 0.382) and at 240 minutes (84.92 ± 42.00 vs 140.67 ± 36.31 p = 0.324).

The data in table 29 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the plasma concentration expressed as ng/ml over time were examined.

Interaction over time: there was a highly significant increase in the presence of ticagrelor parent compound observed over time, for both the STEMI and NSTEMI groups, F(2,27) = 6.930 p = 0.004.

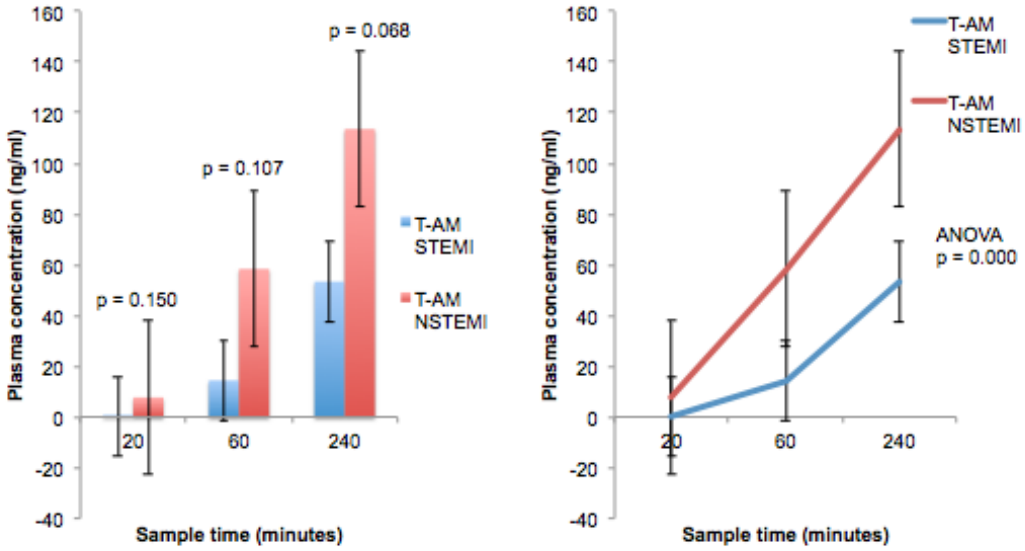
Interaction between disease state by time: there was a non-significant difference between the effect of disease state on the mean plasma concentration over time, F(2,27) = 0.339, p = 0.715.

7.6.2.2 Liquid Chromatography/Mass Spectrometry Results – Ticagrelor Active Metabolite (T-AM).

Table 30. Ticagrelor active metabolite plasma concentration (ng/ml)

Active metabolite plasma concentration (ng/ml) post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	15	0.16	-7.76 [-18.00, 10.19]	0.63	0.16	0.150
	NSTEMI	15	7.72		19.74	5.10	
60 minutes	STEMI	15	14.43	-43.97 [-98.12, 10.19]	28.21	7.28	0.107
	NSTEMI	15	58.40		98.42	25.41	
240 minutes	STEMI	15	53.38	-60.21 [-125.12, 4.70]	97.62	25.21	0.068
	NSTEMI	15	113.59		74.38	19.21	

Figure 26. Mean plasma concentration of ticagrelor active metabolite (ng/ml) (and standard error) following administration of a loading dose in STEMI vs NSTEMI patients.



7.6.2.2.1 Summary

Following administration of a 180mg loading dose of ticagrelor, this data indicates a non-significant difference in mean active metabolite plasma concentration values between STEMI and NSTEMI samples taken at 20 minutes (0.16 ± 0.16 vs 7.72 ± 5.10 p = 0.150), at 60 minutes (14.43 ± 7.28 vs 58.40 ± 25.41 p = 0.107) and at 240 minutes (53.38 ± 25.21 vs 113.59 ± 19.21 p = 0.068). The data in table 30 were analysed using a two way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the mean plasma concentration expressed as ng/ml over time were examined. Interaction over time: there was a highly significant increase in the presence of ticagrelor active metabolite generated over time, for both the STEMI and NSTEMI groups, F(2,27) = 16.219 p = 0.000. Interaction between disease state by time: there was a non-significant difference

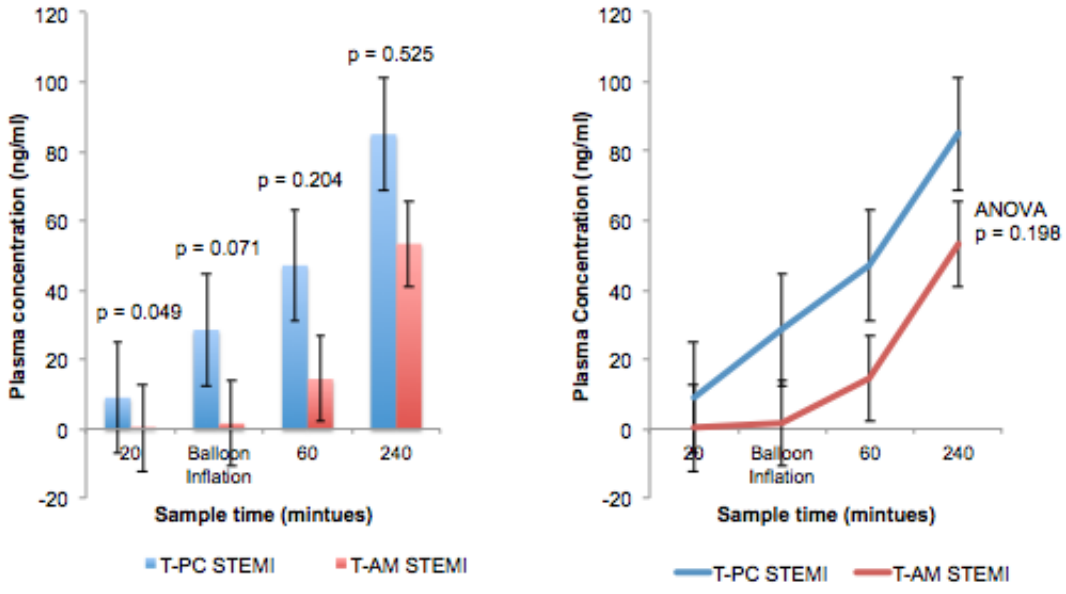
between the effect of disease state on the mean plasma concentration over time, F(2,27) = 2.655, p = 0.089.

7.6.2.3 Relationship between the mean plasma concentration of ticagrelor parent compound and active metabolite in STEMI patients.

Table 31. Mean plasma concentration (ng/ml) ticagrelor parent compound vs active metabolite in STEMI patients

Plasma concentration (ng/ml) post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	T-PC	15	9.04	8.88 [0.44, 17.32]	15.95	4.12	0.049
	T-AM	15	0.16		0.63	0.16	
Balloon Inflation	T-PC	9	47.60	39.39 [-4.19, 82.97]	56.01	18.67	0.071
	T-AM	3	8.22		7.85	4.53	
60 minutes	T-PC	15	47.13	32.70 [-18.85, 84.24]	93.28	24.09	0.204
	T-AM	15	14.43		28.21	7.28	
240 minutes	T-PC	15	84.92	31.54 [-68.79, 131.88]	162.66	42.00	0.525
	T-AM	15	53.38		97.62	25.21	

Figure 27. Mean plasma concentration (ng/ml) (and standard error) of T-PC vs T-AM in STEMI patients following the administration of a 180mg loading dose.



7.6.2.3.1 Summary

The data in table 31 were analysed using a two way analysis of variance test in which the degree of absorption and subsequent metabolism of ticagrelor and its active metabolite as measured by the plasma concentration expressed as ng/ml over time were examined in STEMI patients.

Interaction over time: there was a non-significant increase in the presence of ticagrelor parent compound and its active metabolite over time, $F(3,8) = 1.965$ $p = 0.198$.

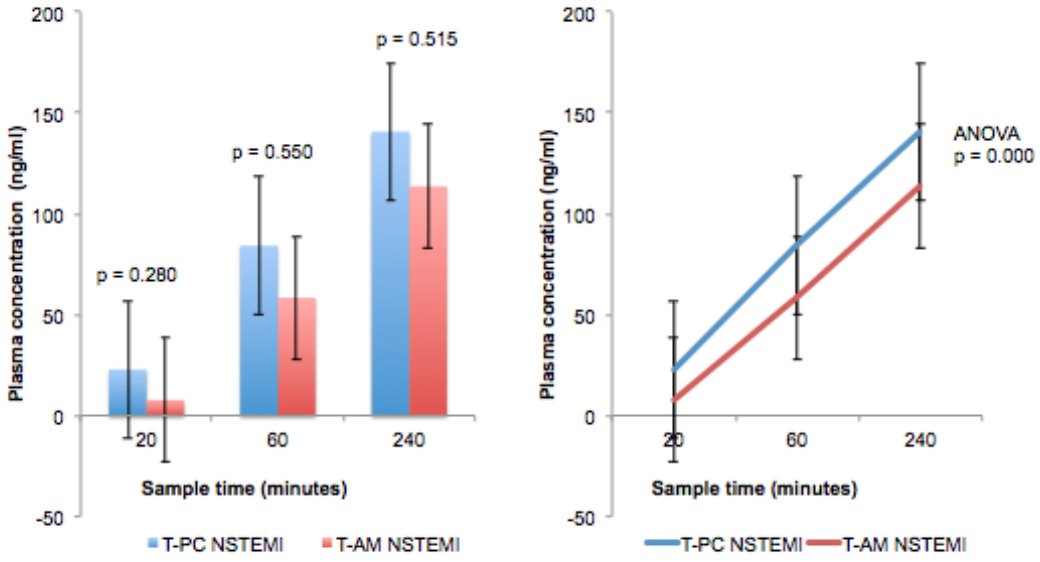
Interaction between disease state by time: there was a non-significant difference in mean plasma concentration (ng/ml) between the presence of the parent compound and active metabolite over time, $F(3,8) = 2.544$, $p = 0.129$.

7.6.2.4 Relationship between the mean plasma concentration of ticagrelor parent compound and active metabolite in NSTEMI patients.

Table 32. Mean plasma concentration (ng/ml) ticagrelor parent compound vs active metabolite in NSTEMI patients.

Plasma concentration (ng/ml) post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	T-PC	15	22.78	15.07 [-8.43, 38.55]	39.78	10.27	0.280
	T-AM	15	7.72		19.74	5.10	
60 minutes	T-PC	15	84.13	25.73 [-61.26, 112.72]	131.78	34.03	0.550
	T-AM	15	58.40		98.42	25.41	
240 minutes	T-PC	15	140.67	27.09 [-57.05, 111.22]	140.61	36.31	0.515
	T-AM	15	113.59		74.38	19.21	

Figure 28 Mean plasma concentration (ng/ml) (and standard error) of ticagrelor parent compound vs active metabolite in NSTEMI patients following the administration of a 180mg loading dose.



7.6.2.4.1. Summary

The data in table 22 were analysed using a two way analysis of variance test in which the degree of absorption and subsequent metabolism of ticagrelor and its active metabolite as measured by the plasma concentration expressed as ng/ml over time were examined in NSTEMI pateints.

Interaction over time: there was a highly significant increase in the presence of ticagrelor parent compound and its active metabolite over time, F(2,27) = 19.254 p = 0.000.

Interaction between disease state by time: there was a non-significant difference in mean plasma concentration (ng/ml) between the presence of the parent compound and active metabolite over time, F(2,27) = 0.085, p = 0.918.

Table 33. Correlation the mean plasma concentration (ng/ml) of T-PC compared with T-AM following the administration of a ticagrelor 180mg loading dose

Plasma concentration T-PC (ng/ml)	Plasma concentration T-AM (ng/ml)			
	STEMI		NSTEMI	
	r	p-value	r	p-value
20 minutes post loading	0.744*	0.022	0.002	0.996
At Balloon Inflation	0.901**	0.000	x	x
60 minutes post loading	0.850**	0.001	0.891**	0.000
240 minutes post loading	0.909**	0.000	0.449	0.107

* Correlation is significant at the 0.05 level (2-tailed)
* * Correlation is significant at the 0.01 level (2-tailed)

The results of the Pearson correlation support the independent t-tests and ANOVA tests which indicate that there is no significant difference in the mean plasma concentrations of T-PC when compared with T-AM. This supports the strong positive correlation, which is highly statistically significant, between T-PC and T-AM plasma concentrations at all time points in the STEMI group. The relationship between T-PC and T-AM in the NSTEMI group also demonstrates a positive correlation, although overall, this was found to be non-significant.

Using these results as a baseline, rather than quote both T-PC and T-AM in the remainder of the thesis, I will refer only to T-PC plasma concentrations. Previous pharmacokinetic studies have demonstrated that the maximum plasma concentration (Cmax) of T-PC is approximately 594ng/ml with the time to maximum concentration (Tmax) being 3.1 hours. The Cmax for T-AM is 135ng/ml with a Tmax of 3.7 hours (Husted, Emanuelsson et al. 2006, Wallentin 2009). Although these data are from patients with stable coronary

artery disease, it does provide some insights into the drug handling following oral ingestion and the ratios in which T-PC and T-AM appear.

7.6.3 Correlation between platelet function assays (VerifyNow and VASP-PRI) and ticagrelor plasma concentration as assessed using LC-MS/MS.

A Pearson's Correlation Co-efficient test was also undertaken to determine the strength of the relationship between the two platelet function assays utilised during the study; VerifyNow and VASP phosphorylation assay. Since all VerifyNow samples were collected and assessed at the base hospital and the results generated are more reliable, this test has been used as the baseline against which to compare the VASP-PRI (%). In order to determine the correlation between the degree of platelet inhibition and plasma concentrations of ticagrelor, a Pearson's correlation co-efficient test was also undertaken between the VerifyNow and ticagrelor parent compound LC-MS/MS results.

Table 34. Correlation the degree of platelet inhibition observed using VerifyNow compared with VASP-PRI following the administration of a ticagrelor 180mg loading dose

	VASP-PRI			
	STEMI		NSTEMI	
VerifyNow	r	p-value	r	p-value
20 minutes post loading	0.264	0.527	0.089	0.850
At Balloon Inflation	0.192	0.650	x	x
60 minutes post loading	0.546	0.160	0.946**	0.004
240 minutes post loading	0.853*	0.007	-0.044	0.925

* Correlation is significant at the 0.05 level (2-tailed)
* * Correlation is significant at the 0.01 level (2-tailed)

Both VerifyNow (expressed as PRU) and VASP-PRI (%) results provide an indication as to the degree of platelet inhibition achieved following the oral

administration of a ticagrelor loading dose to both STEMI and NSTEMI patients.

Table 35. Correlation between the degree of platelet inhibition observeusing VerifyNow compared with ticagrelor parent compound plasma concentration following the administration of a 180mg loading dose.

VerifyNow (PRU)	Ticagrelor parent compound plasma concentration (ng/ml)			
	STEMI		NSTEMI	
	r	p-value	r	p-value
20 minutes post loading	-0.482	0.069	-0.646*	0.017
At Balloon Inflation	-0.424	0.115	x	x
60 minutes post loading	-0.438	0.103	-0.455	0.188
240 minutes post loading	-0.606*	0.017	0.120	0.683

* Correlation is significant at the 0.05 level (2-tailed)

Based on the mechanism of action of ticagrelor and the nature of the platelet function assays, one would expect a negative correlation between the degree of platelet inhibition (PRU) and the amount of active metabolite generated in both the STEMI and NSTEMI groups.

7.7 Discussion

Table 25 describes the baseline characteristics of the ticagrelor group. In terms of the patient population recruited to the ticagelor arm of our study, all are well matched in terms of their age at presentation, co-morbidities and risk factors. The only exception being a statistically significant difference in the incidence of hyperlipidaemia between the NSTEMI and STEMI groups ($p = 0.003$). In addition, a statistically significant greater number of patients in the STEMI group received treatment with opioid based analgesia compared to the

NSTEMI group (p = 0.001). This has been explained and justified in previous chapters (5 and 6).

7.7.1 Pharmacodynamic Analysis - VerifyNow

The observed VerifyNow data (table 27) indicates that at the time of PPCI and for at least 60 (and maybe 120) minutes after the administration of a 180mg loading dose in STEMI patients, ticagrelor does not provide sufficient levels of platelet inhibition. The PRU value at 20 minutes is > 230, which is indicative of HRPR and is > 208 at 60 minutes, indicating reduced antiplatelet effect and an increased risk of thrombotic complications.

When comparing the STEMI results against the NSTEMI results, a statistically significant difference in PRU values is seen at each data collection time point. NSTEMI patients display adequate levels of platelet inhibition at 20 minutes and 60 minutes, however, the PRU at 240 minutes is very low indicating almost complete inhibition of platelet activity and an increased risk of bleeding complications.

When determining the degree of platelet inhibition over time, there was a significant increase in IPA as shown in figure 25 (p = 0.000). In terms of the effect of disease state on IPA, our ANOVA results indicate that the condition of STEMI does not have a significant impact on IPA when compared to NSTEMI (p = 0.076), however, this is in contrast to our t-test data in which a significant difference at each time point is observed.

The reported VerifyNow data indicates that in the context of STEMI, despite being directly acting and able to provide faster and greater levels of IPA,

ticagrelor is still subject to HRPR limiting its clinical efficacy. A possible explanation for this may lie in the reduction in gastric motility and subsequent reduction in gastric absorption that occurs during a STEMI, which will limit the amount of ticagrelor absorbed and able to exert its therapeutic antiplatelet effect.

7.7.2 Pharmacodynamic analysis – VASP-PRI (%)

The VASP-P pharmacodynamic results expressed as %PRI, in the STEMI group indicate an inadequate antiplatelet effect at all time points post loading as evidenced by %PRI values > 50%. As explained in chapter 5, a %PRI > 50% is associated with increased risk of further MACCE and complications such as stent thrombosis. In comparison the NSTEMI group display increased levels of platelet reactivity at 20 minutes only with adequate levels of platelet inhibition at 60 minutes and 240 minutes. The closer the %PRI value is to 0%, the greater the bleeding risk.

The VASP-P data (table 28) indicates inadequate levels of platelet inhibition in our STEMI group, in comparison our NSTEMI group display inadequate levels at 20 minutes only, adequate levels at 60 minutes and excessive levels of platelet inhibition at 240 minutes.

In terms of the degree of platelet inhibition over time, the data indicates a statistically significant increase in IPA (decrease in %PRI) over time, $p = 0.005$. However, the effect of disease state on the degree of platelet inhibition according to our VASP-PRI results indicates that there is no difference between the two groups ($p = 0.143$), as shown in figure 26.

7.7.3 Pharmacokinetic Analysis – LC-MS/MS

T-PC and T-AM are equipotent; since T-AM is present in plasma at a concentration that is a third of that of the parent compound, the majority of the antiplatelet effect seen is due to the activity of the T-PC (Husted, Emanuelsson et al. 2006). For the remainder of the thesis, all LC-MS/MS information regarding ticagrelor will relate to T-PC. The subsequent sections demonstrate that although the final plasma concentrations are lower than those quoted in the literature, the trend in terms of T-PC:T-AM production are also observed in our STEMI and NSTEMI groups. In addition, our data supports the rationale to quote T-PC plasma concentrations only, as outlined below.

Plasma concentrations of T-PC and T-AM, indicate that levels in the region of 549 ng/ml after 3.1 hours and 135ng/ml after 3.7 hours respectively in patients with SCAD can be achieved following the administration of a 180mg loading dose (Wallentin 2009). At the time at which the ticagrelor patient group was recruited to the current study, there were no data relating to plasma concentrations of T-PC or T-AM in the context of STEMI against which the observed LC-MS/MS data could be benchmarked. However, since completion of this study, a paper has been published in which the plasma concentrations of ticagrelor are investigated in STEMI patients. This study was designed to provide a pharmacokinetic and pharmacodynamic comparison of crushed vs integral tablets in STEMI patients (Alexopoulos, Barampoutis et al. 2015). The T-PC and T-AM plasma concentrations reported by Alexopoulos et al (2015) at 1 hour were 70ng/ml and 4.3ng/ml respectively (Alexopoulos, Barampoutis et al. 2015).

This is not too dissimilar to the T-PC and T-AM results from this study, which in comparison demonstrate plasma concentrations of 47ng/ml and 14.3 ng/ml at 1 hour.

This study data therefore is in line with recently reported pharmacodynamic data, which indicates, reduced plasma concentrations of intact ticagrelor tablets in STEMI patients, which is most likely secondary to delayed gastric absorption and the co-administration of morphine (Alexopoulos, Barampoutis et al. 2015, Kubica, Adamski et al. 2015). Although this study was not adequately powered to determine the significance of the effect of morphine on the efficacy of ticagrelor, there is a growing body of evidence with pharmacodynamic data derived from both healthy volunteers and those who present following a STEMI, which indicates that this a drug-drug interaction that is of potential clinical significance (Kubica, Adamski et al. 2015, Parodi, Bellandi et al. 2015).

7.7.3.1 Ticagrelor-Parent Compound (T-PC)

Since, ticagrelor undergoes rapid and complete gastrointestinal absorption following oral ingestion, we would expect the STEMI plasma concentrations to more closely resemble the NSTEMI.

In the STEMI group, the plasma concentrations at 20, 60 and 240 minutes are all less than 100ng/ml, comparatively, in the NSTEMI group, the plasma concentrations are numerically greater, but there is no statistical difference observed at each time point when comparing the two groups.

The amount of T-PC detected in plasma over time increased significantly; the plasma concentration at 240 minutes compared to 20 minutes was statistically

significantly greater in both groups (p = 0.004). However, when assessing the effect of STEMI of plasma concentrations/gastrointestinal absorption, our data indicates no significant difference between the two groups (p = 0.715)

7.7.3.2 Ticagrelor-Active Metabolite (T-AM)

As for theT-PC results, a statistically significant difference was not seen in the plasma concentration of T-AM between the STEMI and NSTEMI groups at each sample collection time point.

However, in terms of the plasma concentration over time, a highly statistically significant increase in T-AM was seen at 240 minutes compared to 20 minutes (p = 0.000). When comparing the effect of STEMI against NSTEMI on the degree of T-AM present over time, we did not see a statistically significant difference between the two groups (p = 0.089).

7.7.3.3 Correlation between T-PC and T-AM

A Pearson's correlation was undertaken to determine the strength of the relationship between the plasma concentration of T-PC and T-AM. A positive correlation was observed between the plasma concentrations of T-PC and T-AM in each disease state and at each time point that was of statistical significance (table 33).

On examining the relationship between the pharmacokinetic and pharmadynamic profiles generated following the administration of a ticagrelor 180mg loading dose, the data in table 35, indicates a very high negative correlation between the degree of platelet inhibition as assessed by VerifyNow and the plasma concentration of T-PC, that is of high statistical significance at each time point in each disease state. Our data indicates that

as the plasma concentration of T-PC increases, the antiplaletet effect of ticagrelor increases and the degree of inhibition of platelet reactivity and aggregation increases as evidenced by the reduction in PRU over time.

Table 36. Pharmacokinetic/Pharmacodynamic trend of ticagrelor over time

	STEMI	NSTEMI
VerifyNow PRU	↓	↓
VASP-P %PRI	↓	↓
LC-MS/MS plasma concentration (ng/ml)	↑	↑

7.8 Conclusion

The reported pharmacodynamic data from this study indicates that even ticagrelor is subject to HRPR; there is a sufficient enough delay between the oral ingestion of ticagrelor and the onset of its therapeutic antiplatelet effect that reduces its efficacy in patients who present with a STEMI. Since ticagrelor is directly acting, it does not rely on metabolic activation in order to execute its antiplatelet effect, however, in STEMI gastrointestinal absorption is impaired sufficiently enough to reduce the amount ticagrelor available to act as a P2Y12 inhibitor.

So, despite ticagrelor being directly acting, in the context of STEMI it does not achieve adequate or sufficient levels of platelet inhibition at the time when it is most desirable in STEMI patients; at the time of angioplasty and coronary artery stent implantation.

STEMI is a condition that precipitates heightened platelet reactivity and increased platelet turnover (Alexopoulos, Xanthopoulou et al. 2013). The lag

time between the oral ingestion of ticagrelor and its ability to undergo gastrointestinal absorption and the subsequent onset of action and its antiplatelet effect is sufficiently long enough to allow for the phenomenon of HRPR to take place, as is apparent from our pharmacodynamic VerifyNow and VASP-P data. So despite claims of an improved pharmacodynamic profile with faster onset of action and greater levels of IPA, the observed STEMI data indicates a lack of adequate antiplatelet action for at least 2 hours following administration. This data is supported by other pharmacodynamic studies whose results were published as this study was in progress; these data also indicate reduced antiplatelet efficacy in the immediate period post loading indicating that at least 2 to 4 hours is required to achieve adequate levels of platelet inhibition (Alexopoulos, Xanthopoulou et al. 2012, Parodi, Valenti et al. 2013).

In summary, the observed ticagrelor data demonstrates that the degree and time course of platelet inhibition observed reduces over time in both our STEMI and NSTEMI groups (based on VerifyNow and VASP-P pharmacodynamic results). Depsite being directly acting, ticagrelor does not provide adequate levels of platelet inhibition at the time of angioplasty and coronary artery stent implantation. From which we can conclude that the delay in gastric emptying and subsequent reduction in GI absorption adversely impacts on the speed of onset and clinical efficacy of ticagrelor. When compared to NSTEMI, the disease state of STEMI does adversely affect the degree of platelet inhibition observed following the administration of a ticagrelor loading dose. NSTEMI patients are not subject to the same

physiological changes or the co-adminstration of opioids as are STEMI patients, these differences most likely account for the improved gastrointestinal absorption and enhanced antiplatelet effect seen.

Chapter 8 - A Pharmacodynamic Comparison of all Three Oral P2Y12 Inhibitors in the context of STEMI vs NSTEMI in Patients Undergoing Percutaneous Coronary Intervention (PCI)

8.1 Introduction

Chapter 1 and the results presented in chapters 5,6 and 7 provide some initial insights into the clinical efficacy and drug handling of the three oral P2Y12 inhibitors administered to patients who present following an ACS.

