Study of Two Dose Regimens of Ticagrelor Compared with Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention for Stable Coronary Artery Disease (STEEL PCI)

**Dr Rachel Orme**

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Department of Infection, Immunity and Cardiovascular Disease

University of Sheffield

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Primary supervisor: Robert F. Storey, Professor of Cardiology, Department of Infection, Immunity and Cardiovascular Disease

University of Sheffield

Secondary supervisor: Kenny P. Morgan, Consultant Cardiologist, Department of Cardiology, Sheffield Teaching Hospitals



**ABSTRACT**

Percutaneous coronary intervention (PCI) is the most common form of revascularisation in the management of ischaemic heart disease. PCI is a prothrombotic process requiring antithrombotic therapy to prevent the formation of stent thrombosis. The standard oral therapy for preventing stent thrombosis is the combination of aspirin and a platelet P2Y12 receptor antagonist (‘P2Y12 inhibitor’). Clopidogrel is an oral thienopyridine P2Y12 inhibitor and produces an active metabolite that binds irreversibly to the P2Y12 receptor. However, clopidogrel is limited by wide interindividual variation in pharmacodynamic efficacy, partly related to genetic variation in activity of cytochrome P450 (CYP) 2C19. Clopidogrel was the standard-of-care in all PCI patients for the last 2 decades until the adoption of ticagrelor in 2012. Ticagrelor is a potent oral P2Y12 inhibitor that binds reversibly to the receptor and, unlike thienopyridines, has a weak effect on cellular adenosine uptake. Ticagrelor is used first-line, at a dose of 90 mg twice daily, in patients with acute coronary syndromes and is also used, at the lower dose of 60 mg twice-daily, in patients who have hade a myocardial infarction more than 1 year previously and are at high risk of further ischaemic events. However, ticagrelor has not previously been studied in patients with stable coronary artery disease (CAD) undergoing PCI, in whom clopidogrel remains the standard-of-care, despite its pharmacodynamic limitations. I, therefore, performed the Study of Two Dose Regimens of Ticagrelor Compared with Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention for Stable CAD (STEEL PCI) to characterise ticagrelor at its available doses in comparison to standard therapy with clopidogrel.

STEEL PCI included 180 aspirin-treated stable CAD patients who were planned to undergo elective PCI. Patients were randomised 1:1:1 to either a standard clopidogrel regimen or one of two regimens of ticagrelor, either 90mg or 60mg twice-daily, both with 180mg loading dose. The primary endpoint was the extent of adenosine uptake within 15 seconds in whole blood using an *in vitro* assay. Adenosine uptake assay, plasma adenosine concentration and platelet reactivity were assessed at the time of the procedure and pre- and post-maintenance dose at 1 month. High-sensitivity troponin T (hs-TnT) was measured pre- and 18-24 hours post-PCI to assess PCI-related myocardial injury. Plasma levels of ticagrelor and an active metabolite were assessed at the same time as platelet reactivity in the ticagrelor-treated patients. Genetic analyses were performed to assess the effects of variants that influence either clopidogrel active metabolite formation or ticagrelor pharmacokinetics. Adverse events were recorded.

No effect of ticagrelor on *in vitro* adenosine uptake or plasma adenosine concentration was seen at any timepoint (all P > 0.4). Both maintenance doses of ticagrelor achieved more potent and consistent platelet inhibition than clopidogrel (VerifyNow P2Y12 % inhibition at 1 month, mean ± SD, pre-dose: ticagrelor 60mg 73 ± 20%, ticagrelor 90mg 83 ± 17%, and clopidogrel 21 ± 17%; post-dose: ticagrelor 60mg 86 ± 13%, ticagrelor 90mg 90 ± 9%, and clopidogrel 32 ± 22%; all P < 0.0001 for ticagrelor vs clopidogrel). The more consistent platelet inhibition with ticagrelor was seen even in those who were predicted, through genetic testing, to have normal CYP2C19 activity. Mean (± SD) hsTnT increase was 50 ± 73 ng/l for the clopidogrel group, 82 ± 157 ng/L for the ticagrelor 60-mg group and 48 ± 76 ng/L for the ticagrelor 90-mg group (all P = ns). There were no PLATO-defined major or minor bleeds and no cases of MACE or stent thrombosis in any of the groups. One non-cardiac death occurred in the ticagrelor 90mg group.

In conclusion,both regimens of ticagrelor achieved greater and more consistent platelet inhibition than clopidogrel, regardless of genotype, but had no detectable effect on cellular adenosine uptake or troponin release following PCI.

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**Publications arising from this thesis**