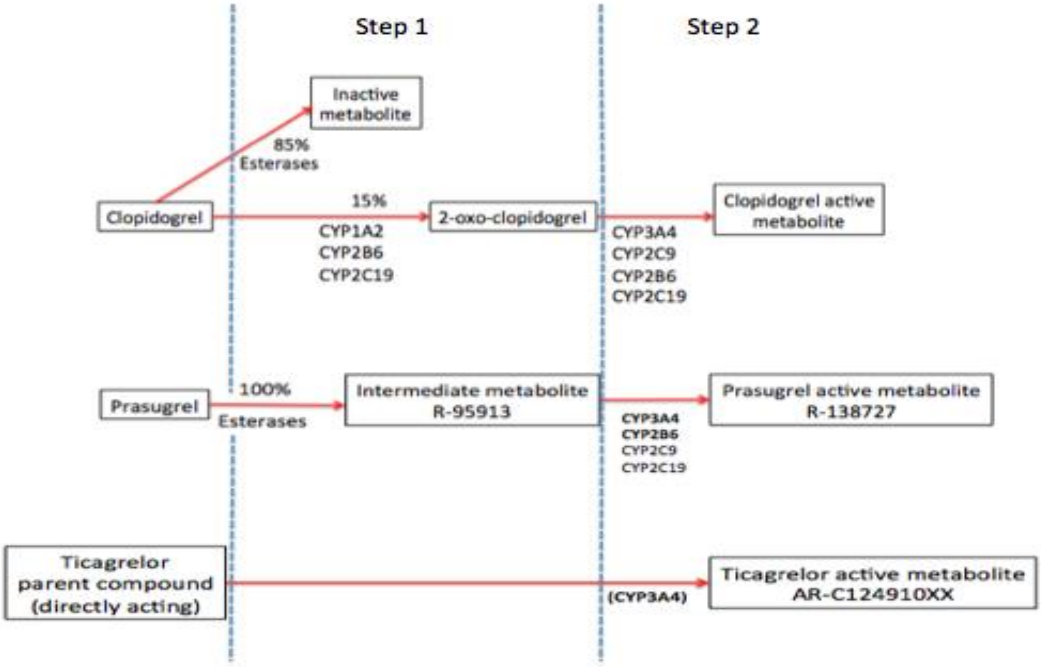
Clopidogrel, although supported by a robust evidence base is accompanied by a number of inherent sub-optimal characteristics that limit its use in clinical practice. As shown in figure 5, and described in chapter 1, clopidogrel following oral administration is subject to two pathways which dictate its pharmacological profile; a major pathway which is driven by human carboxylesterases and leads to inactivation of 85% of the orally administered dose (Varenhorst, James et al. 2009, Floyd, Passacquale et al. 2012). A second minor pathway is also involved and is a two-step process that is reliant upon the cytochrome P450 isoenzymes; 3A4 and 2C19 in particular (Floyd, Passacquale et al. 2012). As a consequence, clopidogrel is relatively slow in terms of its onset of action and is subject to significant interindividual variability in response, secondary to genetic polymorphisms in the 2C19 allele (Tapp, Shantsila et al. 2010).

Prasugrel, on the other hand, following oral ingestion, undergoes rapid and complete gastroinstestinal absorption and is subject to only a single major pathway prior to conversion of the inactive prodrug to its active metabolite (figure 6, chapter 1).

Ticagrelor also undergoes rapid and complete gastrointestinal absorption, however, it differs to the thienopyridines in that it does not require metabolic bioactivation; the parent compound is directly acting and able to exert an antiplatelet effect (figure 7, chapter 1).

Initial study findings indicate that clopidogrel (chapter 5), prasugrel (chapter 6) and ticagrelor (chapter 7) demonstrate reduced antiplatelet efficacy in STEMI patients and that the disease state of STEMI does impact on their pharmacokinetic and pharmacodynamic profiles when compared to NSTEMI patients. With this in mind, I aimed to provide a direct head to head comparison of all three agents and compare their relative effectiveness in both STEMI and NSTEMI patients.

Figure 29. Comparative pathways describing the absorption and metabolic biotransformation of oral P2Y12 inhibitors, clopidogrel, prasugrel and ticagrelor (adapted from Floyd et al 2012).



Step 1 - gastrointestinal absorption. Step 2 - metabolic biotransformation.

8.2 Objectives

This chapter aims to compare the degree and time course of platelet inhibition observed following the administration of clopidogrel 600mg, prasugrel 60mg and ticagrelor 180mg loading doses in STEMI and NSTEMI patients. In addition, we will also determine whether the state of STEMI per se adversely effects the clinical efficacy of clopidogrel, prasugrel and ticagrelor.

8.3 Ethical Considerations and Consent

All ethical considerations and procedures for patient consent and recruitment are described in Chapter 4.

8.4 Materials and Methods

All materials and methods are described in Chapter 4.

8.5 Statistics and Data Analysis

The methods for data collection and statistical analyses are outlined in chapter 4.

8.6 Results

Table 37. Baseline characteristics of study population – STEMI vs NSTEMI

Characteristic	STEMI (n = 43)	NSTEMI (n = 44)	*P-value*
Age (yrs)	65.4 ± 14.4	61.5 ± 11.0	0.223
Sex			
Female	13	6	0.073
Risk Factors			
Diabetes Mellitus	6	14	0.073
Hypertension	20	20	1.000
Current Smoker	9	8	0.792
Ex Smoker	16	20	0.517
Hyperlipidaemia	12	25	0.009
Familial History of CAD	23	21	0.831
Previous MI	4	10	0.143
Previous PCI	3	8	0.196
Previous CABG	1	0	0.494
Previous stroke/TIA	2	0	0.241

Table 38. Baseline characteristics - STEMI cohort

Characteristic	Clopidogrel (n =13)	Prasugrel (n = 15)	Ticagrelor (n =15)	*P-value*
Age (years)	78.00 ±9.70	56.00±12.9	63.73 ±11.59	<0.001
Sex				
Female	6 (46)	3 (20)	4 (27)	0.302
Risk Factors				
Diabetes Mellitus	0 (0)	4 (27)	2 (13)	0.127
Hypertension	6 (46)	7 (47)	7 (47)	1
Current Smoker	2 (15)	3 (20)	4 (27)	0.761
Ex Smoker	4 (31)	8 (53)	4 (27)	0.271
Hyperlipidaemia	4 (31)	5 (33)	3 (20)	0.691
Familial History of CAD	6 (46)	9 (60)	8 (53)	0.765
Previous MI	2 (15)	2 (13)	0 (0)	0.302
Previous PCI	2 (15)	1 (7)	0 (0)	0.280
Previous CABG	0 (0)	1 (7)	0 (0)	0.310
Previous stroke/TIA	1 (8)	0 (0)	1 (7)	0.566
Analgesia (opioid)	10 (77)	13 (87)	13 (87)	0.729

Table 39. Baseline Characteristics - NSTEMI cohort.

Characteristic	Clopidogrel (n =14)	Prasugrel (n = 15)	Ticagrelor (n =15)	*P-value*
Age (years)	62.41 ± 5.40	61.07 ± 7.63	62.0 ±13.9	0.957
Sex				
Female	2 (14)	2 (13)	2 (13)	0.996
Risk Factors				
Diabetes Mellitus	2 (14)	7 (47)	4 (27)	0.068
Hypertension	7 (50)	6 (40)	7 (47)	0.858
Current Smoker	3 (21)	1 (7)	3 (20)	0.303
Ex Smoker	7 (50)	7 (47)	6 (40)	0.858
Hyperlipidaemia	8 (57)	5 (33)	12 (80)	0.036
Familial History of CAD	6 (43)	8 (53)	8 (53)	0.811
Previous MI	6 (43)	3 (20)	1 (7)	0.064
Previous PCI	3 (21)	4 (27)	1 (7)	0.332
Previous CABG	0 (0)	0 (0)	0 (0)	1.000
Previous stroke/TIA	0 (0)	0 (0)	0 (0)	1.000

Table 40. Baseline characteristics of study population – Clopidogrel vs Prasugrel vs Ticagrelor

	Clopidogrel			Prasugrel			Ticagrelor		
Characteristic	STEMI (n = 13)	NSTEMI (n = 14)	P-value	STEMI (n = 15)	NSTEMI (n = 15)	P-value	STEMI (n = 15)	NSTEMI (n = 15)	P-value
Age (Years)	78.00 ± 9.70	62.41 ± 5.40	0.005	56.00 ± 12.9	61 ± 7.63	0.214	63.73 ± 11.59	62.00 ± 13.91	0.714
Sex									
Female	6 (46)	2 (14)	0.103	3 (20)	2 (13)	1.000	4 (27)	2 (13)	0.651
Risk Factors									
Diabetes Mellitus	0 (0)	2 (14)	0.481	4 (27)	7 (47)	0.450	2 (13)	4 (27)	0.651
Hypertension	6 (46)	7 (50)	1.000	7 (47)	6 (40)	1.000	7 (47)	7 (47)	1.000
Current Smoker	2 (15)	3 (21)	1.000	3 (20)	1 (7)	0.598	4 (27)	3 (20)	1.000
Ex-smoker	4 (31)	7 (50)	0.44	8 (53)	7 (47)	1.000	4 (27)	6 (40)	0.700
Hyperlipidaemia	4 (31)	8 (57)	0.252	5 (33)	5 (33)	1.000	3 (20)	12 (80)	0.003
Family History of CAD	6 (46)	6 (43)	1.000	9 (60)	8 (53)	1.000	8 (53)	8 (53)	1.000
Previous MI	2 (15)	6 (43)	0.209	2 (13)	3 (20)	1.000	0 (0)	1 (7)	1.000
Previous PCI	2 (15)	3 (21)	1.000	1 (7)	4 (27)	0.330	0 (0)	1 (7)	1.000
Previous CABG	0 (0)	0 (0)	1.000	1 (7)	0 (0)	1.000	0 (0)	0 (0)	-
Previous Stroke/TIA	1 (8)	0 (0)	0.481	0 (0)	0 (0)	-	1 (7)	0 (0)	1.000
Patient therapy on admission									
Analgesia Comprising of:	10 (77)	7 (57)	0.236	13 (87)	7 (47)	0.050	13 (87)	3 (20)	0.001
Morphine	10	2	0.002	13	2	0.000	13	0	0.000
GTN	0	5	0.041	0	5	0.017	0	3	0.224

8.6.1 Pharmacodynamic analysis of the degree of platelet inhibition following aspirin loading (300mg) STEMI vs NSTEMI

Aspirin is administered at the point of first medical contact either by ambulance staff or medical staff in A&E/emergency admissions unit as soon as a diagnosis of myocardial infarction is confirmed. As for the P2Y12 inhibitors, the pharmacodynamic profile of aspirin can also be assessed using the VerifyNow point of care platelet function assay.

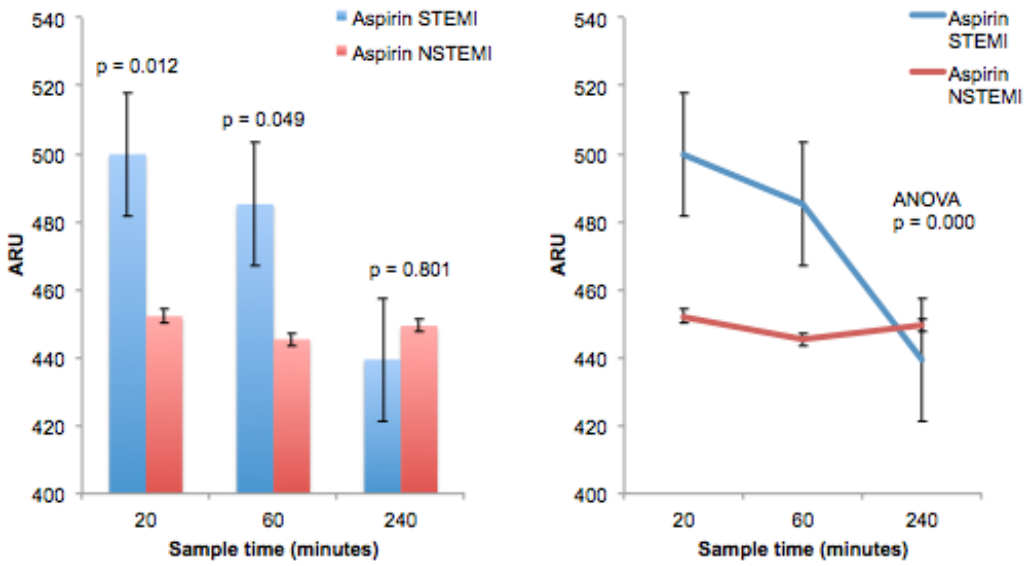
The Aspirin Reaction Unit (ARU) is used as a measure of the degree to which thromboxane A2-mediated platelet activation and aggregation is inhibited following the administration of a 300mg loading dose of aspirin. An ARU of 550 units is assigned as the cut off to determine whether the administration of aspirin yields a sufficient antiplatelet effect. An ARU ≥ 550 is indicative of a lack of antiplatelet effect following the administration of aspirin 300mg orally. An ARU < 550 indicates therapeutic benefit following the administration of oral aspirin. An ARU < 350, is indicative of an excessive antiplatelet effect, which may predispose patients to an increased bleeding risk. The therapeutic window for aspirin following oral ingestion as indicated by the ARU is 350 - 550 (Nielsen, Kristensen et al. 2008, Accumetrics 2016).

8.6.1.1 VerifyNow Results – Aspirin

Table 41. Aspirin - VerifyNow (ARU) STEMI vs NSTEMI

Verify Now result expressed as ARU (post P2Y12 inhibitor loading)	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	STEMI	28	504.89	42.82 [9.77, 75.88]	72.27	13.66	0.012
	NSTEMI	58	462.07		72.22	9.48	
60 minutes	STEMI	28	489.00	35.19 [0.221, 70.16]	83.29	15.74	0.049
	NSTEMI	58	453.81		72.93	9.58	
240 minutes	STEMI	26	447.42	4.20 [-28.92, 37.31]	71.64	14.05	0.801
	NSTEMI	58	443.22		70/04	9.20	

Figure 30. VerifyNow mean ARUs (and standard error) after administration of aspirin in STEMI vs NSTEMI patients.



8.6.1.2. Summary

Following the administration of a 300mg loading dose of aspirin this data indicates a statistically significant difference in mean ARU values between STEMI and NSTEMI samples taken at 20 minutes (504.89 ± 72.27 vs 462.07

± 72.24 p < 0.012) and a marginally significant difference at 60 minutes (489.00 ± 83.29 vs 453.81 ± 72.93 p < 0.049).

At four hours following the administration of a 300mg loading dose of aspirin, there is no statistically significant difference in the mean ARU values observed between the STEMI and NSTEMI groups (447.42 ± 71.62 vs 443.22 ± 70.04 p = 0.087).

The data in table 41 were analysed using a two-way analysis of variance test in which the effect of disease state (STEMI vs NSTEMI) on the degree of platelet inhibition (PRU) over time was examined (figure 30). There was no significant difference between the effect of disease state on the degree of platelet inhibition, $F(2,81) = 2.516$, $p = 0.087$. However, the observed reduction in the degree of platelet inhibition over time (e.g at 240 minutes compared to 20 minutes) for both disease states was significant, $F(2,81) = 9.072$, $p = 0.000$.

The reported data indicates that the disease state of STEMI has a minor impact on the degree of platelet inhibition observed following the administration of aspirin 300mg at 20 minutes and 60 minutes. The ARU values reported indicate that aspirin is exerting an antiplatelet effect and the response to treatment is somewhat diminished in STEMI patients. However, irrespective of disease state, a significant reduction in ARU and increase in platelet inhibition is observed over time.

As for the P2Y12 inhibitors, this data indicates that the state of STEMI and the accompanying reduction in gastric motility, particularly during the acute phase of presentation are sufficient enough to impair the onset of action of aspirin. The degree of platelet inhibition following aspirin administration may be further reduced by the co-administration of opioid-based analgesia, which would be administered by the ambulance staff. This is purely hypothetical and not proven, since much of the current data in which the opioid-antiplatelet drug interaction has been investigated involves morphine plus a P2Y12 inhibitor in either healthy subjects or in STEMI patients (Heestermans, van Werkum et al. 2008, Hobl, Stimpfl et al. 2014, Hobl, Reiter et al. 2015, Parodi, Bellandi et al. 2015).

The NSTEMI data indicates more consistent levels of platelet inhibition over time; from which we can postulate that the extent of gastrointestinal absorption in NSTEMI patients remains unchanged over time. In contrast, in STEMI patients, once they have undergone mechanical reperfusion and have rested, the physiological changes that accompany the condition are no longer manifest. In addition, further doses of opioid-based analgesia will not have been administered, and previously administered doses may have been metabolised and excreted. So, in our STEMI patients, gastrointestinal motility returns to normal once PPCI has taken place and adequate myocardial perfusion has been restored.

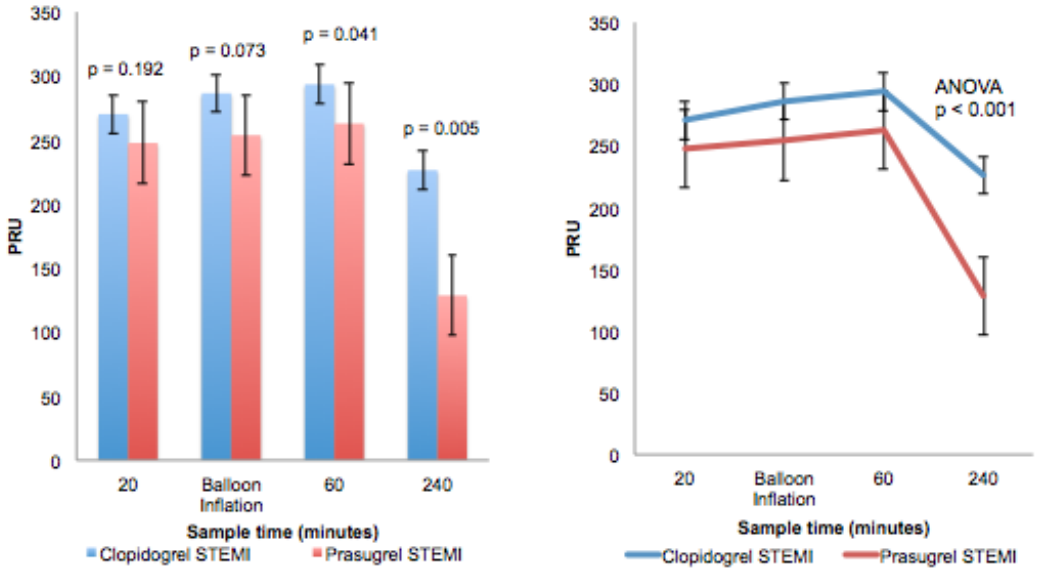
8.6.2 Analysis of the degree of platelet inhibition following clopidogrel loading (600mg) compared with prasugrel loading (60mg) in STEMI and NSTEMI patients.

8.6.2.1 VerifyNow Results – Clopidogrel vs Prasugrel STEMI

Table 42. VerifyNow (PRU) – Clopidogrel vs Prasugrel STEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	13	270.23	22.50 [-12.05, 57.05]	38.56	10.69	0.192
	Prasugrel	15	247.73		48.78	12.60	
Balloon inflation	Clopidogrel	13	286.46	32.73 [-3.25, 68.70]	33.12	9.19	0.073
	Prasugrel	15	253.73		57.18	14.76	
60 minutes	Clopidogrel	13	293.46	30.60 [1.27, 59.92]	31.68	8.79	0.041
	Prasugrel	15	262.87		43.43	11.21	
240 minutes	Clopidogrel	12	226.42	97.77 [32.23, 163.32]	69.44	20.05	0.005
	Prasugrel	14	128.64		89.16	23.83	

Figure 31. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs prasugrel in STEMI patients.



8.6.2.1.1 Summary

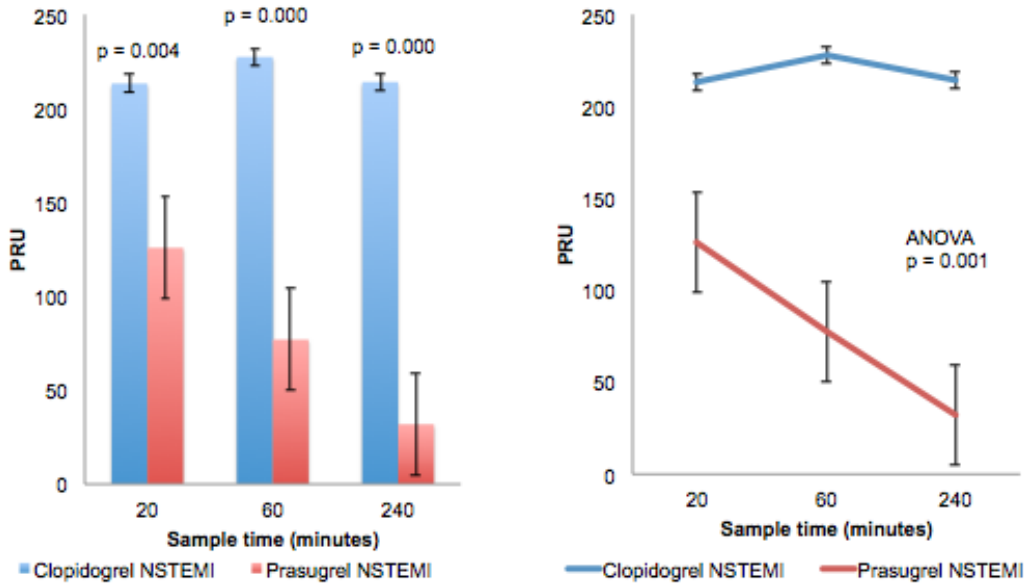
In STEMI patients, the observed pharmacodynamic data (table 42) indicates a non-significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 60mg loading dose of prasugrel for samples taken at 20 minutes (270.2 ± 10.69 vs 247.73 ± 12.60 p<0.192). A significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 60mg loading dose of prasugrel in STEMI patients at balloon inflation (286.46 ± 9.19 vs 253.73 ± 14.76p < 0.073) and 60 minutes (293.46 ± 8.79 vs 262.87 ±11.21 p < 0.041) was observed (figure 31). At four hours following the administration of a 600mg loading dose of clopidogrel vs a 60mg loading dose of prasugrel, a highly statistically significant difference in the mean PRU values was observed (226.4 ± 20.05 vs 128.62 ± 23.83 p = 0.005). The time by group interaction as assessed by ANOVA (the difference in mean PRU reduction over time between clopidogrel vs prasugrel) indicates a non-significant relationship (p = 0.140). However, irrespective of the drug administered, the degree of platelet inhibition at 240 minutes is significantly less than that at 20 minutes (p = 0.000).

8.6.2.2. VerifyNow Results – Clopidogrel vs Prasugrel NSTEMI

Table 43. VerifyNow (PRU) – Clopidogrel vs Prasugrel NSTEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	14	213.21	87.41 [87.04, 213.81]	51.74	13.83	0.004
	Prasugrel	15	125.80		89.34	23.08	
60 minutes	Clopidogrel	14	227.36	150.42 [87.04, 213.81]	61.19	16.36	0.000
	Prasugrel	15	76.93		99.24	25.62	
240 minutes	Clopidogrel	14	214.00	182.13 [136.34, 227.93]	69.98	18.70	0.000
	Prasugrel	15	31.87		45.52	11.75	

Figure 32. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs prasugrel in NSTEMI patients.



8.6.2.2.1 Summary

In NSTEMI patients, the reported data (table 43) indicates a highly significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 60mg loading dose of prasugrel for samples taken at 20 minutes (213.21 ± 13.83 vs 125.80 ± 23.07 p<0.004), at 60

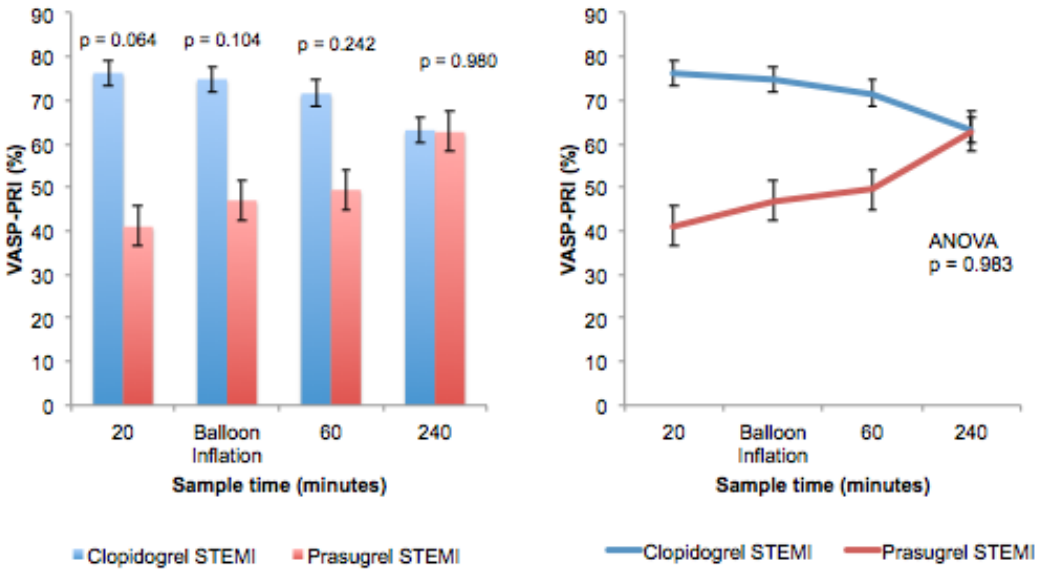
minutes (227.36 ± 16.36 vs 76.93 ± 25.62 p<0.000) and at 240 minutes (214.00 ± 18.70 vs 31.87 ± 11.75 p < 0.000). The data in table 43 were analysed using a two way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of clopidogrel vs prasugrel in NSTEMI patients. There was a statistically significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel at each time point (time*group interaction), F(2,26) = 10.224, p = 0.001. The observed reduction and difference in the degree of platelet inhibition over time between both drugs was also highly significant, F(2,26) = 8.612, p = 0.001.

8.6.2.3 VASP-PRI% Results – Clopidogrel vs Prasugrel STEMI

Table 44. VASP-PRI% – Clopidogrel vs Prasugrel STEMI

VASP %PRI post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	7	76.29	35.27 [-2.34, 72.91]	23.21	8.77	0.064
	Prasugrel	7	41.00		39.35	14.87	
Balloon inflation	Clopidogrel	7	74.86	27.86 [-6.62, 62.34]	23.81	9.00	0.104
	Prasugrel	7	47.00		34.44	13.02	
60 minutes	Clopidogrel	7	71.57	22.14 [-17.03, 61.32]	30.91	11.68	0.242
	Prasugrel	7	49.43		36.16	13.67	
240 minutes	Clopidogrel	7	63.14	0.429 [-35.78, 36.64]	35.08	13.26	0.980
	Prasugrel	7	62.71		26.51	10.02	

Figure 33. Mean VASP-PRI(%) (and standard error) following the administration of clopidogrel vs prasugrel in STEMI patients.



8.6.2.3.1 Summary

In STEMI patients, the reported data indicates a non-significant difference in mean VASP-PRI% values following the administration of a 600mg loading dose of clopidogrel and a 60mg loading dose of prasugrel for samples taken at 20 minutes (76.29 ± 8.77 vs 41.00 ± 14.87 p = 0.064), at the time of balloon inflation (74.68 ± 9.00 vs 47.00 ± 13.02 p = 0.104), at 60 minutes (71.57 ± 11.68 vs 49.43 ± 13.67 p = 0.242) and at 240 minutes (63.14 ± 13.26 vs 62.71 ± 10.02 p = 0.980).