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Abbreviations

AA Arachidonic Acid

ACS Acute Coronary Syndrome

ADP Adenosine Diphosphate

AE Adverse Event

ARU Aspirin Reaction Units

ATP Adenosine Triphosphate

AU Aggregation units

AUC Area Under Curve

BARC Bleeding Academic Research Consortium

BD Twice daily

BMS Bare Metal Stent

CABG Coronary Artery Bypass Grafting

CAD Coronary Artery Disease

COX Cyclooxygenase

CPA The Cone and Plate(let) Analyzer

CV Cardiovascular

CVA Cardiovascular Accident

CVD Cardiovascular Disease

CVRU Cardiovascular Research Unit

CYP Cytochrome P450

DAPT Dual Antiplatelet Therapy

DES Drug-Eluting Stent

DM Diabetes Mellitus

DNA Deoxyribonulcleic Acid

EDTA Ethylenediaminetetraacetic acid

ELISA Enzyme-Linked Immunosorbent Assay

EMA European Medicines Agency

ENT Equilibrative Nucleoside Transporter

ESC European Society of Cardiology

FBC Full Blood Count

FDA Food and Drug Administration

FFR Fractional Flow Reserve

GOF Gain Of Function

GP Glycoprotein

HPLC High Performance Liquid Chromatography

HS-TnT High Sensitivity Troponin T

HTPR High On-Treatment Platelet Reactivity

ISR In-Stent Restenosis

IQR Interquartile Range

LOF Loss Of Function

LMS Left Main Stem

LTA Light Transmittance Aggregometry

MACE Major Adverse Cardiovascular Events

MEA Multiple Electrode Platelet Aggregometry

MI Myocardial Infarction

NICE National Institute for Health and Care Excellence

NO Nitric oxide

NSTE-ACS Non-ST-Elevation Acute Coronary Syndrome

NSTEMI Non-ST-Elevation Myocardial Infarction

OD Once Daily

OMT Optimal Medical Therapy

PAC Plasma Adenosine Concentration

PAR Protease Activated Receptor

PAU Platelet Aggregation Units

PCI Percutaneous Coronary Intervention

PFA-100 Platelet Function Analyser-100

PFT Platelet Function Test

PGE1 Prostaglandin E1

PON1 Paraoxonase 1

PPCI Primary Percutaneous Coronary Intervention

PPP Platelet-Poor Plasma

PRI Platelet Reactivity Index

PRP Platelet-Rich Plasma

PRU P2Y12 Reaction Units

PTCA Percutaneous Transluminal Coronary Angioplasty

RNA Ribonucleic Acid

ROC Receiver Operating Characteristic

SAE Significant Adverse Events

SAT Subacute Stent Thrombosis

SD Standard Deviation

ST Stent Thrombosis

STEMI ST-Elevation Myocardial Infarction

TIMI Thrombolysis in Myocardial Infarction

TRAP Thrombin Receptor-Activating Peptide

TXA2 Thromboxane A2

U&E Urea and Electrolytes

UK United Kingdom

VASP Vasodilator-Stimulated Phosphoprotein

VWF Von Willebrand factor

WB Whole blood

# CHAPTER 1: INTRODUCTION

## Introduction to STEEL PCI

STEEL PCI is a study of two P2Y12 receptor inhibitors that are routinely used in the treatment and management of patients with coronary artery disease (CAD).Clopidogrel, at a maintenance dose of 75mg, is usually used in patients with stable CAD undergoing elective percutaneous coronary intervention (PCI) and ticagrelor at a maintenance dose of 90mg bd is used in patients with acute coronary syndrome (ACS). In addition to aspirin, a second anti-platelet is routinely prescribed for six months following an elective PCI procedure or twelve months following PCI for ACS. In some ACS cases, ticagrelor may be continued for a further three years or long term at a reduced dose of 60mg bd (1).

STEEL PCI compared clopidogrel with ticagrelor at both the 60mg and 90mg twice-daily dose regimens in patients undergoing elective PCI for treatment of stable CAD. Ticagrelor at the 60mg and 90mg twice-daily dose regimens has not been studied in the setting of stable CAD treated by PCI, hence there is a lack of platelet function data in this particular cohort of patients. There are also limited data concerning the effect of ticagrelor 90 mg twice-daily on cellular adenosine uptake in different patient populations and no data on the impact of the ticagrelor 60mg twice-daily dose on this. The primary objective of the study is to assess the effects of a single loading regimen of ticagrelor (180 mg) and two different maintenance regimens of ticagrelor on whole blood adenosine reuptake compared to a standard regimen of clopidogrel. The secondary objectives include the assessment of two regimens of ticagrelor compared to clopidogrel on platelet aggregation in stable patients with CAD undergoing treatment with PCI.

STEEL PCI also looked at the influence of genetic variability in *CYP2C19* on the antiplatelet efficacy of clopidogrel and two regimens of ticagrelor in stable CAD patients managed with PCI. Finally an exploratory objective of the study determined the relationship between adenosine reuptake inhibition and plasma levels of ticagrelor and its active metabolite.

The primary safety objective of the study was to estimate the incidence of PLATO-defined major and minor bleeding at one month (see appendix 1 for definition of PLATO-defined bleeding). All adverse events were recorded, including the incidence of dyspnoea, which is a commonly reported side effect of treatment with ticagrelor.

Sixty patients were recruited to each of the three treatment groups and followed up at one month following their PCI.

### The Pathophysiology of Atherosclerosis and Atherothrombosis

Coronary artery disease is caused by atherosclerosis, a disease primarily affecting the intima of large- and medium-sized arteries. The process results in the formation of plaques within the arteries and a subsequent reduction in the vessel lumen. The condition progresses throughout life, influenced by a number of well-validated risk factors including hypercholesterolaemia, hypertension, cigarette smoking and diabetes mellitus (DM) (2). The clinical sequelae of atherosclerosis include stable angina and acute coronary syndrome, which is an umbrella term used for patients presenting to hospital with ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) or unstable angina. Revascularisation by either coronary artery bypass grafting (CABG) or PCI forms an integral part of the management of these patients, especially those who present with an ACS or who have stable angina with on-going symptoms despite optimal medical therapy (OMT). By early adult life, most people living in developed countries will have some degree of coronary artery plaque formation (3). Within the plaque, there is an accumulation of extracellular lipid, lipid within foam cells and collagen produced by smooth muscle cells.

One of the initial features of hyperlipidaemia is the adherence of circulating monocytes to endothelial cells of the arterial wall. Monocyte-endothelial binding is facilitated by the adhesion molecules Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1; increased expression of endothelial P-selectin also contributes to monocyte recruitment. The adherent monocytes enter the arterial intimal layer, stimulated by a number of chemo-attractants (4). The monocytes present in the intima undergo differentiation into macrophages, which then accumulate large amounts of lipid and are referred to as ‘foam cells’. As these foam cells form a fatty streak, they undergo functional changes that include the production of numerous cytokines that are mediators of inflammation. Smooth muscle cell migration from the media to intima occurs under the stimulus of platelet-derived growth factor released from endothelial cells; lipoprotein lipase also promotes cell proliferation. A number of steps occur that result in switching off gene expression for contractile proteins and switching on genes controlling extracellular matrix synthesis, contributing to stable plaque formation (5). Change within the smooth muscle cells leads to formation of a fibrous cap, which may be thick, offering plaque stability, or thin and liable to rupture. The coronary artery affected by formation of the fatty streak and subsequent early plaque is able to increase its external diameter to accommodate the plaque, thereby initially preventing narrowing of the arterial lumen (6). However, as the plaque grows, it encroaches on the arterial lumen and symptoms may manifest when the resulting stenosis becomes flow-limiting.

Atherothrombosis is a combination of atherosclerotic plaque disruption and adherent arterial thrombosis at the site of the coronary artery lesion. The thin-capped, high-risk coronary lesions consist of a central lipid core with high-density accumulation of macrophages and decreased numbers of smooth muscle cells in comparison to the thick-capped fibrous plaque that is collagen-rich. The rupture or erosion of an atherosclerotic plaque leads to exposure of the lipid core to the circulating blood, provoking formation of thrombus. Consequent vessel occlusion results in the development of an ACS that encompasses the clinical presentations of unstable angina, NSTEMI and STEMI. The formation of thrombus depends on many factors, several of which are well-known cardiovascular disease (CVD) risk factors, including elevated plasma levels of low-density lipoprotein cholesterol, smoking and DM (7).

Platelets are central to the formation of arterial thrombosis. They play an integral part in the formation of thrombus to achieve haemostasis after tissue injury but, in CAD, this process may lead to vessel occlusion. Endothelial cell function is crucial to either maintaining blood circulation or activating thrombus formation when needed by the synthesis of inhibitors or activators of platelet function. The main trigger of platelet-rich thrombus formation is exposure of the lipid core to the circulation and the response of platelets to this, involving platelet adhesion, activation and aggregation.

## The Role of Platelets in Cardiovascular Disease

### **Platelet Biology**

Platelets are produced in the bone marrow from megakaryocytes derived from haemopoietic stem cells under the control of thrombopoietin (8). As the megakaryocyte grows and develops, it undergoes fragmentation leading to the production of platelets that enter the circulation. Platelets do not have a nucleus and so do not contain DNA but contain messenger RNA and are therefore capable of protein synthesis (9). The main roles of the platelet are to stop bleeding and promote healing and so their primary function is that of haemostasis in response to tissue and vascular injury (10).

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| Platelet Adhesion and Activation Platelet adhesion occurs at the site of vessel wall injury facilitated by von Willebrand factor (VWF) bridges between exposed collagen and the glycoprotein (GP) Ib/V/IX receptor that is expressed on the surface of platelets (11). Adhesion also occurs due to the interaction between collagen and GP VI and IIa receptors. Following adhesion of platelets to the sub-endothelium, platelet activation is initiated via Ib/V/IX and GP VI, and then amplified by autocrine and paracrine mediators that include adenosine diphosphate (ADP), thrombin, epinephrine and thromboxane A2 (TXA2). These mediators not only amplify platelet activation but also lead to sustained activation and promote the formation of a platelet-rich plug. The final pathway for all agonists of platelet aggregation is activation of the GP IIb/IIIa receptor, which allows fibrinogen and VWF to provide bridging links between platelets and stabilise the thrombus (12). Activated platelets undergo shape change, cytoskeleton rearrangement and centralisation of their organelles. The platelets transform from biconcave discs to spreading cells with filopodia extrusions, resulting in a significant increase in surface area. The release of ADP, adenosine triphosphate (ATP) and serotonin from dense granules occurs, followed by release of fibrinogen, fibronectin, VWF and P-selectin from the α-granules. Arachidonic acid (AA) is released from the platelet phospholipid membranes via the action of phospholipase A2 and metabolized to TXA2 (13). Platelet Activation Pathways There are two platelet surface ADP receptors, P2Y1 and P2Y12. ADP stimulation of the P2Y12 receptor leads to intracellular signaling cascades that amplify the platelet activation mediated by other intracellular signaling pathways and this results in thrombus growth and stabilisation (14). This amplification role of the P2Y12 receptor modulates the responses to numerous agonists including serotonin, TXA2 and thrombin (15). Thrombin is the most potent of the platelet agonists and activates platelets by cleaving the protease-activated receptor (PAR) -1 and PAR-4 on the platelet surface, which leads to the exposure of a tethered ligand that binds to and activates the receptor. Thrombin additionally mediates the generation of fibrin from fibrinogen and thereby contributes to both cellular and acellular aspects of thrombus formation and haemostasis (16).  TXA2 is produced from AA via cyclooxygenase-1 (COX-1) and thromboxane synthase and then released by adherent platelets, subsequently binding to platelet TP receptors and amplifying platelet activation. All of these platelet-signaling cascades converge towards activation of the GP IIb/IIIa receptor, resulting in the cross-linking of fibrinogen or VWF and consequent platelet aggregation. Outside-in signaling through GP IIb/IIIa binding of these ligands promotes further platelet activation and the recruitment of platelets to thrombus at the site of vessel wall injury. Platelet Inhibition Pathways The vascular endothelium inhibits platelet reactivity via three pathways, i) the AA-prostacyclin pathway, ii) the L-arginine-nitric oxide pathway and iii) the ecto-ADPase pathway. These pathways work to prevent the formation of thrombus. Prostacyclin is a product of AA metabolism and is a potent inhibitor of platelet aggregation (17). The enzyme that synthesises prostacyclin is localised in the vascular endothelial layer. Prostacyclin inhibits platelet aggregation via stimulation of adenylate cyclase, causing an increase in cyclic adenosine monophosphate within platelets. In contrast, in platelets AA is converted via thromboxane synthase to TXA2, a potent vasoconstrictor and facilitator of platelet aggregation. Nitric oxide (NO) is involved in the regulation of vasodilation, vascular permeability, and neurotransmission, and also inhibits platelet activation (18). NO synthesis occurs in a number of cells, including vascular endothelium, macrophages, and platelets; it has a relatively short half-life and so acts locally to inhibit platelet activation and aggregation. NO stimulates soluble guanylate cyclase leading to inhibition of platelet activation through raised cytoplasmic cyclic GMP levels (19). Ecto-ADPase also metabolises ADP released from activated platelets and thus prevents activation of platelet P2Y1 and P2Y12 receptors (20). |