The data in table 44 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs prasugrel in STEMI patients.

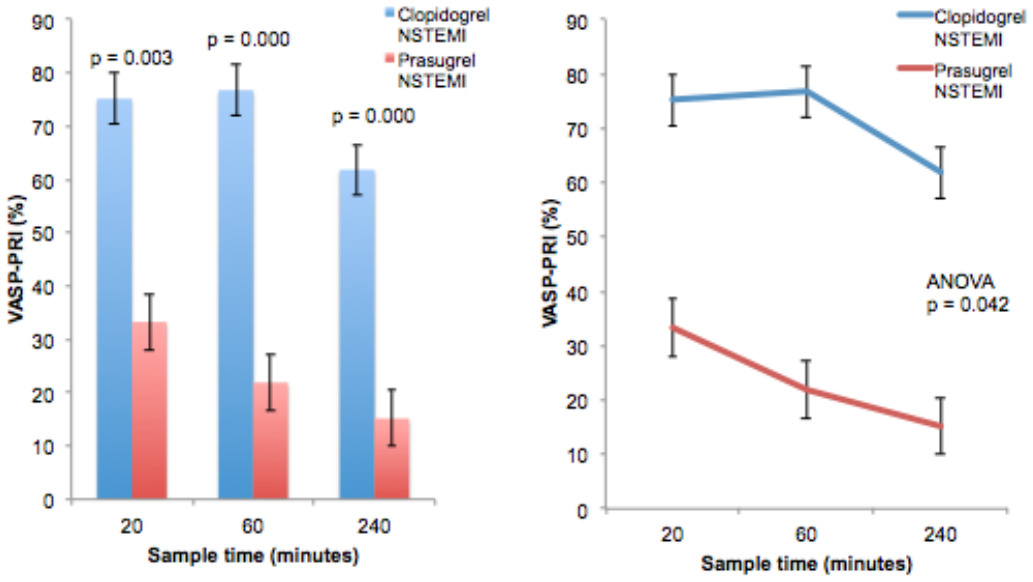
There was no significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel at each time point (time*group interaction), F(3,10) = 1.564, p = 0.259. The observed reduction and difference in the degree of platelet inhibition over time between both drugs was also not significant, F(3,10) = 0.54, p = 0.983.

8.6.2.4 VASP-PRI% Results – Clopidogrel vs Prasugrel NSTEMI

Table 45. VASP-PRI% – Clopidogrel vs Prasugrel NSTEMI

%PRI	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	12	75.17	41.89 [17.75, 66.04]	9.48	2.74	0.003
	Prasugrel	11	33.27		35.36	10.66	
60 minutes	Clopidogrel	12	76.75	54.84 [33.34, 76.35]	15.08	4.35	0.000
	Prasugrel	11	21.91		32.23	9.72	
240 minutes	Clopidogrel	12	61.83	46.65 [29.46, 63.84]	20.16	5.82	0.000
	Prasugrel	11	15.18		19.40	5.85	

Figure 34. Mean VASP-PRI(%) (and standard error) following the administration of clopidogrel vs prasugrel in NSTEMI patients.



8.6.2.4.1 Summary

In NSTEMI patients, the reported data indicates a significant difference in mean VASP-PRI% values following the administration of a 600mg loading dose of clopidogrel and a 60mg loading dose of prasugrel for samples taken at 20 minutes (75.17 ± 2.74 vs 33.27 ± 10.66 p = 0.003) and a highly significant difference at 60 minutes (76.75 ± 4.35 vs 21.91 ± 9.72 p = 0.000) and 240 minutes (631.83 ± 5.82 vs 15.18 ± 5.85 p = 0.000).

The data in table 45 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs prasugrel in NSTEMI patients. There was no significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel at each time point (time*group interaction), $F(2,20) = 1.519$, $p = 0.243$. However, the observed reduction and difference in the degree of platelet inhibition over time, at 20 minutes compared to 240 minutes, between both drugs was significant, $F(2,20) = 3.741$, $p = 0.042$.

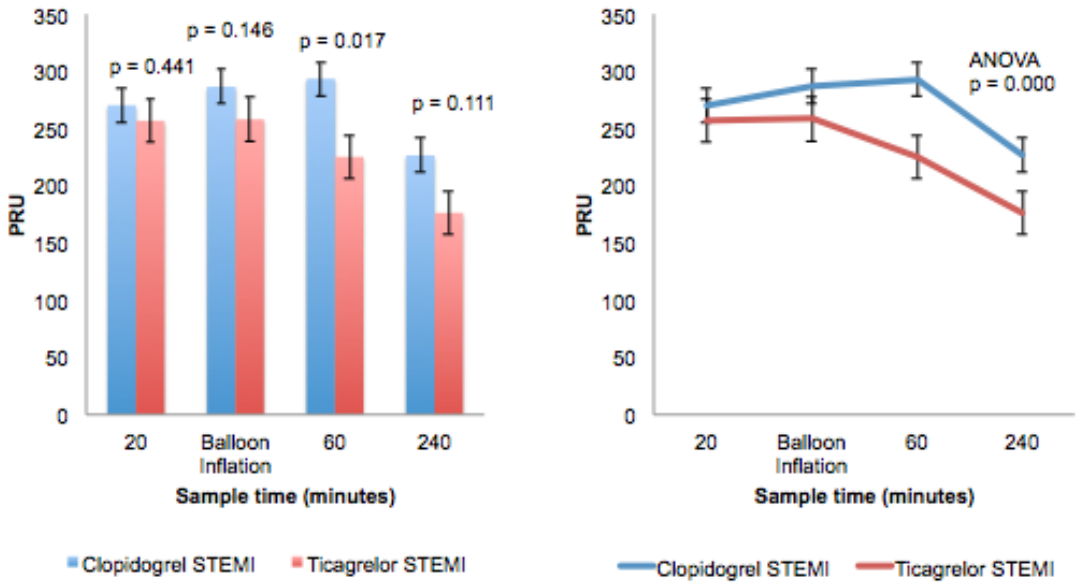
8.6.3 Analysis of the degree of platelet inhibition following clopidogrel loading (600mg) compared with ticagrelor loading (180mg) in STEMI and NSTEMI patients.

8.6.3.1 VerifyNow Results – Clopidogrel vs Ticagrelor STEMI

Table 46. VerifyNow (PRU) – Clopidogrel vs Ticagrelor STEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	13	270.23	13.50 [-21.32, 48.32]	38.56	10.69	0.441
	Ticagrelor	15	256.73		50.81	13.12	
Balloon inflation	Clopidogrel	13	286.46	28.53 [-10.58, 67.61]	33.12	9.19	0.146
	Ticagrelor	15	257.93		61.12	15.71	
60 minutes	Clopidogrel	13	293.46	68.26 [19.85, 116.67]	31.68	8.79	0.017
	Ticagrelor	15	225.20		82.70	21.35	
240 minutes	Clopidogrel	12	226.42	22.50 [=12.05, 57.05]	69.44	20.05	0.111
	Ticagrelor	15	176.27		84.92	21.93	

Figure 35. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs ticagrelor in STEMI patients.



8.6.3.1.1.Summary

In STEMI patients, the reported data indicates a non-significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (270.23 ± 10.69 vs 256.73 ± 13.12 p<0.441) and at balloon inflation (213.21 ± 13.83 vs 125.80 ± 23.07 p<0.004). A significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 180mg loading dose of ticagrelor in STEMI patients at 60 minutes (293.46 ± 8.79 vs 225.20 ± 21.30 p < 0.017) was observed. At 240 minutes a non-significant difference between clopidogrel and ticagrelor was noted (226.42 ± 20.05 vs 176.27 ± 21.93 p < 0.111).

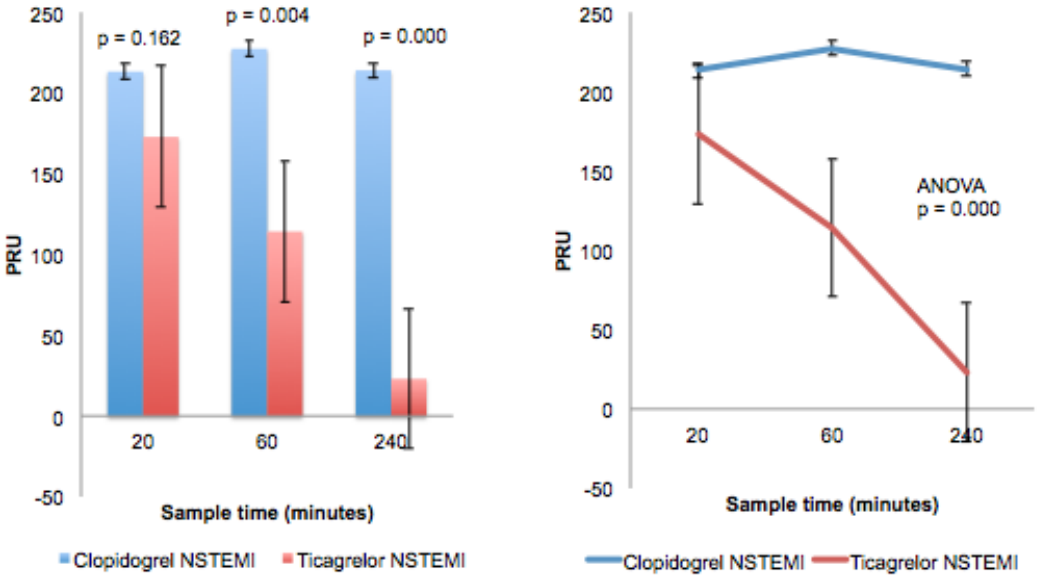
The data in table 46 were analysed using a two-way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of clopidogrel vs ticagrelor in STEMI patients. There was a non-significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel at each time point (time*group interaction), $F(2,23) = 2.143$, $p = 0.122$ (figure 35). The observed reduction and difference in the degree of platelet inhibition over time between both drugs was also highly significant, $F(3,23) = 8.770$, $p = 0.000$ (figure 35).

8.6.3.2 VerifyNow Results – Clopidogrel vs Ticagrelor NSTEMI

Table 47. VerifyNow (PRU) – Clopidogrel vs Ticagrelor NSTEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	14	213.21	40.41 [-17.30, 98.13]	51.74	13.83	0.162
	Ticagrelor	15	172.80		92.54	23.90	
60 minutes	Clopidogrel	14	227.36	113.16 [39.22, 187.09]	61.19	16.36	0.004
	Ticagrelor	15	114.20		122.22	31.56	
240 minutes	Clopidogrel	14	214.00	191.00 [149.64, 232.26]	69.98	18.70	0.000
	Ticagrelor	15	23.00		18.94	4.89	

Figure 36. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs ticagrelor loading doses in NSTEMI patients.



8.6.3.2.1 Summary

In NSTEMI patients, the reported data indicates a non-significant difference in mean PRU values following the administration of a 600mg loading dose of clopidogrel and a 180mg loading dose of ticagrelor for samples taken at 20

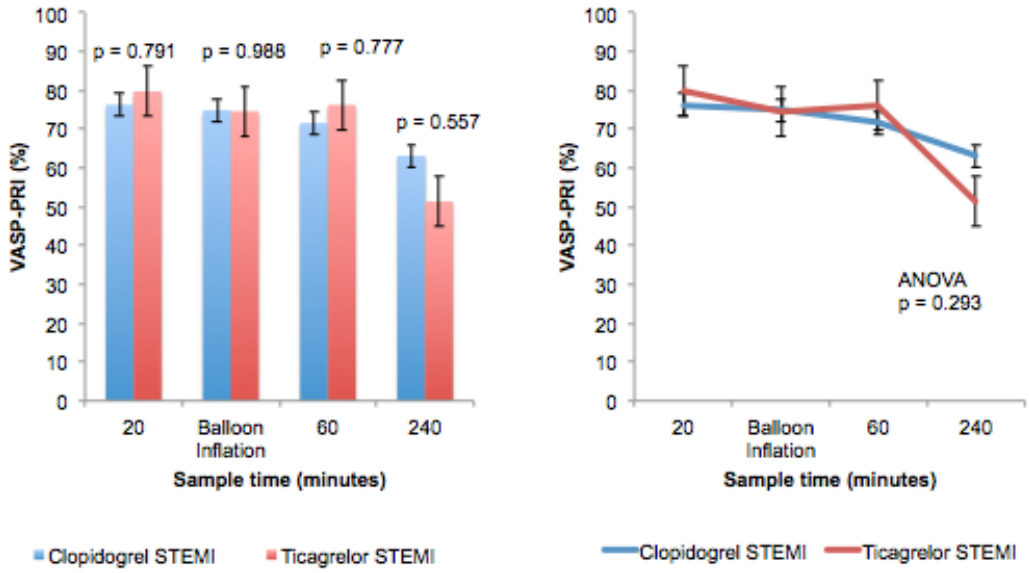
minutes (213.21 ± 13.83 vs 172.80 ± 23.90 p<0.162). A statistically significant difference in mean PRU values was observed for sample taken at 60 minutes (227.36 ± 16.36 vs 114.20 ± 16.36 p<0.004) and at 240 minutes (214.00 ± 18.70 vs 23.00 ± 4.89 p < 0.000). The data in table 47 were analysed using a two-way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of clopidogrel vs ticagrelor in NSTEMI patients. There was a significant difference in the degree of platelet inhibition following the administration of clopidogrel vs ticagrelor at each time point (time*group interaction), F(2,26) = 19.052, p = 0.000 (figure 36). The observed reduction and difference in the degree of platelet inhibition over time between both drugs was also highly significant, F(2,26) = 16.998, p = 0.000 (figure 36).

8.6.3.3 VASP-PRI% Results – Clopidogrel vs Ticagrelor STEMI

Table 48. VASP-PRI% – Clopidogrel vs Ticagrelor STEMI

%PRI	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	7	76.29	-3.46	23.21	8.77	0.791
	Ticagrelor	8	79.75	[-31.15, 72.90]	26.01	9.20	
Balloon inflation	Clopidogrel	7	74.86	0.23	23.81	9.00	0.988
	Ticagrelor	8	74.63	[-31.85,32.31]	32.30	11.42	
60 minutes	Clopidogrel	7	71.57	-4.68	30.91	11.68	0.777
	Ticagrelor	8	76.25	[-39.57, 30.21]	31.46	11.12	
240 minutes	Clopidogrel	7	63.14	11.77	35.08	13.26	0.557
	Ticagrelor	8	51.38	[-30.37, 53.90]	39.78	14.07	

Figure 37. Mean VASP-PRI(%) (and standard error) following the administration of clopidogrel vs ticagrelor in STEMI patients.



8.6.3.3.1 Summary

In STEMI patients, this reported data indicates a non-significant difference in mean VASP-PRI% values following the administration of a 600mg loading dose of clopidogrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (76.29 ± 8.77 vs 79.75 ± 9.20 p = 0.791), at the time of balloon inflation (74.68 ± 9.00 vs 74.63 ± 11.42 p = 0.998), at 60 minutes (71.57 ± 11.68 vs 76.25 ± 11.12 p = 0.777) and at 240 minutes (63.14 ± 13.26 vs 51.38 ± 14.07 p = 0.557).

The data in table 48 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs ticagrelor in STEMI patients. There was no significant difference in the degree of platelet inhibition following the administration of clopidogrel vs ticagrelor at each time point (time*group

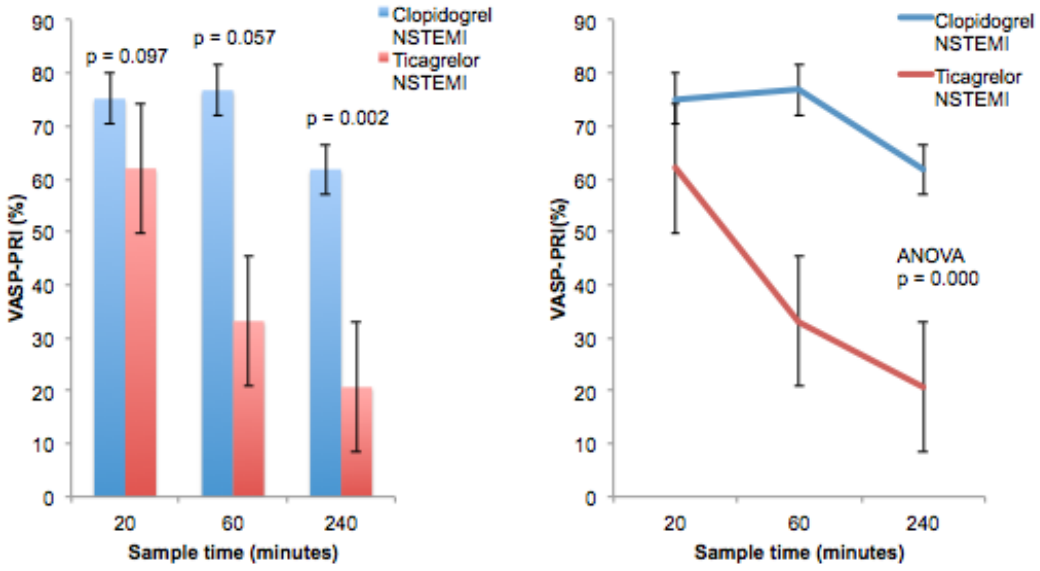
interaction), F(3,11) = 0.361, p = 0.782 (figure 37). The observed reduction and difference in the degree of platelet inhibition over time, at 20 minutes compared to 240 minutes, between both drugs was not significant, F(3,11) = 1.405, p = 0.293 (figure 37).

8.6.3.4 VASP-PRI% Results – Clopidogrel vs Ticagrelor NSTEMI

Table 49. VASP-PRI% – Clopidogrel vs Ticagrelor NSTEMI

%PRI	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Clopidogrel	12	75.17	13.17 [-2.66, 29.00]	9.48	2.74	0.097
	Ticagrelor	7	62.00		23.25	8.79	
60 minutes	Clopidogrel	12	76.75	43.58 [14.63, 72.53]	15.08	4.35	0.057
	Ticagrelor	6	33.17		43.44	17.74	
240 minutes	Clopidogrel	12	61.83	41.12 [17.92, 64.32]	20.16	5.82	0.002
	Ticagrelor	7	20.71		27.74	10.48	

Figure 38. Mean VASP-PRI(%) (and standard error) following the administration of clopidogrel vs ticagrelor in NSTEMI patients.



8.6.3.4.1 Summary

In NSTEMI patients, the observed data indicates a non-significant difference in mean VASP-PRI% values following the administration of a 600mg loading dose of clopidogrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (75.17 ± 2.74 vs 62.00 ± 8.79 p = 0.097). At 60 minutes a marginally significant difference in mean VASP-PRI% values was observed (76.75 ± 4.35 vs 33.17 ± 17.73 p = 0.057) and a highly significant difference was seen at 240 minutes (61.83 ± 5.82 vs 20.17 ± 10.40 p = 0.002).

The data in table 49 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs ticagrelor in NSTEMI patients. There was a highly significant difference in the degree of platelet inhibition following the administration of clopidogrel vs ticagrelor at each time point (time*group interaction), $F(2,15) = 9.982$, $p = 0.002$ (figure 38). The observed reduction and difference in the degree of platelet inhibition over time, at 20 minutes compared to 240 minutes, between both drugs was also highly significant, $F(2,15) = 19.199$, $p = 0.000$ (figure 38).

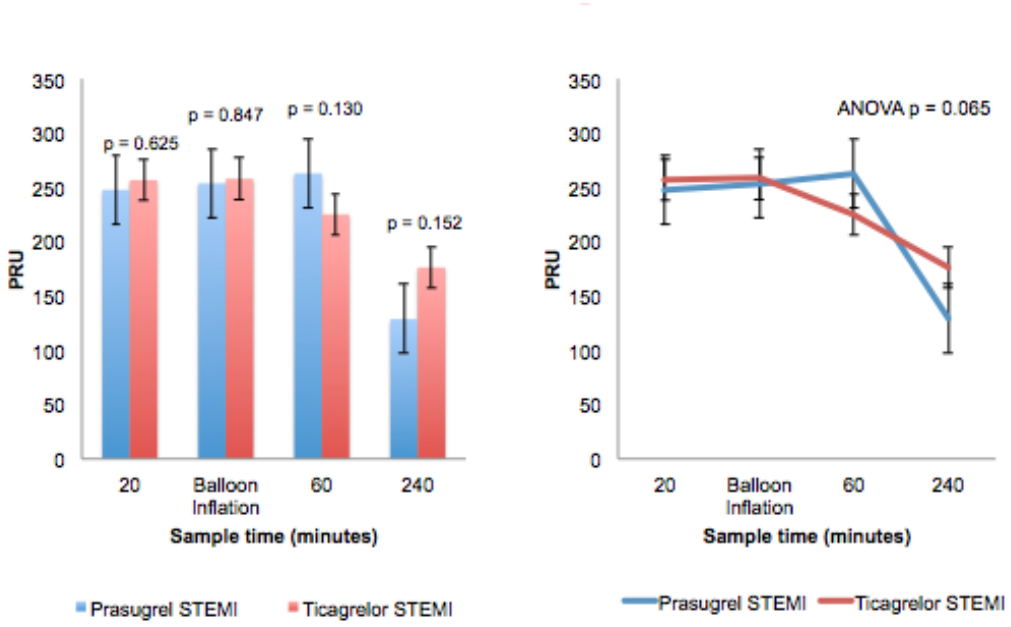
8.6.4 Analysis of the degree of platelet inhibition following prasugrel loading (60mg) compared with ticagrelor loading (180mg) in STEMI and NSTEMI patients.

8.6.4.1 VerifyNow Results – Prasugel vs Ticagrelor STEMI

Table 50. VerifyNow (PRU) – Prasugrel vs Ticagrelor STEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Prasugrel	15	247.73	-9.00 [-46.26, 28.26]	48.78	12.60	0.625
	Ticagrelor	15	256.73		50.81	13.12	
Balloon inflation	Prasugrel	15	253.73	-4.20 [-48.47, 40.07]	57.16	14.76	0.847
	Ticagrelor	15	257.93		61.12	15.78	
60 minutes	Prasugrel	15	262.87	24.12 [-11.74, 87.07]	43.43	11.21	0.130
	Ticagrelor	15	225.20		82.70	21.35	
240 minutes	Prasugrel	14	128.64	-47.62 [-113.95, 18.70]	89.16	23.83	0.152
	Ticagrelor	15	176.27		84.92	21.93	

Figure 39. VerifyNow mean PRUs (and standard error) following the administration of prasugrel vs ticagrelor loading doses in STEMI patients.



8.6.4.1.1 Summary

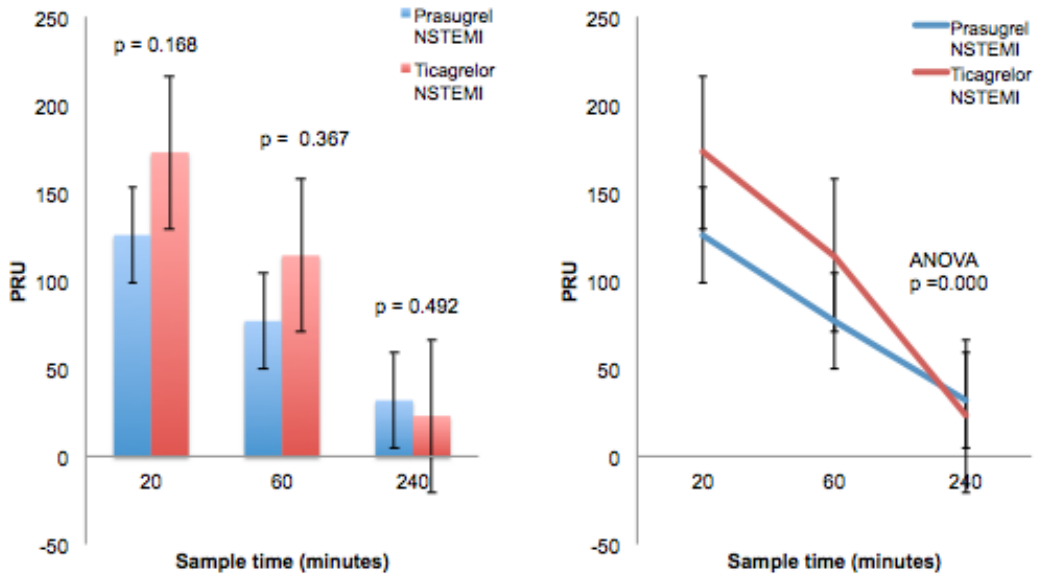
In STEMI patients, the observed data indicates a non-significant difference in mean PRU values following the administration of a 60mg loading dose of prasugrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (242.73 ± 12.60 vs 256.73 ± 13.12 p = 0.625), at balloon inflation (253.73 ± 14.72 vs 257.93 ± 15.78 p = 0.847), at 60 minutes (262.87 ± 11.21 vs 225.20 ± 82.70 p = 0.130), and at 240 minutes (128.64 ± 23.83 vs 176.27 ± 21.93 p = 0.152). The data in table 50 were analysed using a two-way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of prasugrel vs ticagrelor in STEMI patients. There was a significant difference in the degree of platelet inhibition following the administration of prasugrel vs ticagrelor over time, F(3,25) = 10.692, p = 0.000 (figure 39). The observed reduction and difference in the degree of platelet inhibition at each time point between both drugs was not of significance, F(3,25) = 2.729, p = 0.065 (figure 39).

8.6.4.2 VerifyNow Results – Prasugel vs Ticagrelor NSTEMI

Table 51. VerifyNow (PRU) – Prasugrel vs Ticagrelor NSTEMI

VerifyNow PRU post loading	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Prasugrel	15	125.80	-47.00 [-115.03, 21.03]	89.34	23.07	0.168
	Ticagrelor	15	172.80		92.54	23.90	
60 minutes	Prasugrel	15	76.93	-37.27 [-120.54, 46.00]	99.24	25.62	0.367
	Ticagrelor	15	114.20		122.24	31.56	
240 minutes	Prasugrel	15	31.87	-8.87 [-17.21, 34.94]	45.52	11.75	0.492
	Ticagrelor	15	23.00		18.94	4.89	

Figure 40. VerifyNow mean PRUs (and standard error) following the administration of prasugrel vs ticagrelor loading doses in NSTEMI patients.