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## Antiplatelet Therapy in Coronary Artery Disease

### Aspirin

Acetylsalicylic acid, colloquially known by its original brand name aspirin, was discovered by chemist Felix Hoffmann in 1897 and found to be a potent treatment of pain, fever and inflammation (21). It was importantly, however, one of the first antiplatelet medications discovered. Aspirin is a non-selective COX inhibitor via acetylation of the amino acid serine 529 in the enzyme’s catalytic pocket, which results in irreversible inhibition of platelet-dependent thromboxane production. Aspirin is a 150-200-fold more potent inhibitor of the COX-1 enzyme than COX-2, which explains why low-dose aspirin inhibits COX-1-dependent platelet function whereas higher doses are required for COX-2 inhibition (22).

Aspirin plays an important role in the treatment and management of CAD in both primary and secondary prevention strategies (23),(24). A meta-analysis of primary prevention and secondary prevention trials demonstrated a significant reduction in serious vascular events; after ACS, aspirin achieves a 25-30% relative reduction in cardiovascular mortality (23). Aspirin is not suitable as monotherapy for ACS patients or patients undergoing PCI due to the high risk of vascular events, which are likely to be multi-factorial. Aspirin only inhibits one agonist pathway for platelet activation leaving other pathways uninhibited. There are a number of factors related to health and lifestyle that influence platelet reactivity, including cigarette smoking, cholesterol levels, exercise and posture (25, 26).

### P2Y12 Receptor Inhibitors: The Thienopyridines – Ticlodipine, Clopidogrel and Prasugrel

Ticlodipine was introduced in 1978 as an antiplatelet therapy for patients at high risk of thrombotic events. A member of the thienopyridine class, ticlodipine was primarily used in patients with aspirin intolerance or in addition to aspirin as part of dual antiplatelet therapy (DAPT). It had a number of reported minor side effects but the more serious side effects of neutropaenia, thrombotic thrombocytopenic purpura and fatal blood dyscrasias limited its use (27). Clopidogrel, with its more favourable side effect profile, subsequently replaced ticlodipine. Clopidogrel is an irreversible, second-generation thienopyridine P2Y12 inhibitor that inhibits ADP-induced platelet aggregation. Subsequently, more potent inhibitors of platelet aggregation have been introduced, the oral P2Y12 antagonists prasugrel and ticagrelor and the intravenous inhibitor cangrelor. Prasugrel, like clopidogrel, is a pro-drug that requires conversion to its active metabolite and it is almost exclusively used in the management of ACS treated with PCI (28). Initially DAPT was prescribed for 1-6 months after PCI based on data from early trials (29, 30). The issue of late (30 days – one year after PCI) stent thrombosis that emerged, particularly after the widespread adoption of drug-eluting stents (DES), led to the recommendation of a longer period of DAPT and 6-12 months became the standard length of treatment (31).

### P2Y12 Receptor Inhibitors: The Non-Thienopyridines – Ticagrelor and Cangrelor

Ticagrelor is a novel, oral, reversibly-binding, non-thienopyridine P2Y12 receptor antagonist used almost exclusively in the pharmacological treatment of ACS and long-term secondary prevention after MI. In ACS treatment, a standard loading dose of 180mg is followed by 90mg twice-daily maintenance therapy. This regimen of ticagrelor is used in ACS patients managed medically, with PCI or with CABG. Ticagrelor is usually rapidly absorbed, although the rate of intestinal absorption may be delayed in opiate-treated patients, and undergoes enzymatic degradation to produce an active metabolite, AR-C124910XX, that has similar pharmacodynamic properties to the parent compound (32, 33). Cangrelor is an intravenous, reversibly-binding P2Y12 inhibitor that does not require metabolic conversion to an active metabolite and therefore has an immediate antiplatelet effect. It has an ultra-short half-life so platelet function returns to normal 1-2 hours after cangrelor administration ceases. The intravenous route of administration is particularly useful in patients who are unable to swallow or in those who are likely to have a degree of gastric stasis, such as due to concomitant opiate therapy. The United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) both approved cangrelor in 2015 following the CHAMPION PHOENIX trial that demonstrated a significant reduction in ischaemic events and stent thrombosis in patients undergoing urgent or elective PCI (34).