8.6.4.2.1 Summary

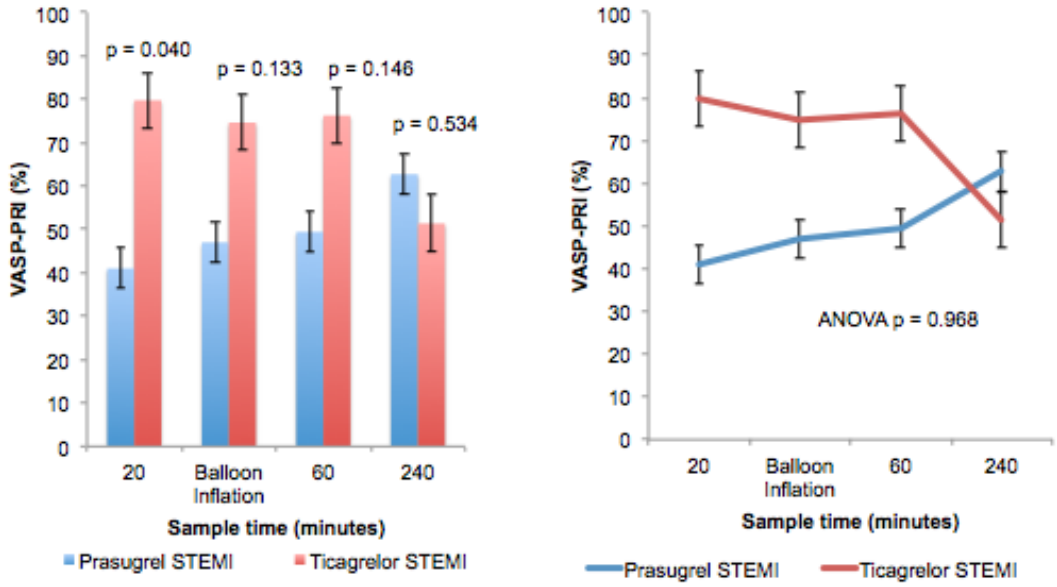
In NSTEMI patients, the observed data indicates a non-significant difference in mean PRU values following the administration of a 60mg loading dose of prasugrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (125.80 ± 23.07 vs 172.80 ± 23.90 p = 0.168), at 60 minutes (76.93 ± 25.62 vs 114.20 ± 31.56 p = 0.367), and at 240 minutes (31.87 ± 11.75 vs 23.00 ± 4.89 p = 0.492). The data in table 51 were analysed using a two-way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of prasugrel vs ticagrelor in NSTEMI patients. There was a non-significant difference in the degree of platelet inhibition following the administration of prasugrel vs ticagrelor at each time point (time*group interaction), F(2,27) = 1.696, p = 0.202 (figure 40). The observed reduction and difference in the degree of platelet inhibition over time for both drugs was highly significant, F(2,27) = 34.162, p = 0.000 (figure 40).

8.6.4.3 VASP-PRI% Results – Prasugrel vs Ticagrelor STEMI

Table 52. VASP-PRI% – Prasugrel vs Ticagrelor STEMI

%PRI	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Prasugrel	7	41.00	-38.74 [-75.47, -2.03]	39.35	14.87	0.040
	Ticagrelor	8	79.75		26.01	9.20	
Balloon inflation	Prasugrel	7	47.00	-27.63 [-64.86, 9.61]	34.44	13.02	0.133
	Ticagrelor	8	74.63		32.30	11.42	
60 minutes	Prasugrel	7	49.43	-26.82 [-64.51, 10.87]	36.16	13.67	0.148
	Ticagrelor	8	76.25		31.46	11.12	
240 minutes	Prasugrel	7	62.71	11.34 [-27.01, 49.69]	26.51	10.02	0.534
	Ticagrelor	8	51.38		39.78	14.07	

Figure 41. Mean VASP-PRI(%) (and standard error) following the administration of prasugrel vs ticagrelor in STEMI patients.



8.6.4.3.1 Summary

In STEMI patients, observed data indicates a significant difference in mean VASP-PRI% values following the administration of a 60mg loading dose of prasugrel and a 180mg loading dose of ticagrelor for samples taken at 20

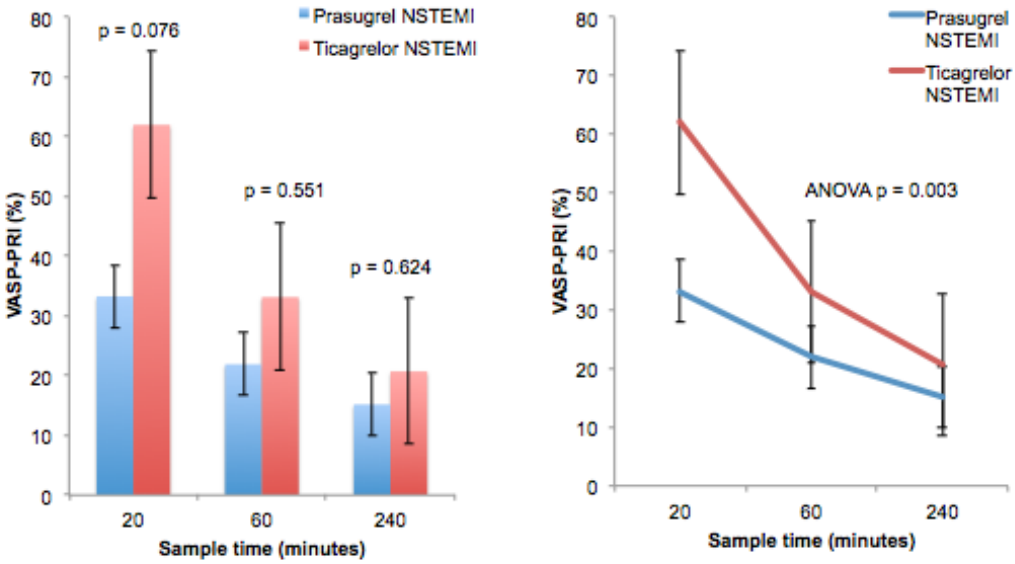
minutes (41.00 ± 14.87 vs 79.75 ± 9.20 p = 0.040). A non-significant difference in mean VASP-PRI% values was observed at the time of balloon inflation (47.00 ± 13.02 vs 74.63 ± 11.42 p = 0.131), at 60 minutes (49.43 ± 13.67 vs 76.25 ± 11.12 p = 0.148) and at 240 minutes (62.71 ± 10.02 vs 51.38 ± 14.07 p = 0.543). The data in table 52 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs ticagrelor in STEMI patients. There was no significant difference in the degree of platelet inhibition following the administration of clopidogrel vs ticagrelor at each time point (time*group interaction), F(3,11) = 1.522, p = 0.256 (figure 41). The observed reduction and difference in the degree of platelet inhibition over time, at 20 minutes compared to 240 minutes, between both drugs was not significant, F(3,11) = 0.083, p = 0.968 (figure 41).

8.6.4.4 VASP-PRI% Results – Prasugrel vs Ticagrelor NSTEMI

Table 53. VASP-PRI% – Prasugrel vs Ticagrelor NSTEMI

%PRI	Group	N	Mean	Mean Difference [95% CI]	Std. Deviation	Std. Error Mean	p-value
20 minutes	Prasugrel	11	33.27	-28.73 [-60.88, 3.43]	35.36	10.66	0.076
	Ticagrelor	7	62.00		23.25	8.79	
60 minutes	Prasugrel	11	21.91	-11.23 [-50.58, 28.07]	32.23	9.72	0.551
	Ticagrelor	6	33.17		43.44	17.73	
240 minutes	Prasugrel	11	15.18	-5.53 [-28.99, 17.92]	19.40	5.85	0.624
	Ticagrelor	7	20.71		27.74	10.48	

Figure 42. Mean VASP-PRI(%) (and standard error) following the administration of prasugrel vs ticagrelor in NSTEMI patients.



8.6.4.4.1 Summary

In NSTEMI patients, the oberserved data indicates a marginally significant difference in mean VASP-PRI% values following the administration of a 60mg loading dose of prasugrel and a 180mg loading dose of ticagrelor for samples taken at 20 minutes (33.27 ± 10.66 vs 62.00 ± 8.79 p = 0.076). A non-significant difference in mean VASP-PRI% values was observed at 60 minutes (21.91 ± 9.72 vs 33.17 ± 17.73 p = 0.551) and at 240 minutes (15.18 ± 5.85 vs 20.71 ± 10.40 p = 0.624).

The data in table 53 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) was examined over time following the administration of clopidogrel vs ticagrelor in NSTEMI patients. There was non-significant difference in the degree of platelet inhibition following the administration of clopidogrel vs ticagrelor at each time point

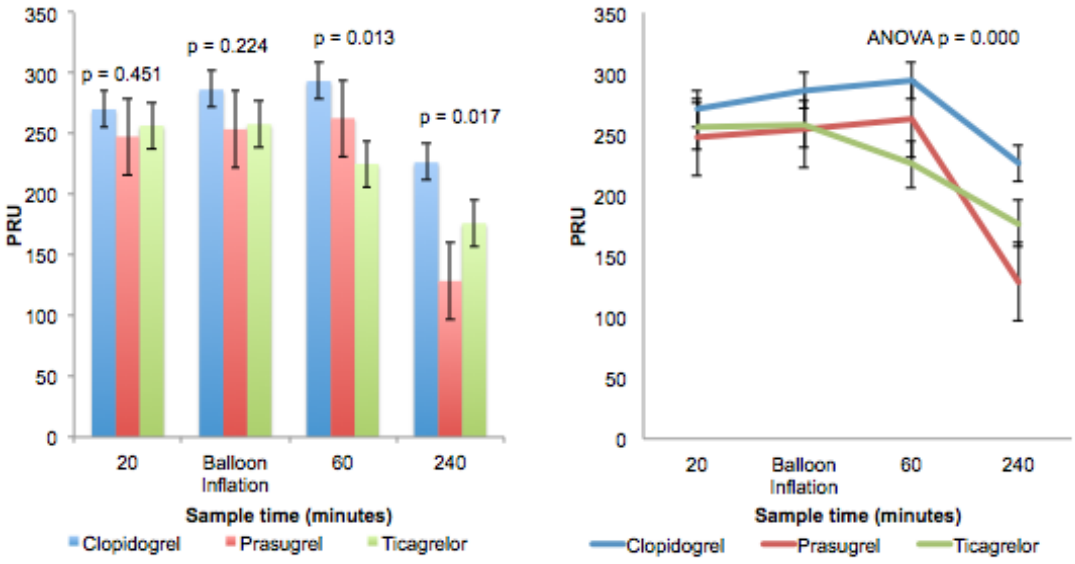
(time*group interaction), F(2,14) = 2.214, p = 0.146 (figure 42). However, the observed reduction and difference in the degree of platelet inhibition over time, at 240 minutes compared to 20 minutes, between prasugrel and ticagrelor was significant, F(2,14) = 9.211, p = 0.003 (figure 42).

8.6.4.7 VerifyNow Results – Clopidogrel vs Prasugel vs Ticagrelor STEMI

Table 54. ANOVA descriptive statistics - Comparison of mean PRU over time following the administration of clopidogrel 600mg vs prasugrel 60mg vs ticagrelor 180mg in STEMI patients.

VerifyNow PRU post loading	N	Clopidogrel (Mean ± SD)	Prasugrel (Mean ± SD)	Ticagrelor (Mean ± SD)	p-value
20 minutes	41	269.42 ± 40.15	240.93 ± 42.60	256.73 ± 50.81	0.451
Balloon inflation	41	286.75 ± 34.58	248.29 ± 55.15	257.93 ± 61.12	0.224
60 minutes	41	296.50 ± 31.05	257.36 ± 39.25	225.20 ± 82.70	0.013
240 minutes	41	226.42 ± 69.44	128.64 ± 89.16	176.27 ± 84.92	0.017

Figure 43. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in STEMI patients.



8.6.4.7.1 Summary

The data in table 54 were analysed using a two-way analysis of variance test

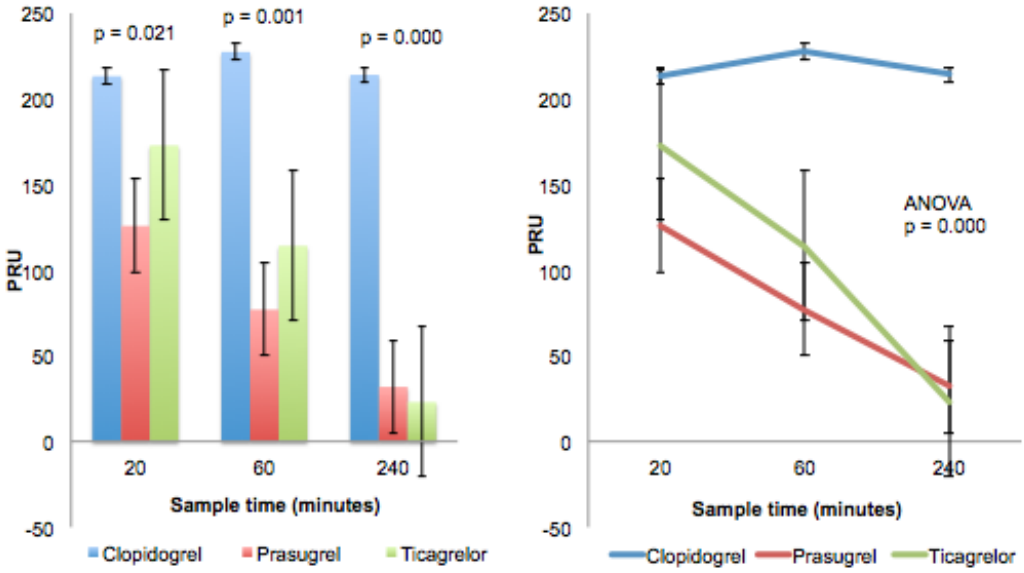
in which the degree of platelet inhibition (PRU) over time was examined following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in STEMI patients. There was a significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel vs ticagrelor at each time point (time*group interaction), $F(6,72) = 2.625$, $p = 0.023$ (figure 43). This significance is driven by clopidogrel and the respective PRU observed in the prasugrel and ticagrelor groups). The observed reduction and difference in the degree of platelet inhibition over time between all three drugs was highly significant, $F(3,36) = 12.282$, $p = 0.000$ (figure 43).

8.6.4.8 VerifyNow Results – Clopidogrel vs Prasugel vs Ticagrelor NSTEMI

Table 55. ANOVA descriptive statistics - Comparison of mean PRU over time following the administration of clopidogrel 600mg vs prasugrel 60mg vs ticagrelor 180mg in NSTEMI patients.

VerifyNow PRU post loading	N	Clopidogrel (Mean ± SD)	Prasugrel (Mean ± SD)	Ticagrelor (Mean ± SD)	p-value
20 minutes	44	213.21 ± 51.74	125.80 ± 89.34	172.80 ± 92.54	0.021
60 minutes	44	227.36 ± 61.19	76.93 ± 99.24	114.20 ± 122.22	0.001
240 minutes	44	214.00 ± 69.98	31.87 ± 45.52	23.00 ± 18.94	0.000

Figure 44. VerifyNow mean PRUs (and standard error) following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in NSTEMI patients.



8.6.4.8.1 Summary

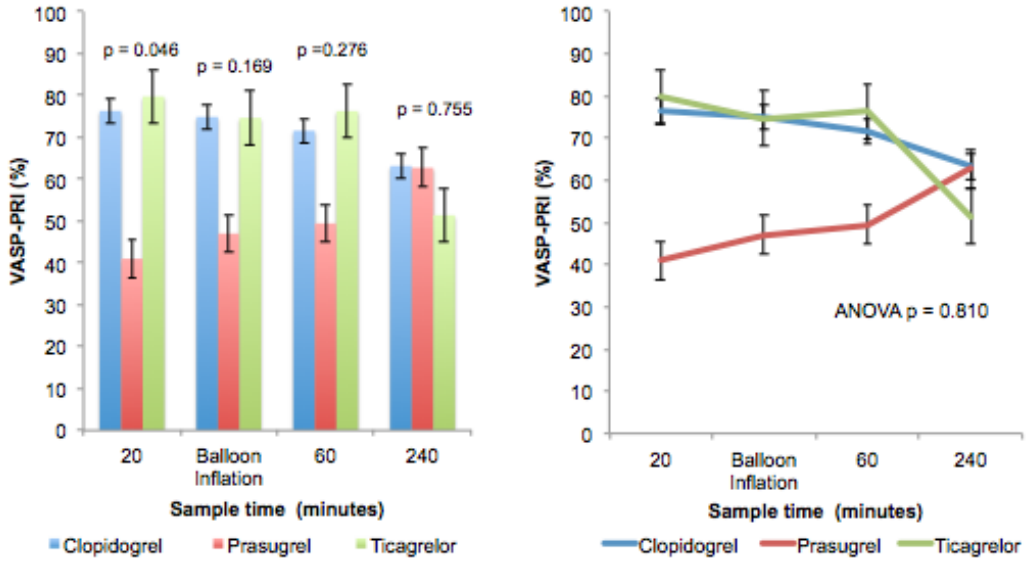
The data in table 55 were analysed using a two-way analysis of variance test in which the degree of platelet inhibition (PRU) over time was examined following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in NSTEMI patients. There was a highly significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel vs ticagrelor at each time point (time*group interaction), F(4,80) = 7.759, p = 0.000 (figure 44). The observed reduction and difference in the degree of platelet inhibition over time between all three drugs was highly significant, F(2,40) = 29.097, p = 0.000 (figure 44).

8.6.4.9 VASP-PRI% Results – Clopidogrel vs Prasugrel vs Ticagrelor STEMI

Table 56. ANOVA descriptive statistics - Comparison of change in mean VASP-PRI% over time following the administration of clopidogrel 600mg vs prasugrel 60mg vs ticagrelor 180mg in STEMI patients.

%PRI post loading	N	Clopidogrel (Mean ± SD)	Prasugrel (Mean ± SD)	Ticagrelor (Mean ± SD)	p-value
20 minutes	22	76.29 ± 23.21	41.00 ± 39.35	79.75 ± 26.01	0.046
Balloon inflation	22	74.86 ± 23.81	47.00 ± 34.44	74.63 ± 32.30	0.169
60 minutes	22	71.57 ± 30.91	49.43 ± 36.16	76.25 ± 31.46	0.276
240 minutes	22	63.14 ± 35.08	62.71 ± 26.51	51.38 ± 39.78	0.755

Figure 45. Mean VASP-PRI% (and standard error) following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in STEMI patients.



8.6.4.9.1 Summary

The data in table 56 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) over time was examined following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in STEMI patients. There was a non-significant difference in the

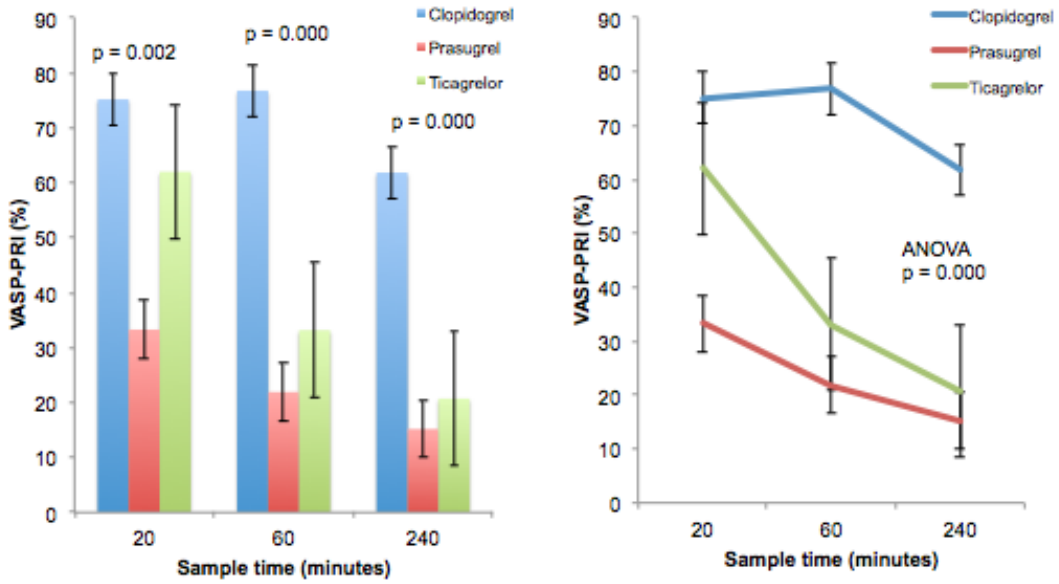
degree of platelet inhibition following the administration of clopidogrel vs prasugrel vs ticagrelor at each time point (time*group interaction), F(6,34) = 20.994, p = 0.445 (figure 45). The observed reduction and difference in the degree of platelet inhibition over time between all three drugs was also non-significant, F(3,17) = 0.321, p = 0.810 (figure 45).

8.6.4.10 VASP-PRI% Results – Clopidogrel vs Prasugrel vs Ticagrelor NSTEMI

Table 57. ANOVA descriptive statistics - Comparison of change in mean VASP-PRI% over time following the administration of clopidogrel 600mg vs prasugrel 60mg vs ticagrelor 180mg in NSTEMI patients.

%PRI post loading	N	Clopidogrel (Mean ± SD)	Prasugrel (Mean ± SD)	Ticagrelor (Mean ± SD)	p-value
20 minutes	29	75.17 ± 9.48	33.27 ± 35.36	69.33 ± 14.04	0.002
60 minutes	29	76.75 ± 15.08	21.91 ± 32.23	33.17 ± 43.44	0.000
240 minutes	29	61.83 ± 20.16	15.18 ± 19.40	19.33 ± 30.12	0.000

Figure 46. Mean VASP-PRI% (and standard error) following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in NSTEMI patients.



8.6.4.10.1 Summary

The data in table 57 were analysed using a two-way analysis of variance test in which the degree of P2Y12 receptor blockade (platelet inhibition) as measured by the platelet reactivity index (%PRI) over time was examined following the administration of clopidogrel vs prasugrel vs ticagrelor loading doses in NSTEMI patients. There was a significant difference in the degree of platelet inhibition following the administration of clopidogrel vs prasugrel vs ticagrelor at each time point (time*group interaction), $F(4,50) = 3.077$, $p = 0.024$ (figure 46). The observed reduction and difference in the degree of platelet inhibition over time (at 240 minutes compared to 20minutes) between all three drugs was highly significant, $F(2,25) = 14.103$, $p = 0.000$ (figure 46).

8.7 Discussion

In terms of the patient population recruited to our study, there are no statistically significant differences between our STEMI and NSTEMI groups (tables 37-40); both groups are well matched in terms of their baseline characteristics, risk factors and co-morbidities. The only exception being that there is a statistically significantly greater incidence of hyperlipidaemia in our NSTEMI group compared with our STEMI group ($p = 0.003$) and the use of opioid based analgesia is significantly greater in our STEMI cohort ($p < 0.000$). The rational behind the difference in opioid use has been explained in chapters 5-7. A possible explanation for the difference in lipid profiles may relate to the time at which blood samples were taken to assess the patient's cholesterol levels. Following a cardiac event lipid levels are known to significantly decline secondary to down regulation of cholesterol biosynthesis

and take approximately 12 weeks to recover (Barth, Jackson et al. 2010). In STEMI patients, a diagnosis is often made on the basis of signs and symptoms at presentation and ECG changes, and not necessarily the presence of cardiac biomarkers. As a result blood samples are not usually requested or taken until the patient has returned to the ward after receiving treatment in the catheter lab. At this time there will be a reduction in cholesterol synthesis, such that the lipid levels recorded may not be a true reflection of the patient's actual baseline prior to presentation. NICE recommendations regarding secondary prevention following a MI advocate aggressive lipid lowering therapy with high intensity statin (atorvastatin 80mg daily), irrespective of the patient's cholesterol levels on presentation (NICE CG 167 2013).

When comparing our STEMI cohort, they are again well matched in terms of co-morbidities and risk factors, however, a statistically significant difference in terms of age is apparent between the three groups ($p < 0.001$) as shown in table 38. In this study, younger patients were recruited to the prasugrel arm and older patients to the clopidogrel arm (56.00±12.9 vs 78.00 ± 9.70, $p < 0.001$). These differences are primarily attributable to the licensing recommendations for prasugrel, which restricts its use to patients under the age of 75 years. As explained in chapter 6, older patients are exposed to greater levels of P-AM compared to younger patients and are therefore at increased risk of experiencing bleeding complications.

Although clopidogrel was the default antiplatelet of choice in patients over the age of 75 years, it's clinical efficacy may be further compromised by age

related physiological changes which may occur in older patients, particularly with regards to reduced gastric emptying, motility and subsequent absorption. Age related physiological changes are known to adversely impact on drug absorption (Mangoni and Jackson 2003).

8.7.1 Clopidogrel vs Prasugrel

Our VerifyNow STEMI data indicates that there are no statistically significant differences in the degree of platelet inhibition achieved between prasugrel and clopidogrel at 20 minutes and balloon inflation and only a moderate difference at 60 minutes. All PRU values > 230, indicating a significantly reduced antiplatelet effect and HRPR, at 240 minutes, however, prasugrel has a therapeutic PRU at 128, whilst the PRU for clopidogrel remains > 208 (table 42). In our NSTEMI group, a statistically significant difference in PRU values between clopidogrel and prasugrel is observed at all time points (table 43). Prasugrel demonstrates a superior pharmacodynamic profile in NSTEMI patients when compared to clopidogrel.

As reported in chapters 5 and 7, our VASP-P data does follow the same trend as our VerifyNow pharmacodynamic data; however, the correlation between the results reported by the two platelet function assays is poor. Although, VASP-P is the gold standard in terms of accurately assessing platelet reactivity, it is a highly specialist technique which requires a considerable degree of expertise to carry out correctly. The inconsistencies in our reported VASP-P results render it inadequate as a reliable indicator of the pharamcocdynamic antiplatelet effect of orally administered P2Y12 inhibitors.

Our VerifyNow STEMI data indicate that neither clopidogrel nor prasugrel provide adequate levels of platelet inhibition at the time of angioplasty and stent implantation; furthermore, both agents are subject to HRPR. Our data is therefore in line with previously reported work in which a reduced antiplatelet effect of clopidogrel and prasugrel in STEMI has been noted (Heestermans, van Werkum et al. 2008, Alexopoulos, Galati et al. 2012, Parodi, Valenti et al. 2013). However, our study is the first to provide a direct head to head comparison of clopidogrel and prasugrel in this context, and demonstrates that there is no difference between the two agents in terms of IPA achieved during the acute phase of a MI.

The differences in IPA observed following the administration of a clopidogrel 600mg loading dose compared with prasugrel 60mg loading dose are driven by the degree to which the parent compound is available for conversion following absorption via the gastrointestinal tract. As described in chapters 5 and 6, 85% of orally administered clopidogrel is converted to an inactive compound following ingestion, in comparison, prasugrel undergoes complete and rapid gastrointestinal absorption facilitating greater generation of its active metabolite (as described in chapter 1, figure 4).