### Use of P2Y12 Inhibitors in the Management of Acute Coronary Syndromes and Percutaneous Coronary Intervention

Over the last twenty years, clopidogrel has shown itself to be an integral part of the pharmacological treatment of CAD and a facilitator of PCI. Clopidogrel is used, in addition to low-dose aspirin, in the treatment of STEMI and non-ST-elevation acute coronary syndromes (NSTE-ACS) and in elective PCI for the treatment of stable CAD. It is, on the whole, well tolerated with few side effects. Pre-treatment with clopidogrel and the duration of DAPT with aspirin and clopidogrel has evolved since its introduction due to the outcomes of several large-scale randomised studies.

CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events) was the first major study using clopidogrel (35). This was a randomised, double-blind study of clopidogrel 75mg once daily (od) compared to aspirin 325mg daily and was designed to assess the benefit of clopidogrel compared to aspirin in patients with prior ischaemic stroke, prior myocardial infarction or peripheral arterial disease. Over a three-year period, 19,185 patients were recruited and followed up for an average of 1.91 years. The primary endpoint was a combination of ischaemic stroke, MI, or vascular death; there were 939 events in the clopidogrel group and 1021 events in the aspirin group. Overall, the relative risk reduction was 8.7% with clopidogrel (95% confidence interval 0.3-16.5), with consistency amongst major subgroups, and there were no major differences in safety between clopidogrel and aspirin.

The Clopidogrel for the Reduction of Events During Observation (CREDO) trial was designed to evaluate the efficacy and safety of a clopidogrel loading dose prior to elective PCI and the efficacy and safety of clopidogrel treatment for twelve months post-PCI (36). Patients undergoing elective PCI for stable CAD were randomised to receive either a 300-mg loading dose of clopidogrel or placebo. Immediately following the PCI procedure, both groups received clopidogrel 75 mg od and aspirin 325 mg od until 28 days post-procedure. After 28 days, the pre-treatment group continued to receive clopidogrel 75 mg od for a total of twelve months. Both treatment groups received standard therapy including aspirin until the end of the 12-month treatment period. The primary one-year outcome was a composite of death, MI and stroke. The primary outcome of interest at 28 days was a composite of death, MI or urgent target vessel revascularization in all randomized patients who underwent PCI. Secondary endpoints included the incidence of major bleeding as defined using the TIMI (Thrombolysis in Myocardial Infarction) bleeding criteria and early discontinuation of study drugs. There was a significant 18.5% relative reduction in the primary endpoint in the pre-treatment group at 28 days. Randomization to long-term treatment was associated with a 26.9% reduction in the relative risk of death, MI and stroke at twelve months (95% confidence interval, 3.9%-44.4%; p=0.02). Patients receiving a clopidogrel loading dose at least 6 hours prior to PCI had a 38.6% relative reduction in the combined endpoint. CREDO helped establish the now widespread practise of pre-treatment with clopidogrel prior to elective PCI.

The Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) was the first large study that demonstrated the benefit of DAPT with clopidogrel and aspirin in patients with NSTE-ACS (37). The addition of clopidogrel reduced the risk of MI and recurrent ischaemia. The primary outcome of death from cardiovascular causes, non-fatal MI, or stroke occurred in 9.3% of the clopidogrel group compared to 11.4% in the placebo group (relative risk 0.80; 95% confidence interval, 0.72 to 0.90; p<0.001). Following this, the PCI-CURE study, a sub-study of CURE, compared clopidogrel versus placebo in patients on aspirin with NSTE-ACS undergoing PCI (38). The study looked at two different antiplatelet treatment strategies, treatment with clopidogrel prior to PCI followed by long-term clopidogrel post-PCI versus no clopidogrel pre-treatment followed by short-term (4 weeks) clopidogrel post-PCI. A total of 2658 patients with NSTE-ACS undergoing PCI were randomised to treatment with clopidogrel (n=1313) or placebo (n=1345) prior to PCI. Following PCI, over 80% of patients in both study groups received open-label thienopyridine P2Y12 inhibitor for around 4 weeks. After four weeks, the study drug was re-started and continued for up to one year. The primary endpoint was a composite of cardiovascular death, MI, or urgent revascularisation within 30 days of PCI. In the clopidogrel group, 59 (4·5%) compared with 86 (6·4%) in the placebo group had a primary endpoint (relative risk 0·70, 95% confidence interval 0·50–0·97, p=0·03). Long-term administration of clopidogrel after PCI was associated with a lower rate of cardiovascular death, MI or any revascularisation (p=0·03). The results of CURE-PCI reinforced the importance of administering a loading dose of clopidogrel, or ensuring adequate clopidogrel pre-treatment, to patients presenting with an ACS treated with PCI.

The CLARITY study enrolled 3491 patients with STEMI treated with a fibrinolytic agent plus aspirin and randomised them to a loading dose of 300mg clopidogrel followed by 75mg od or placebo. The primary endpoint was a composite of an occluded infarct-related artery on angiography, death or MI prior to angiography. There was a significant difference in the rate of the primary efficacy endpoint between the two groups: by 30 days, clopidogrel treatment reduced the odds of the composite endpoint of death from CV causes, recurrent MI or recurrent ischaemia requiring urgent revascularisation by 20% (p=0.03). The rates of major and intracranial bleeding were similar between the two groups (39).

Prasugrel, another thienopyridine P2Y12 receptor antagonist, is used in patients undergoing PCI. The use of prasugrel is contra-indicated in patients with a history of transient ischaemic attack or stroke whilst patients over the age of 75 years or weighing less than 60kg require a reduced maintenance dose.