In this study, sample collection was capped at 4 hours, however, in other reported studies, extending beyond this time, a plateau in IPA is observed with prasugrel; this is a pharmacodynamic limitation secondary to P2Y12 receptor saturation as opposed to a pharmacokinetic limitation of active

metabolite generation, as is the case with clopidogrel (Floyd, Passacquale et al. 2012).

The superior pharmacokinetic/pharmacodynamic profile of prasugrel over clopidogrel presents a number of advantages; there is a reduced period of time period from administration of a 60mg loading dose to conversion to P-AM and subsequent ability to achieve maximal IPA, thereby reducing the incidence and likelihood of HRPR. However, in contrast the PK and PD data reported in chapter 6, indicates that prasugrel is still subject to HRPR, which can limit its immediate effectiveness in the context of STEMI and pre-dispose patients to increased risk of further MACCE or complications such as stent thrombosis.

Clopidogrel is known to demonstrate significant variations in IPA ranging from 0% to 100% (Aleil, Ravanat et al. 2005). However, our data indicate that irrespective of disease state, clopidogrel does not achieve adequate levels of platelet inhibition for at least 4 hours if not longer following the administration of a 600mg loading dose. The pharmacodynamic profile of prasugrel, on the other hand is supposed to be more predictable, with a minimum IPA of 50% at any one time when compared to clopidogrel (Brandt, Payne et al. 2007). Our data very clearly shows that in STEMI patients, the time course of platelet inhibition between the two drugs is similar.

The endovascular injury that occurs during a STEMI and subsequent damage that may arise due to mechanical intervention leads to an increase in platelet turnover and consequently higher levels of circulating immature (juvenille)

platelets (Nylander and Schulz 2016). This increase in platelet turnover contributes to the high prothrombotic environment encountered during a STEMI which increases the thrombotic burden associated with the condition and can subsequently lead to poorer cardiovascular outcomes (Alexopoulos, Xanthopoulou et al. 2013).

The increase in platelet turnover and presence of immature/juvenille platelets may account for the reduced antiplatelet efficacy of clopidogrel and prasugrel seen in STEMI patients despite the administration of adequate loading doses and circulating levels of C-AM that are comparable in potency to P-AM (Ibrahim, Nadipalli et al. 2012).

8.7.2 Clopidogrel vs Ticagrelor

The pharmacodynamic VerifyNow data indicates that when comparing the degree of platelet inhibition between clopidogrel and ticagrelor in our STEMI patients there is no significant difference in the PRU values recorded (table 48). Our data indicates that ticagrelor demonstrates inadequate levels of platelet inhibition early on in treatment and is also subject to HRPR (as shown in chapter 7). As such our data is in line with previously reported pharmacodynamic studies which also demonstrate a delay in the onset of action and a lack of antiplatelet efficacy for at least two hours following the administration of a ticagrelor loading dose (Alexopoulos, Galati et al. 2012, Parodi, Valenti et al. 2013). However, this study is the first to report on a direct head to head comparison of clopidogrel and ticagrelor during the acute phase of an MI; furthermore, our study demonstrates that although ticagrelor

is directly acting and should have a faster onset of action and a more enhanced antiplatelet effect, in the context of STEMI, the degree of IPA achieved is comparable to that seen with clopidogrel and neither agent is particularly effective at the time of angioplasty, with PRU values being > 230 indicating HRPR (table 48).

When comparing the efficacy of clopidogrel against ticagrelor in our NSTEMI patients, our data indicates that ticagrelor provides a level of IPA that is numerically greater at 20 minutes and statistically significantly greater than that seen with clopidogrel at 60 minutes and 240 minutes (table 49).

The reported VASP-P data, very much like the VerifyNow data indicates that there are no differences in the degree of platelet inhibition observed, as expressed by the %PRI between our clopidogrel and ticagrelor STEMI groups. However, all reported %PRI values are > 50% which is indicative of an inadequate antiplatelet effect and HRPR for both drugs over our study period.

As reported in chapters 5 and 7, there is a poor correlation between the results of our two platelet function assays, however, the VASP-P trend observed is very similar to that seen with the VerifyNow results reported in our ticagrelor patients.

As for the VerifyNow NSTEMI data, our VASP-P results indicate a statistically significant difference between the %PRI observed at 60 and 240 minutes for clopidogrel vs ticagrelor and a numerically lower %PRI at 20 minutes.

There are no published pharmacodynamic data comparing the onset of action of clopidogrel against ticagrelor in NSTEMI patients against which we can benchmark our data. But based on what we have already established during our study and our understanding of the mechanisms of action and pathways of activation, ticagrelor is able to provide faster and more consistent levels of IPA in NSTEMI patients secondary to improved gastrointestinal absorption.

8.7.3 Prasugrel vs Ticagrelor

The VerifyNow pharmacodynamic data indicates that there are no statistically significant differences in the degree of platelet inhibition observed following the administration of prasugrel or ticagrelor loading doses in our STEMI patients, and that neither agent demonstrates an adequate or effective level of platelet inhibition for at least the first hour post-loading. As shown in table 54, the PRU values at 20 minutes and at balloon inflation are > 230 for both agents indicating HRPR and an insufficient antiplatelet effect at the time of angioplasty and coronary artery stent implantation. Our study findings are in line with those previously reported by Parodi et al and Alexopoulos et al (Alexopoulos, Xanthopoulou et al. 2012, Parodi, Valenti et al. 2013).
Our VerifyNow NSTEMI data indicates that there is no significant difference in the degree of IPA observed following the administration of either prasugrel or ticagrelor (table 55); both agents achieve adequate levels of platelet inhibition

early after loading with possibly excessive levels IPA at 240 minutes. Such low residual platelet reactivity (LRP) is known to be associated with increased bleeding risk (Montalescot, Collet et al. 2014).

In contrast to the VerifyNow data, the STEMI VASP-P results indicate increasing levels of platelet reactivity (%PRI) over time despite the administration of prasugrel 60mg and decreasing levels of %PRI following the administration of ticagrelor 180mg. Our NSTEMI VASP-P data demonstrates a reduction in %PRI over time, although the values noted are < 50% at all time points, indicating adequate if not slightly excessive levels of platelet inhibition. Our NSTEMI VASP-P results are in line with our VerifyNow results; both agents demonstrate LRPR in the context of NSTEMI.

Of note, both our VerifyNow and VASP-P results indicate that in the NSTEMI patients, prasugrel when compared to ticagrelor results in numerically lower PRU and %PRI values. Since ticagrelor is directly acting, we would expect greater levels of IPA following its administration compared to prasugrel since the latter requires metabolic activation following GI absorption.

As already discussed in our clopidogrel and prasugrel comparisons, the validity of our VASP-P dataset cannot be confirmed, as such these results are not the most reliable upon which to base any conclusions regarding the pharmacodynamic activity of the oral P2Y12 inhibitors used in our study.

The reported VerifyNow results are more reliable, consistent and reproducible and are in line with previously reported findings; they indicate a delay in the onset of action of both prasugrel and ticagrelor in STEMI patients (Alexopoulos, Galati et al. 2012, Parodi, Valenti et al. 2013). Furthermore, our results indicate that despite displaying a superior pharmacodynamic profile to clopidogrel, prasugrel and ticagrelor are still subject to HRPR which limit their effectiveness in STEMI patients (Lemesle, Schurtz et al. 2015).

8.7.4 Clopidogrel vs Prasugrel vs Ticagrelor

When comparing the onset of action and degree of IPA in STEMI patients, our pharmacodynamic data indicates that neither clopidogrel, prasugrel or ticagrelor provide adequate levels of platelet inhibition at all data collection time points as evidenced by PRU values > 230. Both prasugrel and ticagrelor are superior to clopidogrel resulting in a significant reduction in the degree of platelet reactivity over time, however, this significance only becomes apparent after 60 minutes. Although prasugrel and ticagrelor are comparable in terms of inhibition of platelet activity at 20 minutes, time of balloon inflation, 60 minutes and 240 minutes, they still do not achieve the desired levels of platelet inhibition necessary during PPCI (angioplasty and coronary artery stent implantation) as evidenced by PRU > 230.

The reported VerifyNow results indicate that there was a significant difference over time in the degree of platelet inhibition observed between clopidogrel and prasugrel ($p < 0.001$), clopidogrel and ticagrelor ($p = 0.04$) and no statistically significant difference was found when comparing prasugrel with ticagrelor ($p =$

0.065). In the NSTEMI group however, there is a marked and rapid reduction in platelet reactivity for prasugrel and ticagrelor at all time points. Over time there was a significant difference between the effect of both prasugrel ($p < 0.001$) and ticagrelor ($p < 0.001$) in STEMI vs NSTEMI patients. When comparing the effect of clopidogrel on the degree of platelet inhibition over time in STEMI vs NSTEMI patients, there was no significant difference apparent ($p = 0.065$).

The VASP-P data reported does not demonstrate the same degree of reliability or reproducibility as our VerifyNow results. However, some inferences can be drawn from the data; both clopidogrel and ticagrelor show a reduction in %PRI over time, indicating an increase in the level of IPA. In addition, the VASP-P data also indicates reduced antiplatelet efficacy during the acute phase of a STEMI as evidenced by %PRI values > 50%. The VASP-P data indicates that there is a marginal significant difference between the three drugs over time ($p = 0.445$), however, this difference is driven by an increasing %PRI in the prasugrel group. The VASP-P NSTEMI data indicates that as with our VerifyNow, clopidogrel displays higher levels of platelet activity at all data collection time point (%PRI > 50%) and that prasugrel is numerically superior to ticagrelor in terms of platelet inhibition.

This study is the first to report a direct head to head comparison of all three orally administered P2Y12 inhibitors in both STEMI and NSTEMI patients. Our findings demonstrate variability in response, reduced antiplatelet effectiveness and slower onset of action following the administration of

clopidogrel, prasugrel and ticagrelor in STEMI patients when compared with NSTEMI/UA patients.

The results of this thesis demonstrate that, the pathophysiological condition of STEMI has an adverse impact on the clinical effectiveness of all oral P2Y12 inhibitors. The reduction in cardiac output, increase in sympathetic tone, which in turn can lead to mesenteric vasoconstriction, can affect the ability of the gut to facilitate adequate absorption (Agrawal and Bhatt 2013).

Gastrointestinal absorption is of paramount importance to the onset of action and ability of clopidogrel, prasugrel and ticagrelor to exert their antiplatelet effects. Even ticagrelor which is directly acting and doesn't require metabolic activation via first pass metabolism was found to be just as ineffective as prasugrel and clopidogrel in terms of platelet inhibition, particularly at the time of PPCI and for at least 2 hours post administration of the loading dose.

The reported data show that prasugrel and ticagrelor are also subject to HRPR with inadequate levels of early IPA such that neither are effective at the time of PPCI (Lemesle, Schurtz et al. 2015).

Platelets contribute to the inflammatory response exhibited following endovascular injury; upon activation, platelets release pro-inflammatory cytokines in addition to microRNA. MicroRNA can trigger further inflammatory processes as well as contributing to further atherosclerosis and angiogenesis. Platelet inhibition, therefore not only prevents further aggregation but also restricts the pro-inflammatory capability of activated platelets (Nylander and Schulz 2016) Despite administration of clopidogrel, prasugrel or ticagrelor, an

increase in the emerging population of uninhibited (juvenille) platelets occurs; juvenille platelets formed after the administration of an oral P2Y12 inhibitor contribute to thrombus formation and may well account for recurrent ischaemic and thrombotic complications observed in some patients even whilst on antiplatelet therapy (Nylander and Schulz 2016).

There is a growing and compelling body of evidence that support the findings of our work; in that the pharmacodynamic effect of clopidogrel, prasugrel and ticagrelor are impaired and the subsequent onset of action and antiplatelet effect is delayed in STEMI patients (Heestermans, van Werkum et al. 2008, Alexopoulos 2013, Parodi, Valenti et al. 2013). The pharmacodynamic effect of the P2Y12 receptor inhibitors is directly related to and proportional to the presence of the active moiety whether that is the directly acting parent compound or active metabolite.

Unlike ticagrelor, the thienopyridines have very little effect when in the systemic circulation following GI absorption (figure 31 step 1), their antiplatelet effect occurs following metabolic activation in the hepatic circulation, where biotransformation to the active metabolite takes place.

Despite shorter duration of exposure to their active metabolites, the thienopyridines have an overall prolonged duration of action, since recovery of platelet function takes at least 7-10 days following the cessation of prasugrel and 5-7 days with clopidogrel due to the irreversible binding of the AM to the P2Y12 receptor (Price, Walder et al. 2012). Recovery of platelet function returns to almost normal levels after 3-5 days following the discontinuation of

ticagrelor. Due to the reversibility of ticagrelor; the offset or reduction in IPA is more rapid when compared to clopidogrel, such that within 24 hours of discontinuation, a 50% reduction in IPA is seen, hence the need for twice daily dosing (Gurbel, Bliden et al. 2009).

It has been proposed that the presence of ticagrelor within the systemic circulation in its directly acting form should allow for inhibition of these juvenille platelets also; such systemic exposure is not apparent with clopidogrel and prasugrel. In view of this ticagrelor should not be limited by HRPR, since it should be able to inhibit older “established” activated platelets as well as newly formed platelets that appear in increased volumes in response to endovascular injury (Mathur, Robinson et al. 2001, Nylander and Schulz 2016).

However, our pharmacodynamic data demonstrates that despite the pharmacokinetic and pharmacodynamic advantages of ticagrelor over clopidogrel and prasugrel, HRPR is still a limitation to its use in practice, particularly in the immediate period following administration in STEMI patients. A possible reason for this may be attributable to the delay in gastrointestinal absorption that occurs in the setting of a STEMI, so even though ticagrelor is directly acting, the fact that its systemic exposure is reliant on GI absorption which is impaired in STEMI leads to a reduced antiplatelet effect. Such that in the setting of a STEMI, ticagrelor’s onset of action is comparable to that of prasugrel and both are delayed by at least two to fours hours.

8.8 Conclusion

Despite providing faster, greater and more consistent levels of platelet inhibition, in the setting of STEMI, prasugrel and ticagrelor are just as ineffective as clopidogrel and are still subject to HRPR within the first hour following administration of a loading dose. The reported pharmacodynamic data indicates that prasugrel and ticagrelor take at least 4 hours to achieve effective levels of platelet inhibition. As such, all oral P2Y12 inhibitors display a delayed and attenuated effect in the setting of STEMI.

The narrow time frames from symptom onset and mechanical reperfusion coupled with the physiological changes that occur during a STEMI; reduced gastric motility and absorption, increase in intrinsic platelet reactivity in addition to the co-administration of opioid based analgesia, impose immediate barriers that significantly limit the clinical utility of orally administered P2Y12 inhibitors in patients who present following a STEMI.

In contrast, our NSTEMI patients display adequate if not excessive levels of platelet inhibition following the administration of prasugrel and ticagrelor. Clopidogrel despite the administration of a loading dose does not provide adequate levels of platelet inhibition during the study period.

The results of this chapter demonstrate that the degree and time course of platelet inhibition is such that none of the orally administered P2Y12 inhibitors display an inadequate antiplatelet effect at the time of angioplasty and stent implantation in STEMI patients treated with PPCI. When comparing STEMI to NSTEMI patients, both prasugrel and ticagrelor demonstrate an attenuated

antiplatelet effect in STEMI patients and clopidogrel displays a delayed onset of action in both groups. Indicating that the disease state of STEMI does adversely impact on the onset of action and subsequent therapeutic effect of orally administered P2Y12 inhibitors.

Chapter 9 – Overall Summary and Conclusions

The principle aim of this thesis was to provide insights into the pharmacokinetics and pharmacodynamics of orally administered P2Y12 inhibitors during the acute phase of a myocardial infarction and to determine the degree to which the physiological state of a STEMI impacts on drug handling.

In this chapter, the principal findings of my thesis will be discussed, with a particular focus on how they can be applied to influence and/or modify existing clinical practice, in addition to how they may act as a platform for future research projects.

9.1 Summary of key findings

The main findings of this thesis can be summarised as follows:

1. Analysis of the STEMI antiplatelet registry indicates the use of prasugrel and ticagrelor is associated with a mortality benefit when compared with clopidogrel ($p < 0.001$). When comparing survival between prasugrel and ticagrelor, there is no statistically significant difference in outcome ($p = 0.785$). However, when adjusted for confounders, no difference in survival outcomes between the three drugs was seen, indicating that irrespective of improved pharmacological profiles, in the context of AMI, the agent administered has no impact on mortality in the short-term in the study population.
2. STEMI patients undergoing PPCI display sub-optimal levels of platelet inhibition following the administration of clopidogrel 600mg, prasugrel

60mg and ticagrelor 180mg loading doses at 20 minutes, at the time of angioplasty/balloon inflation and at 60 minutes as demonstrated by PRU > 230 and %PRI > 50%.

3. The administration of prasugrel 60mg and ticagrelor 180mg loading doses in NSTEMI patients results in adequate if not optimal levels of platelet inhibition at all times.
4. When comparing STEMI against NSTEMI, the state of STEMI adversely impacts on the pharmacodynamic profile of the oral P2Y12 inhibitor antiplatelet agents administered as demonstrated by the significant differences in PRU values recorded. The degree of platelet inhibition is far less in the STEMI cohort as evidenced by PRU and %PRI values in excess of 230 and 50% respectively, indicating that these patients remain at increased risk of experiencing thrombotic complications or further adverse cardiac events.
5. When comparing the antiplatelet efficacy of prasugrel against ticagrelor, it is apparent that irrespective of disease state, there is no significant difference in the degree of platelet inhibition observed between the two agents following the administration of a loading dose. However, both prasugrel and ticagrelor are superior to clopidogrel.
6. Gastrointestinal absorption appears to be a main contributor to and important determinant of the onset of action and clinically efficacy of the orally administered P2Y12 inhibitors. Delays in gastric emptying and reduced absorption either secondary to the diversion of blood flow away from the gut or as a consequence of opioid administration, introduces significant delays and inter-individual variability in response

to the effects of clopidogrel, prasugrel and ticagrelor in the context of STEMI.

9.2 General Discussion

The STEMI/PPCI pathway has been refined and standardised through audit and national benchmarking to such an extent that the timescales from symptom onset and diagnosis to reperfusion are now very short. Whilst this leads to improved outcomes for the patient, it presents a number of challenges in ensuring the pharmacological management options utilised are effective; the reduced period of time from presentation to reperfusion reduces the time over which orally administered drugs can exert an optimal therapeutic effect.

The improved pharmacokinetic and pharmacodynamic profiles supporting the large phase III studies behind prasugrel and ticagrelor apply data that were taken from healthy patients or those with SCAD. The work presented in this thesis has shown that these findings cannot be extrapolated and applied to STEMI patients; not only do the physiological changes that occur during a major heart attack impose inherent and immediate barriers to onset of action and clinical efficacy of oral P2Y12 inhibitors, the short time scales between presentation, administration of loading doses and mechanical reperfusion are too narrow to allow for a clinically meaningful antiplatelet effect to take place. The pharmacodynamic data reported in this thesis indicates a lack of antiplatelet effect for at least the first hour following administration of an oral loading dose of either clopidogrel, prasugrel or ticagrelor; the mean DTB in our centre for STEMI patients is 26.8 ±12.7 minutes. As such the data

presented in this thesis clearly demonstrates inadequate levels of platelet inhibition at the time of angioplasty and coronary artery stent implantation.

As well as being the first study to report a direct head to head comparison of all three oral P2Y12 inhibitors, this study is also unique in that it is the first to report a direct comparison of the degree and time course of platelet inhibition between STEMI and NSTEMI patients allowing us to quantify the extent by which the efficacy of orally administered antiplatelet agents is impaired by the physiological state of STEMI. The results generated for the STEMI cohort directly correlate with those previously reported in which the majority of STEMI patients require at least 2 to 4 hours to achieve a sufficient degree of platelet inhibition following the administration or prasugrel and ticagrelor loading doses (Alexopoulos, Galati et al. 2012, Parodi, Valenti et al. 2013). The clopidogrel STEMI data indicates a delay in onset of action and reduced degree of platelet inhibition is also in line with previously reported works (Heestermans, van Werkum et al. 2008).

The elevated baseline PRU and %PRI values seen in our STEMI group are indicative of high intrinsic platelet reactivity implying higher activation of platelets in STEMI patients even prior to the administration of an oral antiplatelet loading dose. A direct pharmacodynamic comparison of baseline PRU values between the STEMI and NSTEMI groups demonstrates that a greater period of time is required for clopidogrel, prasugrel and ticagrelor to achieve sufficient levels of platelet inhibition in the context of STEMI. This delay in the onset of action/lag time between administration to absorption

and/or metabolic activation contributes to the development of HRPR and lack of antiplatelet effect.

The results of this thesis suggest that the majority of PPCI procedures were performed without functional levels of platelet inhibition. In contrast to NSTEMI patients, there is a distinct and marked reduction in the PRU values observed for patients treated with prasugrel and ticagrelor. From this we can surmise that the high prothrombotic milieu and physiological changes that occur during a STEMI are not present in NSTEMI patients.

This is supported by the PRU values observed in our NSTEMI cohort; all patients demonstrate low levels of platelet reactivity as evidenced by PRU < 208, indicating adequate and sufficient antiplatelet effects immediately following the administration of prasugrel/ticagrelor loading doses.

This work has highlighted that the safety and efficacy of prasugrel and ticagrelor in NSTEMI patients is largely an underexplored area. The study results demonstrate that when compared with STEMI patients, the NSTEMI cohort achieve adequate if not excessive levels of platelet inhibition, particularly if treated with the more potent prasugrel and ticagrelor. Although this was not an outcome driven study, the recently published meta-analysis by Bavishi et al indicates an increased incidence of TIMI minor and major bleeding in patients who receive pre-treatment with prasugrel or ticagrelor (Bavishi, Panwar et al. 2015).

Bleeding complications in ACS patients are associated with poor clinical outcomes and prognosis in both the short and long term (Becker and Gurbel 2010). The low PRU values observed following the administration of

prasugrel and ticagrelor loading doses at all time points in the our NSTEMI group can be seen as having a strong positive predictive value for bleeding complications in patients pretreated with oral P2Y12 inhibitors (Cuisset, Grosdidier et al. 2013). Based on our results, it would therefore seem reasonable to suggest withholding the administration of oral antiplatelet loading doses in NSTEMI patients until coronary angiography is scheduled or until coronary anatomy is known post angiography. This latter strategy was heavily criticised following the publication of TRITON-TIMI 38 since it was thought to disadvantage clopidogrel in view of its slower onset of action in comparison to prasugrel (Wiviott, Braunwald et al. 2007). Furthermore, such an approach at that time was not reflective of UK practice in which all patients received antiplatelet loading doses upstream of any planned/emergency PCI.

The timing of antiplatelet loading with an oral P2Y12 inhibitor is still under debate; pre-treatment with prasugrel in NSTEMI patients does not lead to a reduction in ischaemic complications, but is associated with a significant increase in bleeding risk (Montalescot, Collet et al. 2014). The findings of ACCOAST-PCI prompted a change in national recommendations regarding the use of prasugrel in NSTEMI patients such that a loading dose should only be administered at the time of PCI if angiography is performed within 48 hours of admission. (NICE TAG 317 2014).

Although, pre-hospital treatment with ticagrelor is associated with a reduction in stent thrombosis without any increase in bleeding complications, this benefit was observed in STEMI patients only (Montalescot and van 't Hof 2014). Study data from this work regarding the degree of platelet inhibition

achieved after the administration of ticagrelor loading doses in NSTEMI patients implies that the low PRU values observed will predispose patients to an increased risk of bleeding complications and as such would support the case to withhold administration in line with the recommendations for prasugrel.

In summary, the pharmacodynamic data presented in this thesis indicates that despite treatment with prasugrel and ticagrelor STEMI patients demonstrate HRPR at the time of PPCI. Increased platelet reactivity is known to adversely impact on angiographic success following PPCI. The co-administration of intravenous antithrombotic therapies must therefore contribute to mitigating against peri-procedural thrombotic complications during this period of inadequate antiplatelet cover.

In contrast, the effectiveness of oral antiplatelet loading in NSTEMI patients is not adversely affected by the limitations observed in STEMI patients. NSTEMI itself is not a highly prothrombotic state and does not stimulate an increase in platelet reactivity, nor is it associated with haemodynamic instability, the administration of catecholamines, significant adrenergic activation or the diversion of blood flow away from the gut/liver, all of which are attributable to the impaired effectiveness of orally administered antiplatelet agents observed in STEMI patients (Alexopoulos, Galati et al. 2012, Parodi, Valenti et al. 2013, Orban, Mayer et al. 2014).

9.2.1 Opioid based analgesia and antiplatelet drug interaction

Opioid-based analgesia, in particular morphine/diamorphine is recommended for pain relief in STEMI patients (Parodi, Bellandi et al. 2015). Additional benefits include an anxiolytic effect, reduction the patient's respiration rate and subsequent myocardial oxygen consumption in addition to vasodilatory effects leading to a reduction in afterload.

Since the use of morphine is associated with increased mortality and poor outcomes in NSTEMI patients, it should be noted that NSTEMI patients are not pre-disposed to the potential drug-drug interaction between antiplatelet agents and opioid-based analgesia (Meine, Roe et al. 2005). This interaction has recently been reported to be of clinical significance when assessed in both healthy subjects and STEMI patients (Hobl, Reiter et al. 2015, Parodi, Bellandi et al. 2015). Morphine as well as being highly emetogenic, is known to cause inhibition of the normal muscular activity of stomach and intestines, causing a reduction in peristalsis of the gut which delays gastric emptying and in turn delays antiplatelet drug absorption as shown by a reduction in IPA (PRU >230 and %PRI >50%) as demonstrated by the studies scrutinised as part of the systematic review in chapter 3 (Parodi, Valenti et al. 2013, Kubica, Adamski et al. 2015, Parodi, Bellandi et al. 2015).

The deleterious effects of opioid administration on gastric absorption on the background of the physiological changes that occur during a STEMI have been discussed from as early as the 1980's. Kumana et al (1982), very eloquently summarise that the absorption and subsequent pharmacological

handling of orally administered drugs is altered and impaired during an acute MI. Pharmacokinetic and pharmacodynamic profiles are altered secondary gastrointestinal hypoperfusion, the presence of nausea and vomiting and the administration of opioid based analgesia such as morphine that can predispose to gastric stasis (Kumana, Rambihar et al. 1982) This concept is further supported by Heestermans who demonstrated that physiological state of STEMI adversely influences intestinal absorption of orally administered clopidogrel (Heestermans, van Werkum et al. 2008).