The TRITON TIMI 38 study compared prasugrel with clopidogrel in patients with an ACS undergoing PCI; 13,608 patients were randomised to receive a loading dose of prasugrel or clopidogrel followed by 6 to 15 months of maintenance therapy. The primary efficacy endpoint of cardiovascular death, non-fatal MI and non-fatal stroke occurred in 12.1% of patients on clopidogrel and 9.9% of patients on prasugrel (p<0.001). There were significant reductions in rates of MI, urgent target vessel revascularisation and stent thrombosis in the prasugrel group (p<0.001). The incidence of life-threatening, non-fatal and fatal bleeding was higher in the prasugrel group (40). In a randomised trial of 7,243 aspirin-treated patients below the age of 75 with medically managed unstable angina or NSTEMI, the TRILOGY ACS investigators evaluated treatment with prasugrel (10mg od) versus clopidogrel. A second group of 2083 patients over 75 years of age received lower-dose prasugrel (5mg od) or clopidogrel. The results showed no statistical difference in the primary endpoint of death from all causes, MI or stroke between the prasugrel and clopidogrel groups in both the under and over 75 cohorts. Similar rates of bleeding were observed (41).

The ACCOAST trial investigated the optimum timing of prasugrel administration in patients with NSTEMI, either at the time of diagnosis or after angiography prior to PCI. Patients were randomised to a prasugrel 30mg loading dose before angiography or placebo, if PCI was planned an additional 30mg was given to the pre-treated group and 60mg to the placebo group. The composite primary endpoint of cardiovascular death, MI, stroke, urgent revascularisation or GP IIb/IIIa inhibitor treatment in the first week was not significantly different but TIMI major bleeding was increased in the pre-treatment group (42).

Ticagrelor provides greater platelet aggregation inhibition compared to clopidogrel and this was shown in a sub-study of DISPERSE 2 (43). The novel P2Y12 antagonist was shown to inhibit platelet aggregation in a dose-dependent fashion and to achieve greater levels of platelet inhibition than clopidogrel. Ticagrelor also provided additional platelet aggregation inhibition in patients who had previously been treated with clopidogrel.

The ONSET/OFFSET study compared ticagrelor against standard clopidogrel treatment in patients with stable CAD (44). Patients pre-treated with aspirin were randomised to receive clopidogrel, ticagrelor or placebo during the study period. Bleeding was defined according to the PLATO criteria. The antiplatelet effect was assessed using light transmittance aggregometry (LTA), VerifyNow P2Y12 assay, VASP phosphorylation assay and measurement of GP IIb/IIIa and P-selectin receptor expression. The onset of ticagrelor’s antiplatelet effect was rapid and markedly greater than with high-loading-dose clopidogrel. The greater antiplatelet effect of ticagrelor continued during maintenance therapy and the offset of effect of ticagrelor was faster than that of clopidogrel.

The Platelet Inhibition and Patient Outcomes (PLATO) study was a multi-centre, randomised, double-blind study that randomised ACS patients to either ticagrelor or clopidogrel(45). The primary endpoint at 12 months of death from vascular causes, MI and stroke occurred less frequently in the ticagrelor-treated group compared to the clopidogrel-treated group (9.8% vs 11.7%, HR 0.84; 95% confidence interval 0.77-0.92, p<0.001). The difference between the two treatment groups was evident within the first thirty days of treatment and the difference continued throughout the study period. Secondary endpoints of death from any cause, MI and stroke were also significantly reduced in the ticagrelor group (10.2% vs 12.3%, p<0.001). MI and ST also occurred less often in the ticagrelor-treated group.

TWILIGHT is a randomised, double-blind, placebo-controlled trial evaluating the efficacy and safety of antiplatelet monotherapy versus DAPT in up to 9000 high risk patients undergoing PCI (46). All patients will be treated with low-dose aspirin (81-100 mg daily) plus ticagrelor (90 mg twice daily) for 3 months. Patients will then be randomised to aspirin or placebo whilst continuing ticagrelor treatment for an additional 12 months. The primary hypothesis is that ticagrelor monotherapy will be superior with respect to the primary endpoint of BARC (Bleeding Academic Research Consortium) type 2, 3 or 5 bleeding, while maintaining non-inferiority for ischemic events compared with ticagrelor plus aspirin.

## Factors Affecting The Clinical Efficacy of The P2Y12 Inhibitors

### Factors Affecting Pharmacokinetics of Clopidogrel

Clopidogrel is first absorbed by the intestine and approximately 15% then undergoes two oxidative steps in the liver to become an active metabolite. In the first step, the cytochrome P450 (CYP) enzymes CYP2C19, CYP1A2 and CYP2B6 convert clopidogrel into 2-oxoclopidogrel, which in turn is converted by CYP2C19, CYP2C9 and CYP3A to become the active metabolite R130964 (47, 48). The antiplatelet effect is slow in onset because of this requirement for conversion of clopidogrel to its active metabolite. Despite the beneficial effects of clopidogrel therapy, there is one significant drawback, clopidogrel non-responsiveness. The degree of platelet inhibition in individual patients is variable and patients with poor response to clopidogrel are at higher risk of ST and other arterial thrombotic events (49).

Medications that inhibit CYP3A4 and CYP2C19 can potentially block the conversion of clopidogrel to its active metabolite. A large number of medications have potential interactions with clopidogrel but commonly co-administered medications are cholesterol-lowering statin therapy and proton pump inhibitors. The hydroxymethyglutaryl-CoA reductase inhibitors (statins) are metabolised in the liver before being excreted by the kidneys, simvastatin has moderate affinity and atorvastatin lower affinity for CYP3A4 cytochrome. Lau et al demonstrated that atorvastatin 40mg od produced a statistically significant reduction in clopidogrel’s antiplatelet effect. Others groups confirmed the same interaction with simvastatin (50). Since then other studies have refuted the relevance of this interaction with statins: the PRONTO, CREDO and MITRA PLUS studies found that use of statins did not compromise clopidogrel’s antiplatelet effect as assessed by platelet function tests (PFTs) and clinical endpoints (36, 51, 52). A number of methodological limitations, including unknown statin dosages within the individual studies, affect the interpretation of the results and, in the absence of robust data, statins and clopidogrel are used concomitantly.

Medications that inhibit CYP2C19 have more conclusively been shown to impair the pharmacodynamic response to clopidogrel. The reduced pharmacodynamic efficacy of clopidogrel with the use of proton pump inhibitors, especially omeprazole and esomeprazole, is well documented. Several studies have shown a reduction in the antiplatelet effect of clopidogrel assessed by the VASP phosphorylation test with concomitant omeprazole treatment (53) (54). However, studies to date have not shown that the diminished antiplatelet effects due to concomitant proton pump inhibitor treatment have an adverse impact on clinical outcomes in clopidogrel-treated patients. Overall the pharmacodynamic studies support the use of the newer proton pump inhibitors that have less inhibitory effect on CYP2C19, such as pantoprazole (55).

### Genetic Factors Affecting Pharmacokinetics and Clinical Efficacy of Clopidogrel

One small study suggested that paraoxonase 1 (PON1), a non-CYP enzyme may also be involved in conversion to clopidogrel active metabolite; however, this has not been replicated in subsequent studies (56) -(57, 58). The CYP2C19 enzyme has a role in both metabolic steps required for the conversion of clopidogrel to its active metabolite and so has become a focus of attention. Loss-of-function (LOF) and gain-of function (GOF) polymorphisms of the gene coding for the CYP2C19 enzyme have been identified and contribute to variability in individual response to clopidogrel (59-61). The LOF *CYP2C19*\*2 allele is found in 25-35% of the Caucasian population and 55-70% of the Asian population (62). Healthy volunteer studies have repeatedly shown that *CYP2C19* LOF allele carriers were significantly associated with lower exposure to clopidogrel active metabolite, when measured by the area-under-the concentration-curve (AUC) or maximal plasma concentration, and lower levels of platelet inhibition (63) (64). The reduced exposure to clopidogrel active metabolite can be partially overcome by increasing the dose of clopidogrel. For example, Horenstein et al demonstrated in healthy volunteers that maintenance doses of up to 300mg clopidogrel daily can achieve similar active metabolite concentrations in *CYP2C19*\*2 homozygous individuals as 75mg in those individuals without LOF alleles (65).

Clopidogrel non-responsiveness is clinically relevant and related to recurrent ischaemic events. One study enrolled 518 patients with ACS (n= 214) and stable angina (n = 304), all of whom went on to have PCI and DAPT with aspirin and clopidogrel (66). The *CYP2C19* genotype was determined in each study participant and platelet reactivity was assessed. Mean platelet reactivity was significantly higher in the *CYP2C19* LOF allele carriers in each of the two groups. Cardiovascular events were higher in the ACS LOF allele group (24.6% vs. 11.1%, p < 0.05) but no significant difference was identified in the stable angina group (14.8% vs. 7.9%, p = 0.078).

Sibbing et al studied 2,485 patients pre-treated with clopidogrel undergoing PCI and determined their genotype. *CYP2C19\*2* carrier status was significantly associated with an increased risk of stent thrombosis and the risk of stent thrombosis was highest in the homozygote group (67). The FAST-MI investigators enrolled 2208 patients with MI and genotyped them for *CYP2C19\*2* and *CYP2C19\*3* LOF alleles. They demonstrated that patients carrying two LOF alleles had a higher rate of adverse cardiovascular events compared to those with no LOF alleles (21.5% vs 13.3%; adjusted HR 1.98; 95% confidence interval 1.7-3.58). In those patients undergoing PCI (n=1535), the rate of cardiovascular events in those with two LOF alleles was considerably higher (95% confidence interval 1.71-7.51) (68).

The largest single study to assess the influence of *CYP2C19* genotype on clinical outcomes was the PLATO genetic substudy. 10,285 patients were genotyped for *CYP2C19* LOF alleles \*2, \*3, \*4, \*5, \*6, \*7, and \*8. The primary efficacy endpoint was a composite of cardiovascular death, MI or stroke and, in the clopidogrel-treated group, the event rate at 30 days was higher in those with LOF alleles (5.7 vs 3.8%, p=0.028) (69). These findings were also confirmed by the smaller TRITON-TIMI 38 trial in 2932 patients with ACS that also demonstrated a higher incidence of the primary endpoint in clopidogrel-treated LOF allele carriers (70).