The importance of gastric emptying as a predictor of pharmacological efficacy and subsequent clinical outcomes is demonstrated by the reduction in IPA, presence of HRPR and increased PRU as seen in the pharmacodynamic data reported in chapters 5-8. The systematic review in chapter 3 also highlights that morphine administration is an important contributing factor to the delays seen in achieving maximal levels of IPA. For example, the adverse impact of morphine on ticagrelor pharmacodynamics was also highlighted during the ATLANTIC-PCI study. Although a directly acting agent that does not require metabolic activation, the co-administration morphine in the ambulance leads to a delay in the onset of action of ticagrelor (Montalescot and van 't Hof 2014). Thereby indicating that ticagrelor is still reliant on gastric absorption in order to exert its therapeutic effect.

As such, the systematic review questions the administration of opioid based analgesia in the context of ACS, since its use has previously been associated with increased mortality in NSTEMI patients (Meine, Roe et al. 2005) and

more recently a delay in the onset of action for clopidogrel, prasugrel and ticagrelor when administered to patients in the setting of STEMI (Heestermans, van Werkum et al. 2008, Parodi, Bellandi et al. 2015)

9.2.2 Alternative Treatment Options – antiplatelet therapy

In summary, this work shows that, despite administration of clopidogrel, prasugrel and ticagrelor loading doses, a significant proportion of patients undergoing PPCI do not achieve optimal levels of platelet inhibition during the acute phase of presentation. Gastrointestinal and hepatic hypoperfusion lead to impaired gastrointestinal absorption and subsequent delays in the metabolic conversion of clopidogrel and prasugrel. Ticagrelor, a directly acting agent that does not require metabolic conversion to its active form, is dependent on gastrointesinal absorption and as such is also be subject to a delayed onset of action in STEMI patients. Consequently, all three agents display sup-optimal levels of IPA in the context of STEMI patients who undergo PPCI.

In the highly prothrombotic state encountered in the setting of STEMI, more rapid and profound platelet inhibition, as achieved by intravenous agents such as cangrelor, may be advantageous in patients undergoing PPCI. Intravenous cangrelor offers the ability to achieve optimal levels of IPA during the narrow door to balloon time window, while the ability to transition to oral prasugrel/ticagrelor will allow for the longer-term benefits that are derived from DAPT (Bhatt, Stone et al. 2013). Cangrelor also has a rapid offset of action, which may be of value in certain patient subsets, for example those

undergoing surgery or with bleeding complications (Angiolillo, Firstenberg et al. 2012).

While there are no head to head trials comparing the effectiveness of intravenous cangrelor with intravenous GPIs (abciximab, eptifibatide, tirofiban), both have proven efficacy in achieving rapid, high levels of platelet inhibition. Previous guideline recommendations for the use of GPIs are based on data derived from clinical trials that precede recent pharmacological advances in oral antiplatelet therapies. A number of studies have questioned the co-administration of GPIs in combination with the more potent oral antiplatelet agents, prasugrel and ticagrelor, particularly in view of the additional bleeding complications that can arise following such a combination (De Luca, Suryapranata et al. 2005, Mehilli, Kastrati et al. 2009). A number of large-scale clinical trials have failed to demonstrate a significant clinical benefit following the administration to GPI +/- UFH in STEMI patients, such that even international guideline recommendations are unable to provide definitive endorsements regarding the utility of GPI in the era of potent oral P2Y12 inhibitors (Stone, Witzenbichler et al. 2008, Mehilli, Kastrati et al. 2009, Steg, James et al. 2012).

Recent trial data has demonstrated that modifying the formulation of prasugrel and ticagrelor administered to STEMI patients provides earlier and more pronounced levels of platelet inhibition (Alexopoulos, Barampoutis et al. 2015, Parodi, Xanthopoulou et al. 2015, Rollini, Franchi et al. 2016). The administration of crushed tablets dispersed in water (unlicensed use) rather

than intact film coated tablets allows for faster and enhanced drug absorption, particularly in the first hour following administration and results in higher plasma levels of prasugrel active metabolite in addition to ticagrelor and its active metabolite and consequently greater reduction in platelet reactivity (Alexopoulos, Barampoutis et al. 2015, Parodi, Xanthopoulou et al. 2015, Rollini, Franchi et al. 2016). While modification of the dosage form represents an unlicensed, off-label use of prasugrel and ticagrelor, administration in such a manner often occurs as part of our routine clinical practice, for example in patients who are unconscious and/or intubated. The works of Rollini, Alexopoulos and Parodi provide some reassurance and demonstrate that crushing oral antiplatelets does not compromise their efficacy or lead to an increase in adverse effects e.g. stent thrombosis (Alexopoulos, Barampoutis et al. 2015, Parodi, Xanthopoulou et al. 2015, Rollini, Franchi et al. 2016).

9.2.3 Alternative Treatment Options – analgesia

The systematic review in this thesis and previous studies undertaken by Parodi, Hobl and Alexopoulos, show that the co-administration of opioid based analgesia with oral antiplatelet medications introduces a clinically significant drug-drug interaction which should not be overlooked (Alexopoulos 2013, Parodi, Valenti et al. 2013, Hobl, Stimpfl et al. 2014, Hobl, Reiter et al. 2015, Parodi, Bellandi et al. 2015). While this study was not adequately powered to determine whether the administration of morphine has a significant impact on the rate at which orally administered P2Y12 inhibitors

undergo gastrointestinal absorption, there is a signal indicating a potential interaction is present.

Although there is little evidence base to support the use of intravenous paracetamol in ACS patients, it does have a place in other cardiac settings e.g. post cardiac surgery, post-transcatheter aortic valve implantation and during renal denervation procedures. Paracetamol is known to be opioid sparing, and evidence from other clinical settings has shown that intravenous paracetamol is comparable in terms of clinical efficacy and effectiveness to intravenous morphine (Remy, Marret et al. 2005, Cattabriga, Pacini et al. 2007, Fassl, Walther et al. 2009). As such, the administration of intravenous paracetamol in STEMI patients due to undergo PPCI may well be a possible avenue of investigation in future studies; providing comparable degrees of pain relief without contributing to further delays in gastric emptying and compromising the onset of action of orally administered medications.

9.3 Limitations

9.3.1 Antiplatelet registry

The data included in the antiplatelet registry relates to STEMI patients only and as such a real world analysis of the use antiplatelet agents in all ACS patients cannot be made. In addition, there are further statistical analyses, which will be undertaken to determine the true effect of antithrombotic therapies, both oral and intravenous on clinical outcomes. Due to the timescales involved, much of this more complex work and analysis will be undertaken after submission of this thesis.

9.3.2 Pharmacokinetic and Pharmacodynamic comparisons of oral P2Y12 inhibitors; STEMI vs NSTEMI

This was a pilot and not outcome/event driven study and is therefore not adequately powered to detect clinical outcomes. Due to the small sample size, the clinical effect of morphine in STEMI patients cannot be determined. However, data and outcomes from recently published studies can be applied to our patient population to indicate that the co-administration of morphine has a deleterious effect on the pharmacodynamic profile of both prasugrel and ticagrelor impairing antiplatelet effectiveness in practice.

The cohorts and patients recruited to each treatment arm are not equally matched in terms of their age and co-morbidities; licensing restrictions resulted in younger and heavier patients being recruited to the prasugrel arm, such patients will have a lower propensity for bleeding.

A significant limitation to the reliability of the results produced in my thesis relate to the platelet function assays utilised and the LC-MS/MS analysis undertaken. I have confidence in the VerifyNow results, since these were obtained using a point of care device within 15-30 minutes of sample collection. However, third parties undertook the VASP-P and LC-MS/MS analyses, the former at the University of Wolverhampton and the latter in the clinical chemistry department at The Royal Wolverhampton Hospital. Since I am not skilled or trained in either technique, there was a necessity to rely on others to complete this work. While the correct method was used to collect and prepare samples for analysis, the results obtained were very inconsistent and not in line with published literature. The methodology utilised for VASP-P and LC-MS/MS is questionable, therefore this limits the reliability of the results

obtained and inferences that can be drawn from them. In future studies, we will move to using VASP-FIX kits, which allow for the samples to be stored at -80c for up to 6 months, rather than 48 hours, thereby facilitating batch analysis rather than the ad hoc analysis undertaken for our current study.

In terms of the LC-MS/MS analysis, it is unlikely we will be able to undertake such an approach again in our own organisation.

During the study baseline platelet reactivity was not determined for patients administered an oral P2Y12 inhibitor. While a baseline sample would have been useful, the absence of one is neither detrimental nor critical to my data interpretation, since the principle aim of my work was to determine antiplatelet efficacy at the time of angioplasty and coronary artery stent implantation. However, if I were to conduct similar work in future, this is certainly a parameter that I would incorporate into my data collection.

9.4 Future Research and Projects

9.4.1 Antiplatelet registry

9.4.1.1 Propensity Score Analysis

The propensity score analysis as developed by Rosenbuam and Ruben in 1983, can be used as a method by which we are able to minimise any bias that may be inadvertently introduced in the dataset, which is collated from non-randomised, retrospective observational data (Becker 2002, Spreeuwenberg, Bartak et al. 2010). Since allocation of study participants to the treatment and control groups is not randomised, the presence of

confounding factors may influence the estimation of treatment effect (Becker 2002). To provide an unbiased review of observational "real-world" data collected regarding pre-defined outcomes following the administration of an oral P2Y12 inhibitor prior to PPCI a propensity score analysis will need to be undertaken. The propensity score will allow for patient characteristics and other pre-specified confounder to be balanced between treatment groups, thereby allowing a direct comparison between equally weighted groups.

9.4.2 Pharmacokinetic/Pharmacodynamic Study

The pharmacokinetic/pharmacodynamic study undertaken has created a platform for further antiplatelet work in which a comparison of the efficacy of intravenous cangrelor against oral ticagrelor can be made. Both agents are directly acting P2Y12 inhibitors that do not require metabolic biotransformation. A direct pharmacodynamic comparison of IV vs PO administration will allow for us to determine degree of platelet inhibition prior to, during and up to 36 hours after PPCI between the two agents. I am a co-investigator in this study.

There is also scope to undertake a prospective study comparing the efficacy of intravenous paracetamol against intravenous morphine/diamorphine.

The antiplatelet registry provides an opportunity to undertake a number of retrospective observational analyses using the information contained within the database; management of out of hospital cardiac arrest patients, the effect of switching between antiplatelets on bleeding and outcomes, the effects of ethnicity, age and gender on call to balloon time and subsequent clinical outcomes.

9.5 Changes in clinical practice

Since we are a tertiary referral cardiac centre for the Black Country, I am in the process of updating our antiplatelet prescribing protocols to reflect not only the findings of this study but also recently reported outcomes which advice against pre-treatment with the newer more potent P2Y12 inhibitors, prasugrel and ticagrelor in NSTEMI patients.

The results of the IV Cangrelor vs PO Ticagrelor study will help to determine whether pre-treatment with prasugrel/ticagrelor in the context of STEMI is still necessary and will help to determine future prescribing protocols. Since pre-treatment with oral P2Y12 inhibitors is a contra-indication to the use of IV cangrelor; withholding pre-procedural loading doses will be a significant change in practice which goes against current national and international guideline recommendations.

9.6 Conclusions

Despite the administration of more potent P2Y12 inhibitors, in-hospital and 30 day mortality in our STEMI group is not affected by the choice of agent administered. Indicating that other factors affect the ability of oral antiplatelet agents to influence clinical outcomes.

This study is the first to characterise and define the pharmacokinetic and pharmacodynamic profile of all three orally available P2Y12 inhibitors in a head to head manner in STEMI patients. This study contributes to the mounting “real world data” that HRPR in the context of STEMI impairs the onset of action and clinical efficacy of even the newer more potent agents,

prasugrel and ticagrelor, such that even they are not effective at the time of PPCI.

This study is the first to quantify the extent to which the disease state can affect the clinical efficacy and onset of action of prasugrel and ticagrelor in ACS patients. The findings of the systematic review, in addition to the pharmacokinetic and pharmacodynamic data collated during during study highlight that interindividual variability in antiplatelet response is not a limitation that is restricted to just clopidogrel, but is manifest with all three orally administered P2Y12 inhibitors; the reasons being predominantly variability in the degree and extent of gastrointestinal absorption, since even directly acting agents (ticagrelor) that do not require metabolic activation, demonstrate a delay in onset of action in the context of STEMI.

This research indicates that the degree and time course of platelet inhibition following the oral administration of clopidogrel, prasugrel and ticagrelor is highly variable and does not provide adequate levels of platelet inhibition that are required at the time of PPCI in STEMI patients

9.7 Key messages

- Despite providing faster, greater and more consistent degrees of inhibition of platelet activity (IPA), prasugrel and ticagrelor in the setting of STEMI are still subject to HRPR and take at least 2 to 4 hours to achieve effective levels of platelet inhibition.
- The disease state of STEMI does contribute to an attenuated antiplatelet effect when comparing the efficacy of orally administered P2Y12 inhibitors between STEMI and NSTEMI patients.

- The administration of morphine impairs the gastrointestinal absorption of all three orally administered P2Y12 inhibitors with a consequent delay in their activity as demonstrated by a reduction in active metabolite generation and increased PRU and %PRI values following their co-administration in STEMI patients.
- Modifying the formulation of the P2Y12 agent administered, either to a crushed tablet/liquid or as an intravenous agent allows for greater levels of platelet inhibition even in the context of STEMI and provides an opportunity to overcome the delay in onset of action seen following the administration of orally administered tablets.

10. Appendices

Appendix 1. Case report form – antiplatelet registry data collection

Year		LVEF category	
Hospital ID		LVEF %	
NHS Number		DES (name)	
Patient surname		BMS (name)	
Patient first name		POBA	
Date and time of operation		Flow IRA (pre)	
DOB		Flow IRA (post)	
Age at presentation		PCI hospital outcome	
Sex		Status at discharge	
Ethnicity		LOS	
Diagnosis		In hospital mortality	
Clinical syndrome		30 day mortality	
Indication for intervention		1 year mortality	
Procedure urgency		DTB	
Cardiogenic shock (pre-procedure)		CTB	
Admission route		PCI for ST	
Cardiac enzymes raised		Pre aspirin/dose	
Previous MI		Pre clopidogrel/dose	
Previous CABG		Pre prasugrel/dose	
Previous PCI		Pre ticagrelor/dose	
Height/Weight/BMI		Peri UFH	
Diabetes		Peri bivalirudin	
PVD		Peri abciximab	
CHD		Post clopidogrel	
Afib		Post prasugrel	
COPD/Asthma		Post ticagrelor	
VHD		Post BB/RLCCB	
Smoking status		Post ACEI/ARB	
FHx		Post statin	
Medical Hx		EF on discharge	
Hypercholesterolaemia		Epler/Spiro	
Tc on admission		Bleeding comp	
Hypertension		Transfusion	
SBP on admission		MACCE Death	
HR on admission		MACCE Stroke	
Hb on admission		MACCE MI	
Glucose on admission		OHCA	
Cr > 200microM			

Appendix 2. Systematic review - Inclusion and Exclusion Criteria

	Inclusion Criteria		**Exclusion Criteria**	
Populations	PD/PK human studies (Phase II, III or dose finding studies)		PD/PK animal studies	
	Adults > 18 years		Children < 18 years	
	Adults < 70 years		Adults > 70 years (co-morbidities make DAPT difficult)	
	Patients diagnosed with/recruited following an MI - STEMI - NSTEMI		Primary prevention or other non-cardiac disease states	
	Patients with unstable angina		Patients with chronic stable angina (stable coronary artery disease)	
			Those who undergo elective PCI	
			Those who are medically managed	
			Doesn't relate to inclusion criteria	
Interventions	Primary PCI		Thrombolysis	
	Antiplatelets - Oral P2Y12 inhibitors: Clopidogrel, Prasugrel, Ticagrelor Pre-procedural loading Intravenous antiplatelet agents:Cangrelor, eptifibatide, abciximab		Oral antiplatelets: Cilostazol, dipyridamole, ticlodipine Oral P2Y12 inhibitor (clopidogrel/prasugrel/ticagrelor) loading post-PCI	
	Other medications administered at the time of PCI – morphine/diamorphine			
Comparators	STEMI vs NSTEMI		Doesn't relate to inclusion criteria Papers on HTPR with clopidogrel and clopidogrel pharmacogenomics.	
	Drug handling - ADME			
	Degree of IPA STEMI vs NSTEMI vs healthy volunteer			
	Clopidogrel, prasugrel, ticagrelor, cangrelor			
	In extremis vs healthy			
Outcomes	%IPA			
	PRI			
	PRU			
	Adverse events (relate back to question)			
	Mortality (relate back to question)			
	Bleeding complications (relate back to question)			
Study Design	RCTs		Abstracts	
	Comparative studies		Case reports	
	Placebo controlled studies			
	English only papers			

Appendix 3. Screening questions to assess the quality and appropriateness of the final articles selected for scrutiny prior to inclusion in the systematic review.

	Yes	Can't tell	No
1. Does the study/paper relate back to the research question? (Is there a clear statement of the aims of the research/paper?)			
Population/problem?			
Intervention?			
Comparator/control?			
Outcomes? (is primary outcome identified?)			
RCT			
2. Was the population randomised? If yes, were the methods appropriate?			
Was allocation to a comparator/group concealed?			
Were the participants/investigators blinded to group allocation?			
Were interventions/comparators well described and appropriate?			
Were the groups similar at the start of the trial?			
Was the sample size sufficient?			
Were participants appropriately accounted for?			
Data analysis – appropriate?			
Results – were outcomes measures reliable and complete?			
Qualitative			
3. Was the qualitative methodology appropriate?			
4. Was the research strategy appropriate to the aims of the research?			
5. Was the recruitment strategy appropriate to the aims of the research?			
6. Was the data collected in a way that addressed the research issue?			
7. Have ethical issues been taken into consideration?			
8. Was the data analysis sufficiently rigorous?			
9. Is there a clear statement of findings?			
10. Are there any major limitations?			
11. How well does the paper/research relate back to your research question?			

	Yes/No	Explanation
Include		
Exclude		

Appendix 4. Case Report Form STEMI

CASE REPORT FORM

Pharmacokinetics and Pharmacodynamics of Platelet $P2Y_{12}$ Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction (P^3-AMI): A Pilot Study

1. DEMOGRAPHIC DATA

Age (yrs):			Sex:	Female			Male	
Height (m):		•						
Weight (Kg):			•					
Body Mass Index (BMI = Wt (kg)/H^2 (M):			•					

2. ANALGESIA GIVEN

Pain relieving agent given:

Total (mg):

Time of dose:

3. P2Y12 AGENT DATA

P2Y12 inhibitory agent given:

Time of loading dose:

Sampling time:	1		2		3		4	

4. RISK FACTORS

Treatment/Condition	*Yes	No	Ex
Diabetes			
Family history			
Hypertension			
Hyperlipidaemia			
Smoker			
Other			

5. PREVIOUS MEDICAL HISTORY

Treatment/Condition	*Yes	No
Myocardial infarction		
CABG		
PCI		
CVA/TIA		

6. CURRENT MEDICATION

Medication	Daily Dose

Medication	Daily Dose

7. ANGIOGRAPHIC/PPCI DATA

Number of vessels diseased:				
Number of stents used:				
Drug eluting stents used:	Yes		No	
Total stent length (mm)				

8. POST PPCI RESULTS

Result	*Yes	No	Figure
TIMI flow			
Slow flow/no reflow			
ST-segment resolution			
Balloon pump			

9. POST PPCI ADVERSE EVENTS

Event	*Yes	No
ITU admission		
Stroke		
Revascularisation		
Death		
TIMI minor bleed		
TIMI major bleed		

Completed by

Name…….

Signature……...

Date……..

Appendix 5. Case Report Form NSTEMI

CASE REPORT FORM

Pharmacokinetics and Pharmacodynamics of Platelet $P2Y_{12}$ Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction (P^3-AMI): A Pilot Study

1. DEMOGRAPHIC DATA

Age (yrs):			Sex:	Female			Male	
Height (m):			•					
Weight (Kg):				•				
Body Mass Index (BMI = Wt (kg)/H^2 (M):				•				

2. ANALGESIA GIVEN

Pain relieving agent given:

Total (mg):

Time of dose:

3. P2Y12 AGENT DATA

P2Y12 inhibitory agent given:

Time of loading dose:

Sampling time:	1		2		3		4	

4. RISK FACTORS

Treatment/Condition	*Yes	No	Ex
Diabetes			
Family history			
Hypertension			
Hyperlipidaemia			
Smoker			
Other			

5. PREVIOUS MEDICAL HISTORY

Treatment/Condition	*Yes	No
Myocardial infarction		
CABG		
PCI		
CVA/TIA		

6. CURRENT MEDICATION

Medication	Daily Dose

Medication	Daily Dose

7. ADVERSE EVENTS

Event	*Yes	No	N/A
Angiography/PCI			
Stroke			
Death			
TIMI minor bleed			
TIMI major bleed			

Completed by

Name……..

Signature……

Date……

VERIFYNOW P2Y12 RESULTS

SAMPLE 1.

SAMPLE 2.

SAMPLE 3.

VERIFYNOW ASPIRIN RESULTS

SAMPLE 1.

SAMPLE 2.

SAMPLE 3.

VERIFYNOW P2Y12 RESULTS

SAMPLE 1.

SAMPLE 2.

SAMPLE 3.

SAMPLE 4.

VERIFYNOW ASPIRIN RESULTS

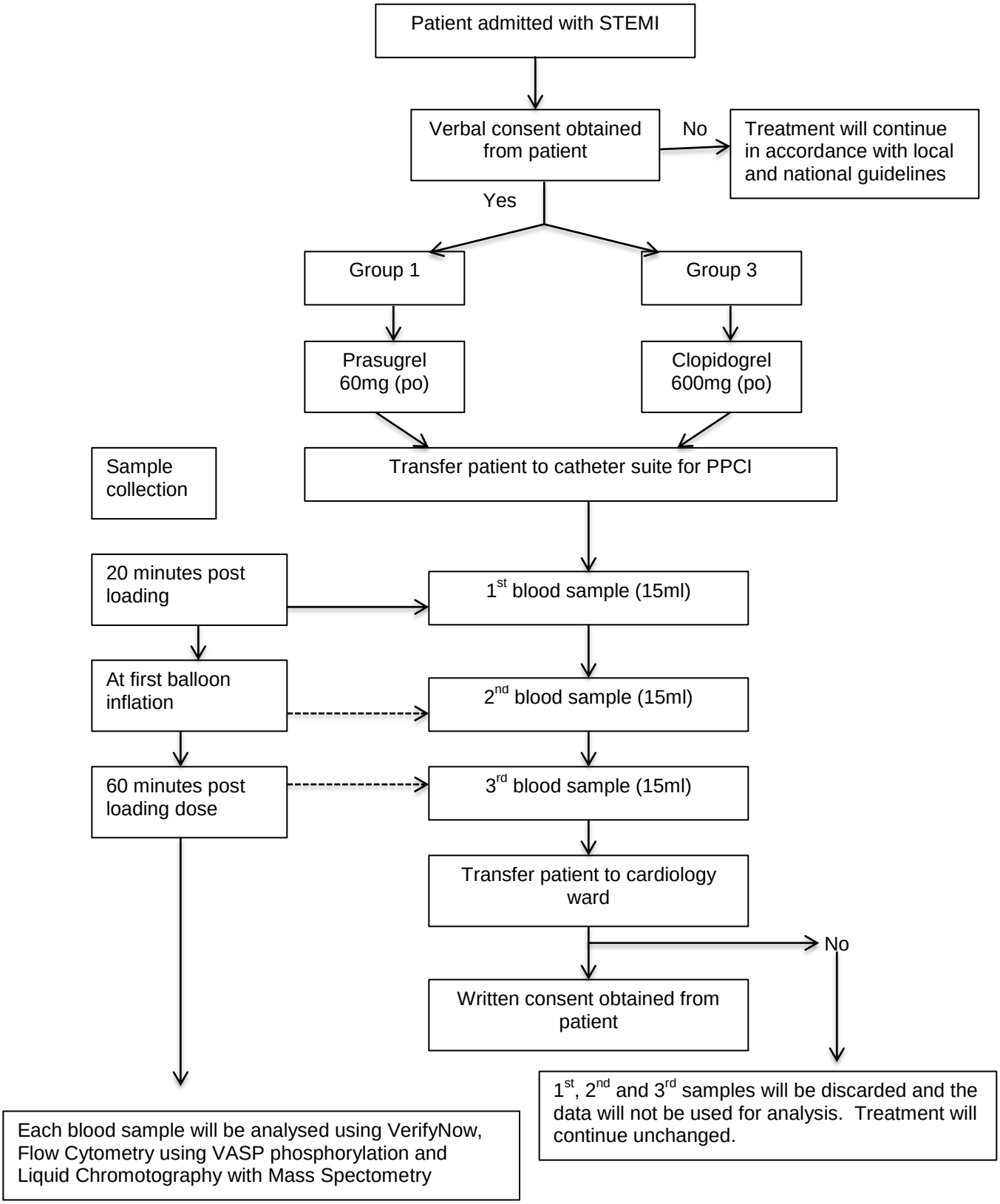
SAMPLE 1.

SAMPLE 2.

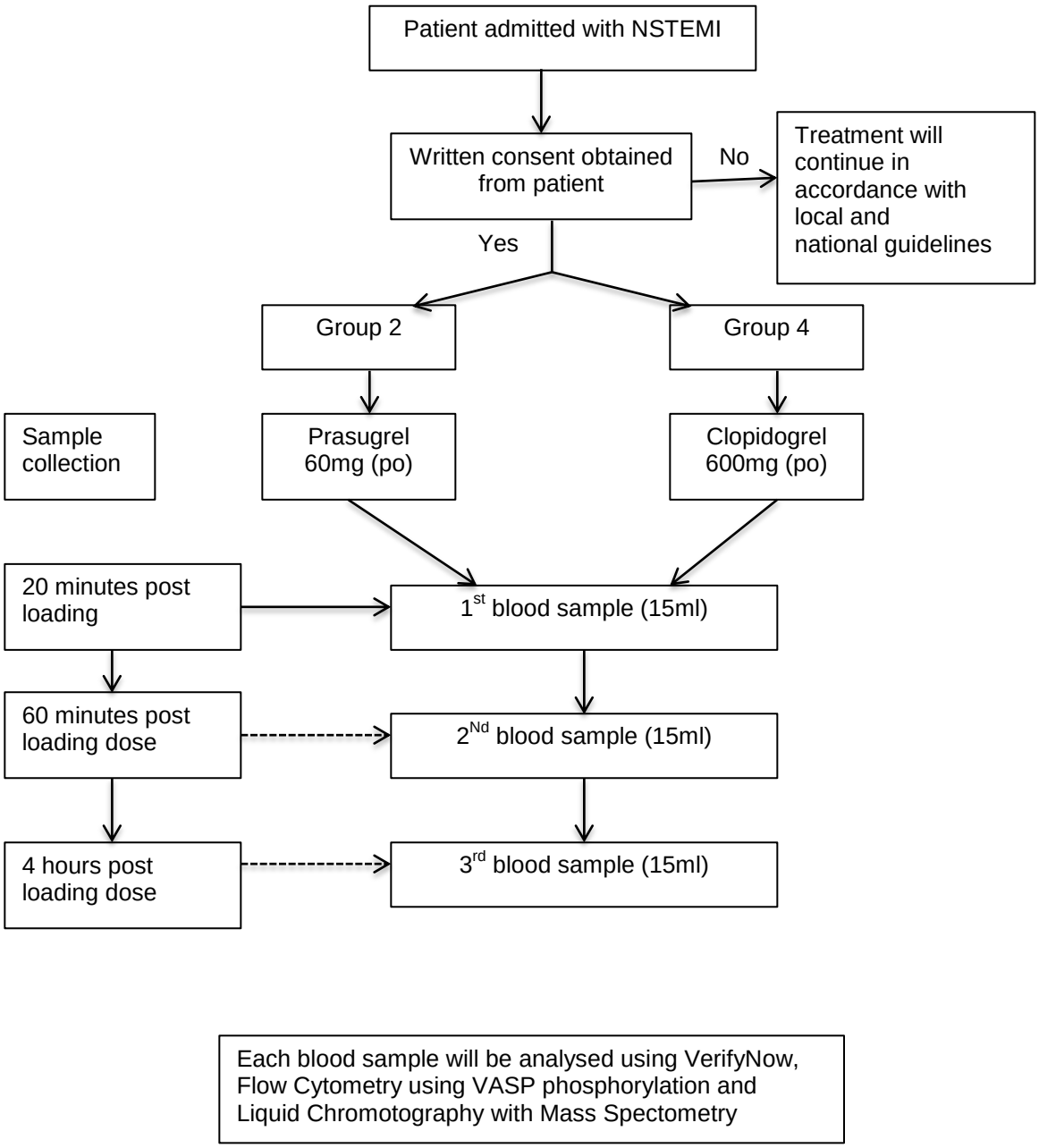
SAMPLE 3.

SAMPLE 4.

Appendix 6. Process for sample collection from patients admitted following STEMI

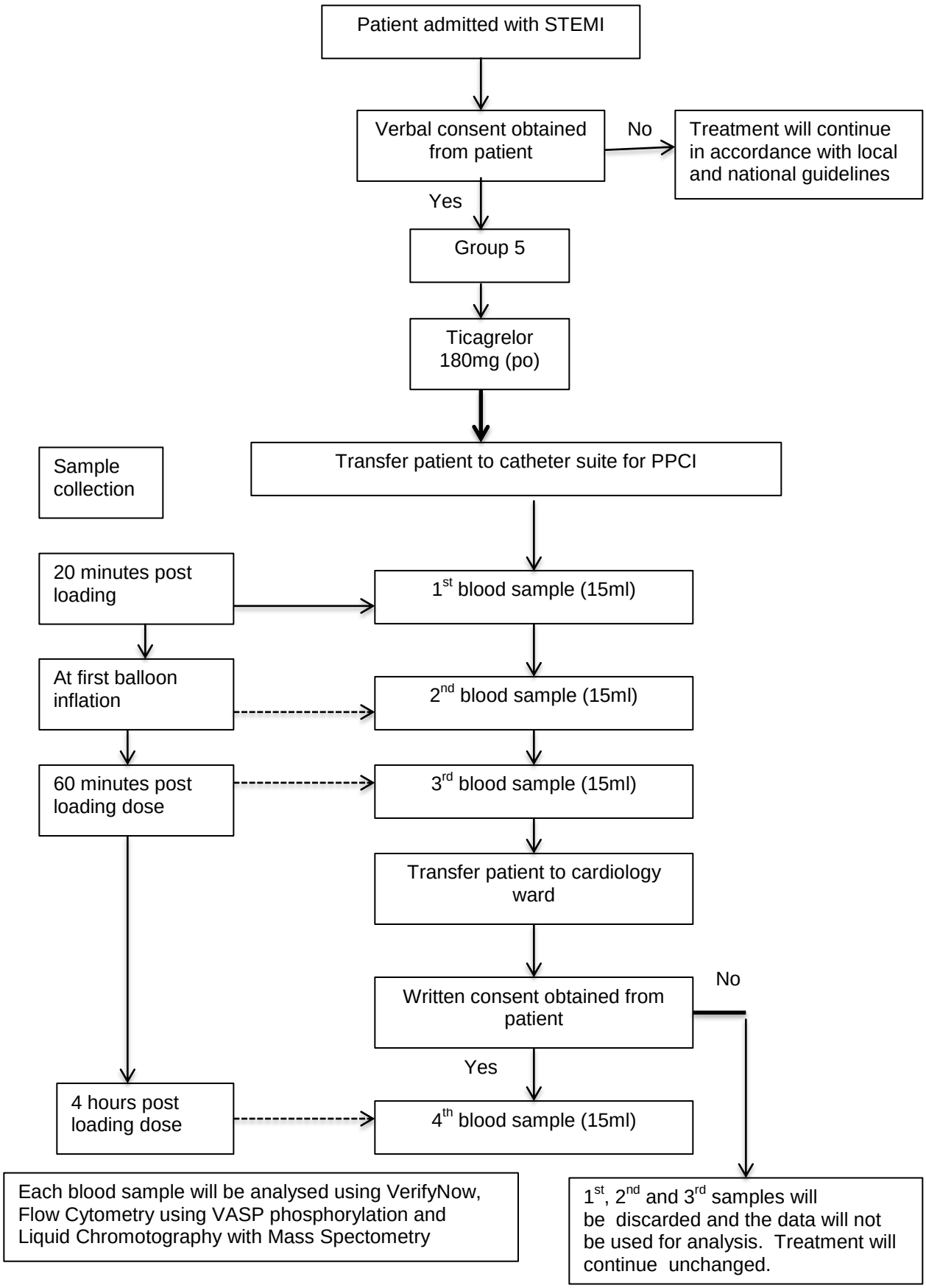


Appendix 7. Process for sample collection from patients admitted following NSTEMI

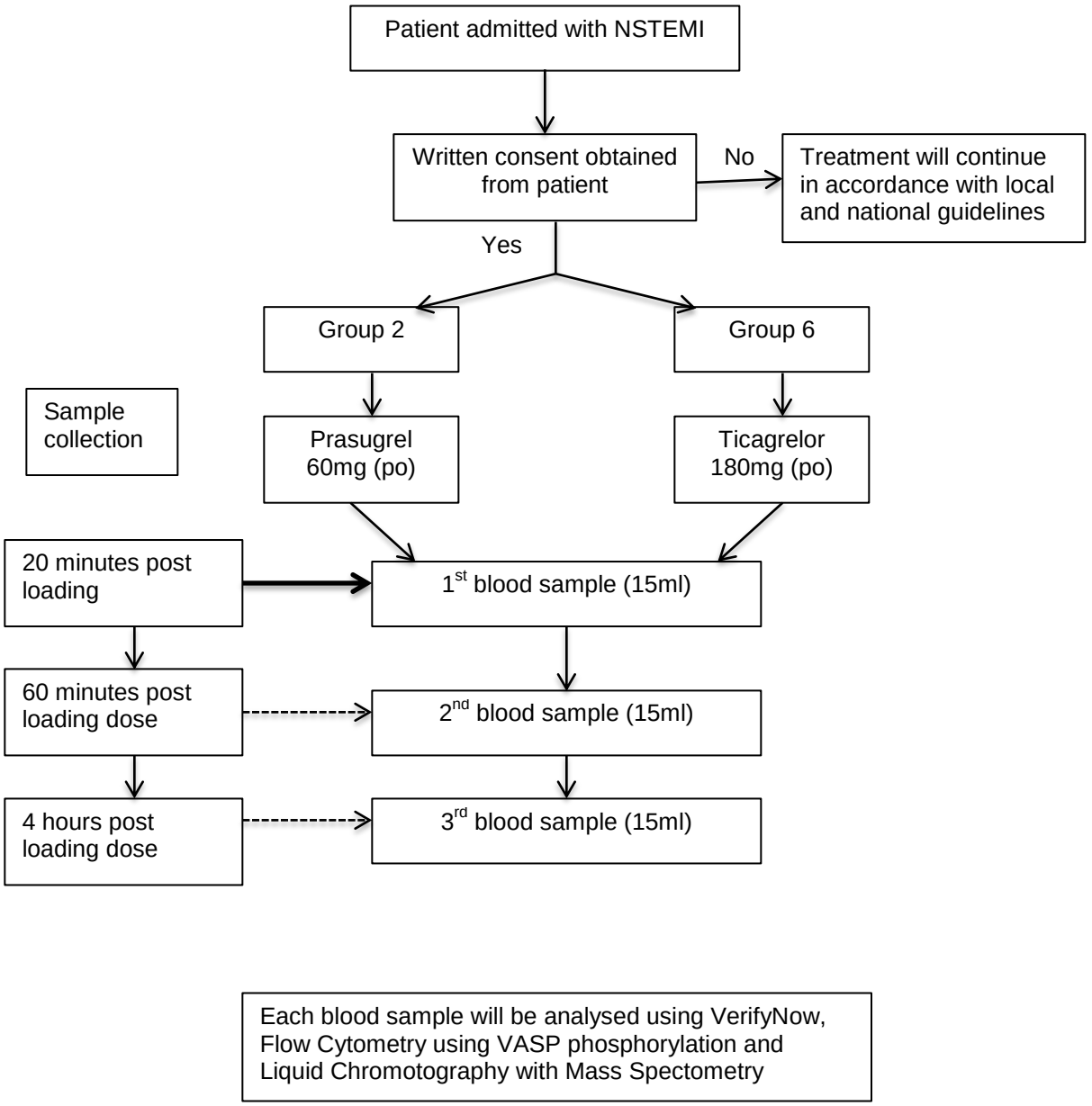


Each blood sample will be analysed using VerifyNow, Flow Cytometry using VASP phosphorylation and Liquid Chromotography with Mass Spectometry

Appendix 8. Following approval of substantial amendment – process for sample collection from patients admitted following a STEMI



Appendix 9. Following approval of substantial amendment - process for sample collection from patients admitted following a NSTEM



Appendix 10 – Shortened Patient Information Sheet to obtain verbal assent STEMI

Patient information sheet

Pharmacokinetics and Pharmacodynamics of Platelet P2Y12 Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction: A Pilot Study

To be <u>read</u> to the patient to gain verbal consent prior to primary angioplasty

You doctor will have explained that you are having a heart attack and require an emergency angioplasty procedure. As part of this procedure you will receive tablets to prevent your blood from clotting as per current guidelines.

We are inviting you to be part of a research study looking at how well these tablets work during and shortly after your angioplasty procedure. The study does not require a change in your medical treatment, but we will take extra blood samples after 20 minutes, during the angioplasty procedure, after 60 minutes, and again 4 hours after taking your tablets. Each sample will be approximately 3 teaspoons of blood which will not harm your health. If you are happy to take part in this study we will give written information after your procedure when you are feeling better, and will ask for your written consent. Your treatment will not be affected should you choose to take part in the study or not.

Thank you for considering this trial.

Appendix 11. Patient Information Sheet STEMI

Patient information sheet (STEMI)

Pharmacokinetics and Pharmacodynamics of Platelet P2Y12 Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction: A Pilot Study

You have been previously invited to take part in this research study. Before you decide whether you would like to continue your participation it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Background

Major heart attacks are caused by a number of factors. The two main causes are furring up of a coronary artery and sudden clot formation in this area leading to a blockage and subsequent interruption of blood flow. The clots that lead to heart attacks are largely made of clotting blood cells (platelets) which, in health are involved in stopping bleeding and the repair damaged blood vessels. When a patient is admitted with a major heart attack, they are first treated medically with aspirin and one of three anti-platelet agents called clopidogrel, prasugrel, and ticagrelor which are taken in tablet form. The combination of aspirin and one of the three anti-platelet agents named above will help to make the platelets less “sticky”. Patients are then transferred to a catheter suite where they undergo emergency primary percutaneous coronary intervention (PPCI), a technique where a wire and balloon are used to reopen the blocked coronary artery and then usually a slotted metal tube or stent is placed to keep the artery open. You have already undergone this procedure.

In a healthy stable patient, it can take up to 2 hours for an anti-platelet agent to develop its full effect. Often the angioplasty procedure is performed urgently, well within this timescale. Furthermore patients who are having a heart attack may not have normal drug absorption with blood being diverted away from the stomach and gut activity being suppressed by other drugs such as morphine.

This research proposes to look at whether having a major heart attack affects the absorption and activity of these three anti-platelet agents and to determine which agent takes effect and works most quickly.

Why me?

You have been chosen because you have been admitted to the hospital after suffering a major heart attack and have been treated with aspirin and the anti-platelet agent ticagrelor.

Do I have to take part?

You have previously given verbal consent to inclusion in the trial; we will also require written consent from you. It is entirely up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you now decline to be in the study we will destroy your blood samples that have been taken and not use any of your data in the trial.

What is involved in the study?

At this stage of the study we will require one further blood sample 4 hours following your first dose. The quantity of blood taken from each sample time is 15 ml (approx. 3 teaspoons in volume). A variety of tests will be used to check the activity of the anti-platelet agent including measuring the concentration of the active component in the blood. Thus this study will allow us to determine if the anti-platelet agent you have been administered is working adequately during the procedure, given the very short timescales involved. You will exit the study after the 4 hour blood test and will have no on-going commitment to the trial

What are the benefits of taking part?

Although you will not directly benefit from taking part in this study, the information gained from the study should help us to understand if the anti-platelet agent you have been administered is the best treatment for future patients and indeed whether these agents are working at the time of emergency angioplasty. As part of the study your care will be closely scrutinised.

What are known risks of the study or the side effects?

It is unlikely you will suffer any detrimental effects if you continue with your participation in the study at this stage. The one extra blood sample to be taken may require 1 extra needle prick; therefore there is a possibility of bruising. We will however endeavour to keep the bruising to a minimum. There are no significant risks associated with participation in the trial. Ticagrelor is licenced to be used as part of the routine treatment for people with your condition. Ticagrelor can be associated with bleeding complications when compared to the standard anti-platelet clopidogrel. Conversely there is evidence of better clinical outcomes with ticagrelor when compared to clopidogrel.

Confidentiality

All information about you that is collected during the course of this research will be kept strictly confidential. Your medical notes will need to be seen by members of the research team so they can collect information about you needed for this research study. The confidentiality of your medical notes will be respected at all times.

Each participant will be allocated a study code, which will be known only to the researcher. All personal and medical details will be stored in accordance with the Data Protection Act (1998). All data will be kept on an encrypted, and password protected computerised storage system.

What will happen to the results of the research study?

This study is being carried out as part of an on-going program of research working towards better outcomes for patients. The results of this study will be submitted to medical journals for peer review and publication or presented at meetings. Again your identity will not be disclosed. Each patient should they wish will receive a feedback sheet which will summarise the results of the study; however individualised results will not be available.

What if there is a problem?

If you have any concerns about any aspect of the study you should speak to the investigator who will do his/her best to answer your questions. If you remain unhappy and wish to make a formal complaint please contact the Patient Advisory and Liaison Service on 01902 696362.

Who reviewed and approved this study?

This study was reviewed by the Coventry and Warwickshire Research Ethics Committee who gave approval for the research team to conduct this study.

Contact for further information

Research Team:
Principal Investigator: Dr James M. Cotton, Consultant Cardiologist.
Tel:

Co-Investigators: Ms Nazish Khan, Principal Pharmacist Cardiac Services
Tel: Bleep
Dr Vincent Amoah, Cardiology Research Fellow
Tel: ext

Thank you again for reading this leaflet and considering taking part in this study

Appendix 12. Patient Information Sheet NSTEMI

Patient information sheet (NSTEMI)

Pharmacokinetics and Pharmacodynamics of Platelet P2Y12 Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction: A Pilot Study

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask me if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

Background

Heart attacks are caused by a number of factors, the main two of which are furring up of a coronary artery and sudden clot formation on this area leading to a blockage and interruption of blood flow to the heart muscle. The clots that lead to heart attacks are largely made of clotting blood cells (platelets) which, in health, are involved in stopping bleeding and the repair damaged blood vessels. When a patient is admitted with a major heart attack, they are first treated medically with aspirin and one of three anti-platelet agents called clopidogrel, prasugrel, and ticagrelor which are taken in tablet form. The combination of aspirin and one of the three anti-platelet agents named above will help to make the platelets less “sticky”. Patients are then transferred to a catheter suite where they undergo a coronary angiogram and where possible an angioplasty technique, during which a wire and balloon are used to reopen the blocked coronary artery and then usually a slotted metal tube or stent, is placed to keep the artery open.

In a healthy stable patient, it takes up to 2 hours for an anti-platelet agent to develop its full effect. In heart attack patients, often the angioplasty procedure is performed urgently well within this timescale. Furthermore patients who are having a heart attack may not have normal drug absorption with blood being diverted away from the stomach and gut activity being suppressed by other drugs such as morphine.

Why me?

You have been chosen because you have been admitted to the hospital after suffering an episode of severe angina or heart attack and are going to be treated with aspirin and one of two anti-platelet agents (prasugrel, or ticagrelor). You do not need emergency angiography and angioplasty but you do need aspirin plus an anti-platelet agent. We would like to study how the anti-platelet agent you have been administered affects your platelets compared to patients having a severe heart attack that requires more urgent treatment. We anticipate that you will have your coronary angiogram investigation on the same day as taking part in the trial (ie today).

Do I have to take part?

It is entirely up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. .

What is involved in the study?

If you decide to take part in this study you will be asked to provide consent. Following this we will gather some clinical information about you and take some of your blood at 20 and 60 minutes after dosing, and again at 4 hours post dosing. The quantity of blood taken from each sampling time will be 15 ml (approx. 3 teaspoons in volume). A variety of tests will be used to check the activity of the anti-platelet agents including measuring the concentration of the active component in the blood. Thus this study will allow us to determine if the anti-platelet agent you have been administered is working adequately given the very short timescale required when treating heart attack patients. On the day of admission you may receive treatment with prasugrel or ticagrelor. If you are treated with prasugrel we may decide to switch to clopidogrel. This switch will be made for clinical reasons only and will not affect the care that you receive nor should it affect your recovery. You will exit the study after the 4 hour blood test and will have no on-going commitment to the trial.

What are the benefits of taking part?

Although you will not directly benefit from taking part in this study, the information gained from the study should help us to understand which agent is the best treatment for future patients. As part of the study your care will be closely scrutinised.

What are known risks of the study or the side effects?

It is unlikely you will suffer any detrimental effects if you enter the study as it only involves small changes in your routine care. The three extra blood samples to be taken after your anti-platelet treatment may require 3 extra needle pricks; therefore there is a possibility of bruising and slight discomfort. We will however endeavour to keep the discomfort to a minimum. All three anti-platelet agents are used as part of routine care for people with your condition.

Prasugrel is licenced for use in patients with your condition. Currently we use this agent in certain groups only in place of clopidogrel. If we treat you with prasugrel as part of the trial, we may then switch you back to clopidogrel if this is the hospital's standard anti-platelet agent for you.

Prasugrel is associated with reduced angina and heart attacks in patients with your condition (which is good), it is also associated with an increase in major (including fatal) bleeding. In clinical trials these major bleeds almost always occurred in the late follow up phase (weeks or months) and therefore if you are treated initially with this agent as part of the trial it is very unlikely that you will suffer from this complication any more than if you were treated initially with clopidogrel.

Confidentiality

All information about you that is collected during the course of this research will be kept strictly confidential. Your medical notes will need to be seen by members of the research team so they can collect information about you needed for this research study. The confidentiality of your medical notes will be respected at all times.

Each participant will be allocated a study code, which will be known only to the researcher. All personal and medical details will be stored in accordance with the Data Protection Act (1998). All data will be kept on an encrypted, and password protected computerised storage system.

What will happen to the results of the research study?

This study is being carried out as part of an on-going program of research working towards better outcomes for patients. The results of this study will be submitted to medical journals for peer review and publication or

presented at meetings. Again your identity will not be disclosed. Each patient should they wish will receive a feedback sheet which will summarise the results of the study; however individualised results will not be available.

Who reviewed and approved this study?

This study was reviewed by the Coventry and Warwickshire Research Ethics Committee who gave approval for the research team to conduct this study.

Contact for further information

Research Team:
Principal Investigator: Dr James M. Cotton, Consultant Cardiologist
Tel:
Co-Investigators: Ms Nazish Khan, Principal Pharmacist Cardiac Services
Tel: Bleep
Dr Vincent Amoah, Cardiology Research Fellow
Tel: Bleep

Thank you again for reading this leaflet and considering taking part in this study

Appendix 13. Consent form

Consent form

Study Title

Pharmacokinetics and Pharmacodynamics of Platelet P2Y12 Inhibitors in Patients Undergoing Percutaneous Coronary Intervention (PCI) for Acute Myocardial Infarction: A Pilot Study

Name of Principal Investigator: Dr James M. Cotton
Tele. **(Secretary)**
E-mail.

Please initial box

1. I confirm that I have read and understand the information sheet dated…… (version ……) for the above study and have had the opportunity to ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time; without giving a reason, without effecting my treatment or my legal rights. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by authorised members of research team at New cross hospital, from regulatory authorities or from the NHS trust, where it is relevant to my taking in his research. I give permission for these individuals to have access to my records. ☐

4. I understand that the project using the samples I give will include a variety of test aimed at understanding the effect of a major heart attack on the digestive system and if this influences the absorption and activity of platelet inhibitory agents. I also understand the results of these investigations are unlikely to have any implications for me personally at this time. ☐

5. I agree that samples I have given and the information gathered about me can be stored and possibly used for future projects in cardiovascular medicine, if these projects are approved by the local research ethics committee. ☐

6. I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test. ☐

7. I agree to take part in the above study. ☐

Thank you for agreeing to participate in this research

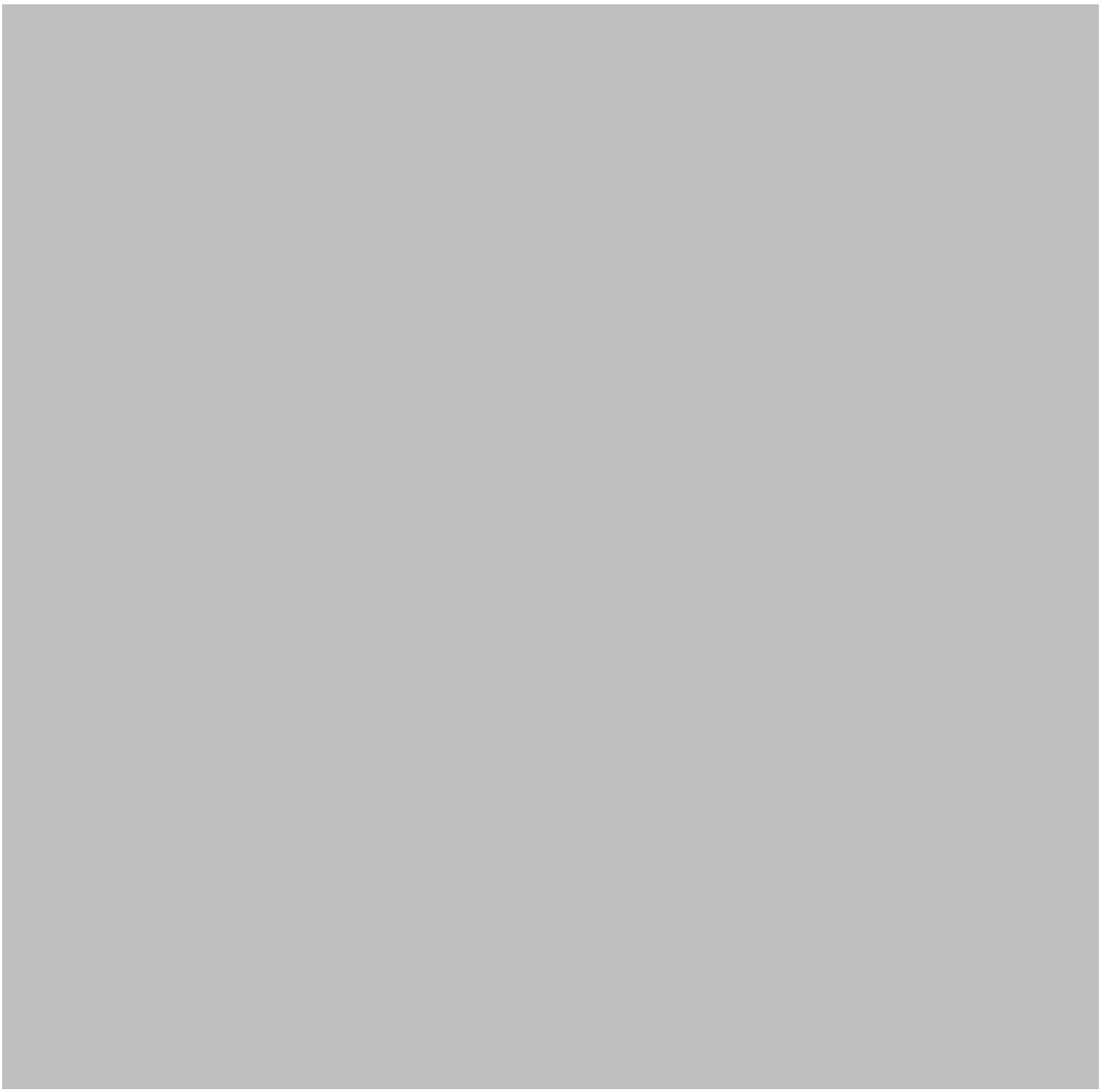
Name of patient	Date	Signature

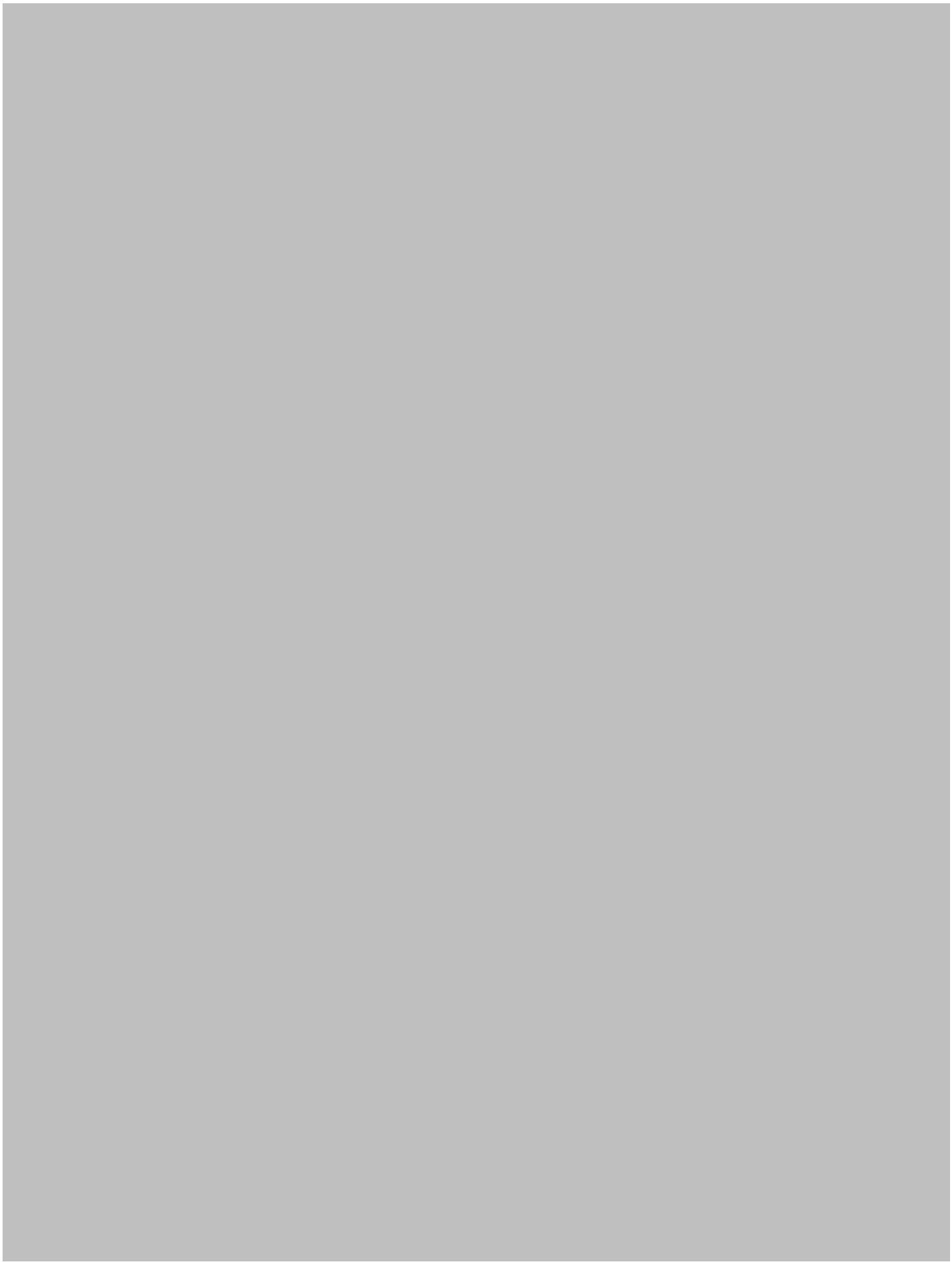
Name of person taking consent (if different from researcher)	Date	Signature

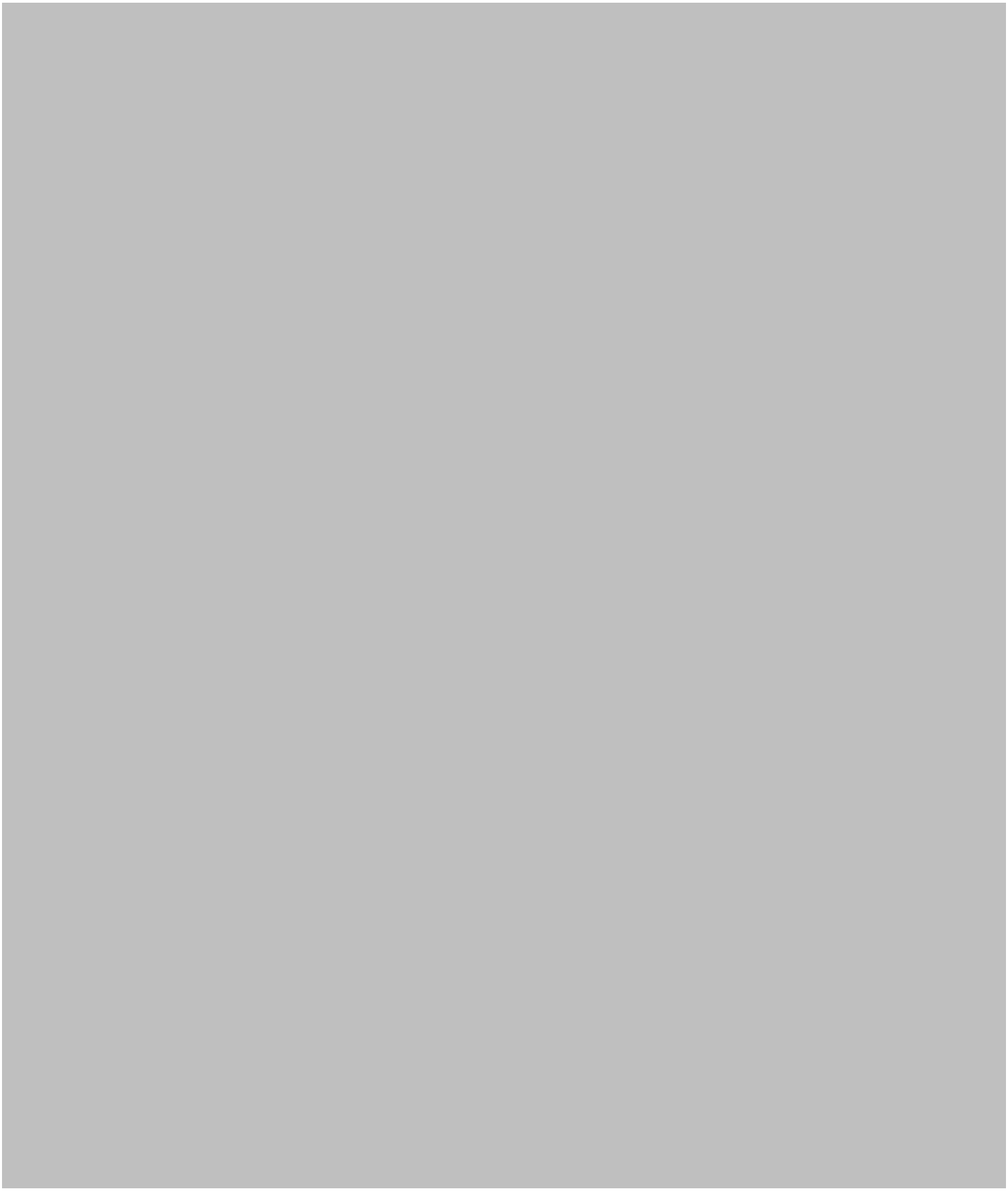
Each individual who signs this document must PERSONALLY date his or her signature.

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

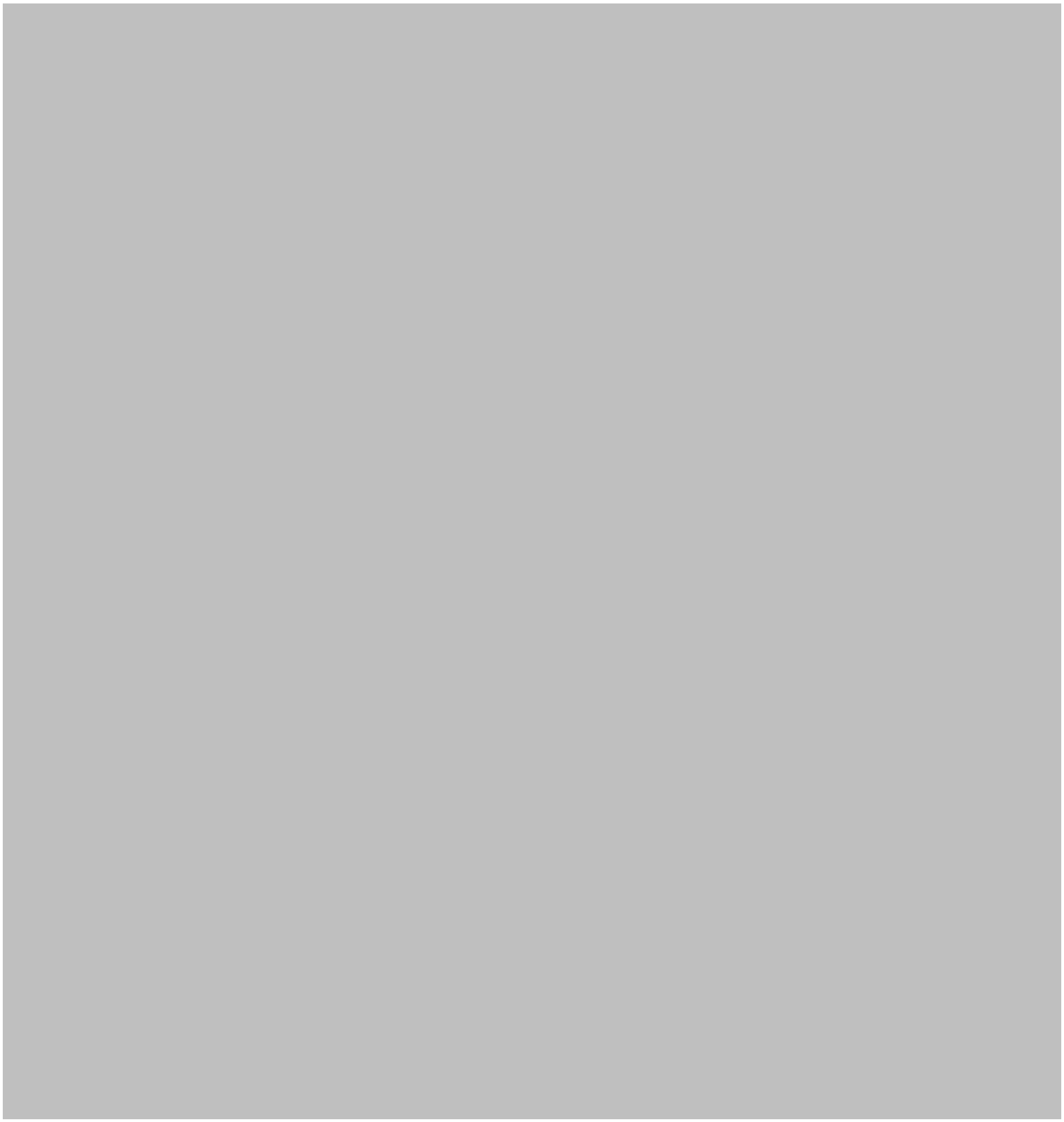
Appendix 14. Research Ethics Committee Approval Letter 2013

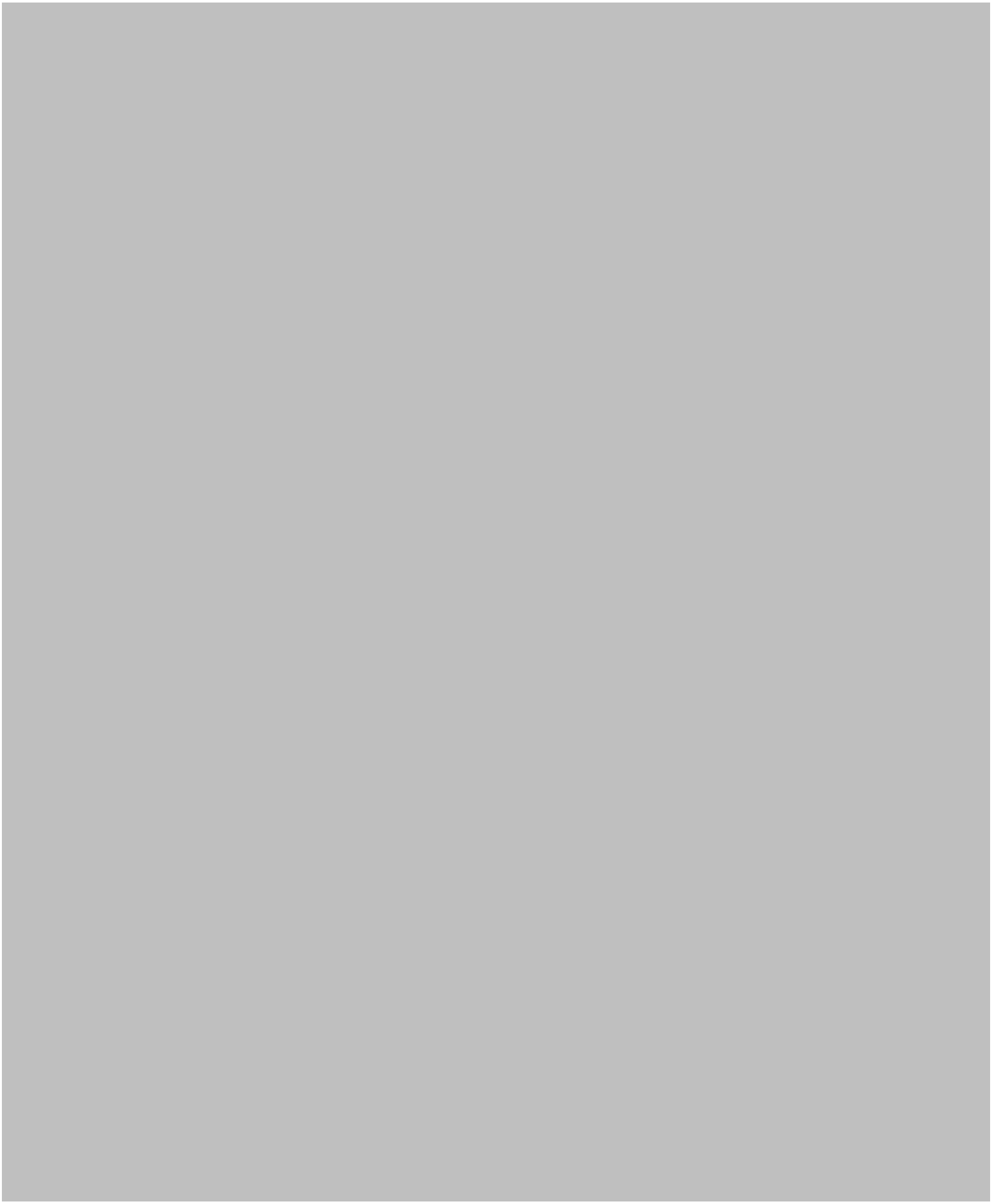


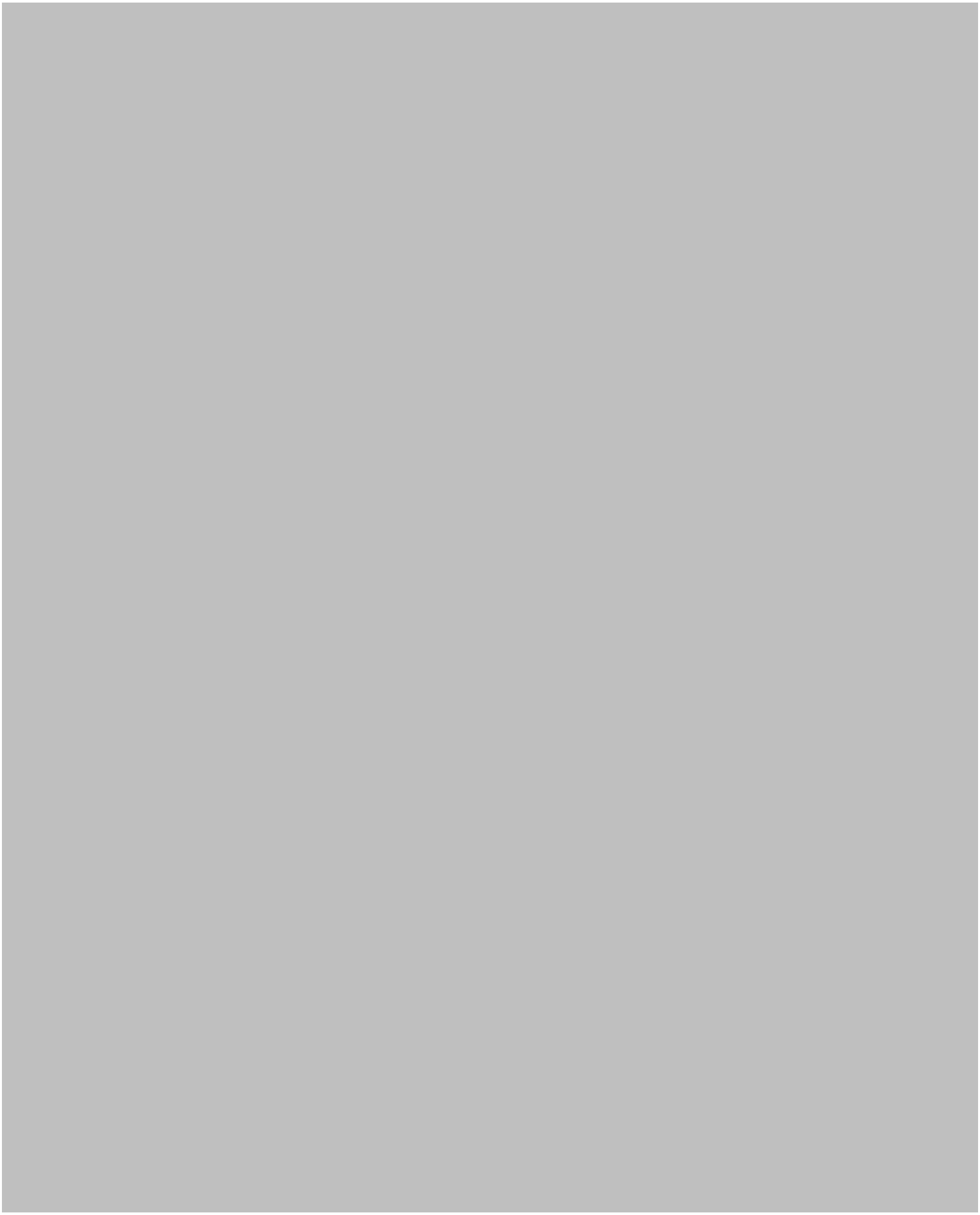


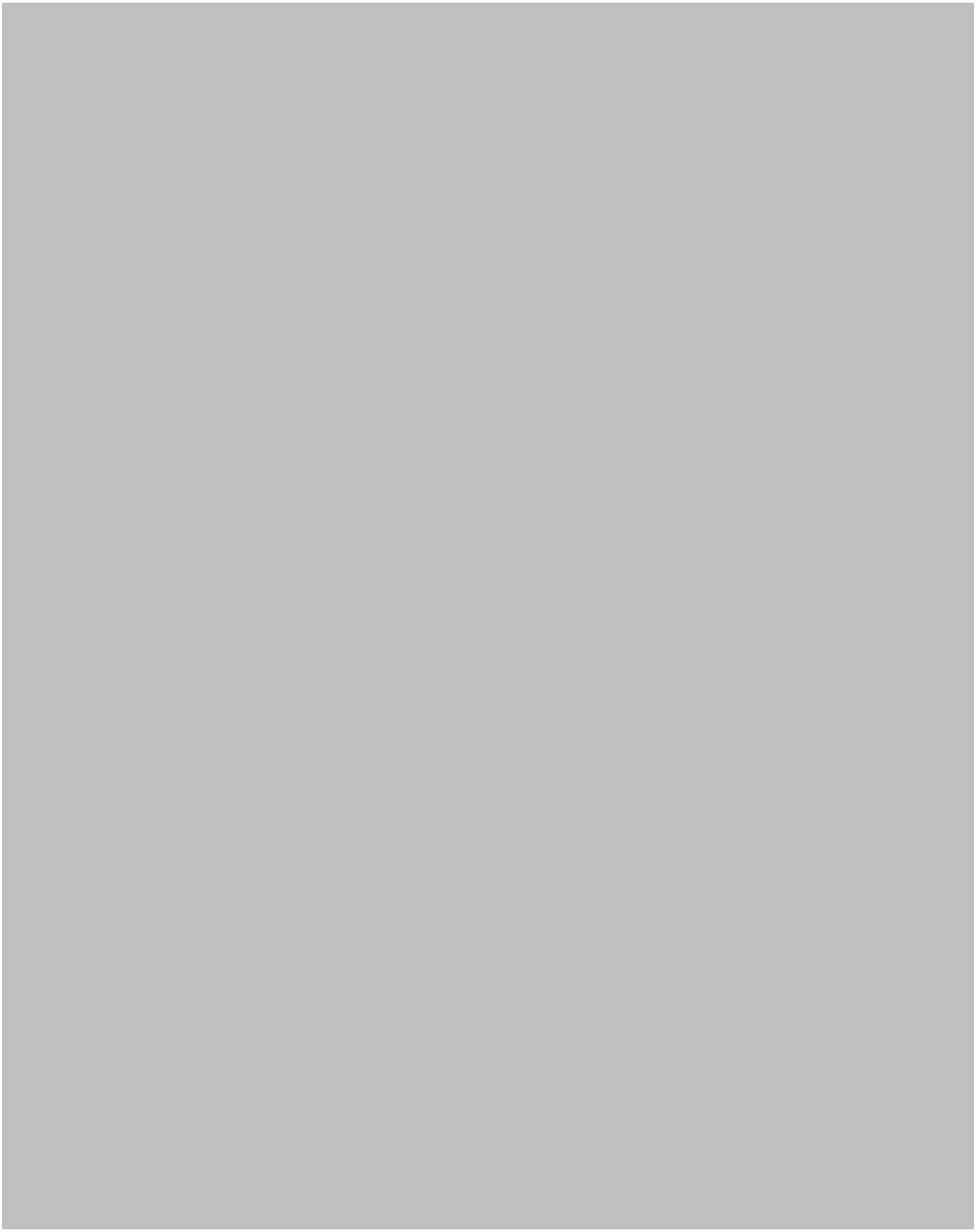


Appendix 15. Research Ethics Committee Approval Letter 2015









Appendix 16. Presentations, Publications and Abstracts

The work included in this thesis has been presented at an international meeting. I have also been invited to present on antiplatelet/antithrombotic therapies at local, regional and national meetings.

Appendix 16.1 Presentations

Title	Forum	Month/Year
An update of the new NICE Atrial Fibrillation Guidelines 2014	UKCPA – Cardiac Group Introduction to Cardiology	September 2014
Advances in Cardiac Disease Management	Association of Pharmacy Technicians UK	May 2014
Variations in ACS Management in the UK	Interventional Cardiology Pharmacists Group	December 2013
Anti-platelet Therapies – An Update	Midlands Cardiothoracic Nurse Practitioner Forum	November 2013
Management of Acute Coronary Syndromes	UKCPA – Cardiac Group Introduction to Cardiology	September 2013
Advances in Anti-platelet Therapy	Society of Cardiothoracic Surgeons – Annual Conference	March 2013

Appendix 16.2 Publications arising from this thesis

The work from this thesis has resulted in the following publications:

Khan N and Cox A. Advances in Antiplatelet Therapies. ***BJ Clin Pharm*** (2014);Jul:1–5
Khan N. Advances in Cardiac Disease Management. ***The Journal of the Association of Pharmacy Technicians UK*** (2014)
Khan N. Developments and risk analysis in anticoagulation. ***British Journal of Cardiac Nursing*** *(2015)* DOI: http://dx.doi.org/10.12968/bjca.2015.10.2.66
Khan N. Risks and benefits of antiplatelet therapies. ***British Journal of Cardiac Nursing*** *(2015)* DOI: http://dx.doi.org/10.12968/bjca.2015.10.5.236
Khan N, Cox AR, Cotton J. Pharmacokinetics and pharmacodynamics of oral P2Y12

inhibitors during the acute phase of a myocardial infarction: A systematic review. PROSPERO (2015): CRD42015023393. Available to view via: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015023393

Khan N, Cox AR, Cotton J. Pharmacokinetics and pharmacodynamics of oral P2Y12 inhibitors during the acute phase of a myocardial infarction: A systematic review. **Thrombosis Research (2016)** DOI: 10.1016/j.thromres.2016.05.019

Appendix 16.3 Abstracts presented following collation of results

The results and outcomes from chapters 5 to 8 have been presented at a national cardiovascular meeting:

British Cardiovascular Society Annual Conference – June 2016

The degree and time course of platelet inhibition following the administration of oral antiplatelet agents in patients presenting with ST-elevation MI.

Khan N[1], Amoah A[1], Wrigley B[1], Munir S[1], Khogali S[1], Martins J[1], Smallwood A[1], Vickers J[2], Nevill AM[2], Cotton J[1]
Department of Cardiology, The Heart and Lung Centre, The Royal Wolverhampton Hospitals NHS Trust, UK[1], School of Applied Science, Research Institute for Healthcare Science, University of Wolverhampton, UK[2]

British Cardiovascular Society Annual Conference – June 2016

Marked differences in the pharmacodynamics of modern P2Y12 inhibitors in patients undergoing treatment for ST segment elevation Myocardial infarction and Non ST segment Elevation MI.

Amoah A[1], **Khan N**[1], Wrigley B[1], Munir S[1], Khogali S[1], Martins J[1], Smallwood A[1], Vickers J[2], Nevill AM[2], Cotton J[1]
Department of Cardiology, The Heart and Lung Centre, The Royal Wolverhampton Hospitals NHS Trust, UK[1], School of Applied Science, Research Institute for Healthcare Science, University of Wolverhampton, UK[2]

Appendix 16.4 Work in Progress - publications in preparation:

Khan N, Nightingale P, Newman C, Buckingham M, Nevill AM, Chen R, Cotton J. Oral P2Y12 Inhibitors Administration and Outcomes in STEMI patients Undergoing PPCI - A Single Tertiary Centre Retrospective Observational Analysis.
Amoah A, **Khan N,** Wrigley B, Munir S, Khogali S, Smallwood A, Vickers J, Nevill AM, Cotton J. Marked heterogeneity in the pharmacodynamics of modern P2Y12 inhibitors depending on acute coronary syndrome presentation.
Khan N, Amoah A, Wrigley B, Munir S, Khogali S, Martins J, Smallwood A, Nevill AM, Cotton J. The degree and time course of platelet inhibition following the administration of oral antiplatelet agents in patients presenting with ST-elevation MI.
Khan N, Amoah A, Wrigley B, Munir S, Khogali S, Smallwood A, Cornes M, Nevill AM, Cotton J. Gastrointestinal absorption as an important determinant of the onset of action and clinical efficacy of ticagrelor.
Khan N, Nightingale P, Newman C, Buckingham M, Cotton J. The impact of ethnicity, age and gender on call to balloon times and subsequent clinical outcomes.
Khan N, Nightingale P, Newman C, Buckingham M, Cotton J. A single centre retrospective observational review of the antiplatelet strategies utlised in patients who present following an out of hospital cardiac arrest; patterns of use and clinical outcomes.

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