The ONSET/OFFSET and RESPOND Genotype studies investigated the effect of *CYP2C19* status on platelet reactivity in patients taking clopidogrel or ticagrelor (71). The study compared platelet reactivity between the treatment groups with specific genotypes. A total of 174 patients underwent genotyping of the *CYP2C19* LOF alleles \*2-8 and the *CYP2C19* GOF allele \*17 as well as *ABCB1*, which is involved in intestinal absorption. In the clopidogrel-treated group, the genotypic influence on PFT was most evident when assessed by the VerifyNow P2Y12 assay, which showed that the *CYP2C19* LOF carriers had higher mean platelet reactivity. This was most clearly seen during the maintenance therapy period. The antiplatelet effect of clopidogrel in the LOF carriers was in keeping with other studies (72-74).There was no influence of *CYP2C19* genotype in the ticagrelor group, either after a loading dose or during maintenance treatment. This study also demonstrated that ticagrelor provided greater platelet inhibition, even in those patients that were classified as clopidogrel ultra-rapid metabolisers (GOF allele carriers) and extensive metabolisers (homozygous for wild-type allele).

### Factors Affecting Pharmacokinetics of Ticagrelor

Ticagrelor is metabolised into active and inactive metabolites by CYP3A4 and CYP3A5 enzymes so concurrent treatment with strong CYP3A4/5 inhibitors, such as ketoconazole, is best avoided. Similarly, strong CYP3A4 inducers (for example itraconazole, ketoconazole and ritonavir) may accelerate ticagrelor metabolism leading to low drug levels. However, a large study did not demonstrate any effect of CYP3A4 genetic variants on clinical endpoints: The genome-wide association study of ticagrelor PK in the ticagrelor-treated ACS patients in PLATO showed modest genetic impact on plasma levels of ticagrelor and its active metabolite, which did not translate into effect on any efficacy or safety endpoints (75).

### Genetic Factors Affecting Pharmacokinetics of Ticagrelor

The ONSET/OFFSET and RESPOND Genotype studies were the first to report the superior pharmacodynamic effect of ticagrelor compared with clopidogrel irrespective of the *CYP2C19* genotype. One hundred and seventy-four patients had genotyping performed, 94 of whom were treated with ticagrelor. Platelet reactivity was lower in the ticagrelor-treated group and *CYP2C19* genotype had no influence on ticagrelor’s antiplatelet effect, consistent with the lack of role of CYP2C19 in the metabolism of ticagrelor. In the ticagrelor-treated group, platelet reactivity was lower than in clopidogrel-treated patients who were defined as ultra-rapid and extensive metabolisers (71). In the PLATO trial, patients were genotyped for *CYP2C19* LOF alleles and GOF alleles and the *ABCB1* single nucleotide polymorphism 3435C->T. The primary outcome in the ticagrelor-treated group was not significantly different regardless of the *CYP2C19* genotype (8.6% vs 8.8% for carriers of LOF allele or non-carriers, respectively) or *ABCB1* genotype (69).

## Diabetes Mellitus and Clinical Efficacy of The P2Y12 Inhibitors

DM is a well-documented risk factor for the development of coronary artery atherosclerosis and symptomatic ischaemic heart disease. Patients with DM have a two- to four-fold increased risk of CAD and those DM patients without a history of MI are at the same risk of an ACS as non-diabetic patients that have had a prior MI. The cause of accelerated atherosclerosis and the increased risk of thrombotic events in DM may result from a combination of dyslipidaemia, endothelial dysfunction, impaired fibrinolysis, platelet hyper-reactivity and abnormal blood flow (76).

Among the factors that contribute to the pro-thrombotic status in DM, altered platelet function plays a crucial role (77). Platelets in DM patients have dysregulation of signalling pathways and demonstrate increased adhesion, activation and aggregation (78). Insulin has a direct inhibitory effect on platelet aggregation, binding to its platelet membrane receptor and reducing responses to thrombin, ADP, AA and collagen. However, platelets in DM patients are less sensitive to insulin (79). There is a decrease in the number of platelet insulin receptors, which suggests that reduced insulin sensitivity may account for platelet hyper-reactivity in patients with DM (80).

Angiolillo et al were the first to investigate the effects of loading and maintenance doses of clopidogrel on platelet function in aspirin-treated DM and non-DM patients (81). The study recruited 52 patients to receive a clopidogrel 300-mg loading dose on the day of PCI and 120 patients with a history of CAD already on long-term clopidogrel maintenance therapy. The results demonstrated significantly greater levels of ADP-induced platelet aggregation and a significantly higher number of clopidogrel non-responders in DM patients following a loading dose compared to non-DM patients. In the maintenance therapy group, DM patients again had greater platelet aggregation levels compared to non-DM patients (p=0.04). In the long-term clopidogrel treatment group, DM patients treated with oral hypoglycaemic agents or treated with insulin did not have a significant difference in platelet function.

The PLATO substudy of ticagrelor versus clopidogrel in patients with ACS and DM studied 4662 patients with pre-existing DM. Ticagrelor therapy significantly reduced rates of the primary composite endpoint (cardiovascular death, MI or stroke), all-cause mortality, MI, and stent thrombosis in patients without DM. In the smaller subgroup of patients with DM, this benefit was consistent with the overall results and the relative risk reductions with ticagrelor were similar in DM and non-DM patients without any significant interaction: there was a reduction in the primary composite endpoint, all-cause mortality and stent thrombosis with no increase in PLATO-defined major bleeding. The PEGASUS TIMI-54 study used the standard ticagrelor 90mg bd dose and the lower ticagrelor 60mg bd versus placebo in aspirin-treated patients commencing 1-3 years following MI. Patients with DM demonstrated higher absolute cardiovascular benefit from long-term DAPT than patients without DM (82). The ticagrelor 60mg dose had not been studied in patients with DM and the PEGASUS platelet substudy investigated whether lower dose ticagrelor would provide similar levels of P2Y12 platelet inhibition as the 90mg dose in patients with DM. Fifty patients in the PEGASUS platelet function substudy had a diagnosis of DM and the results of the platelet function tests (VerifyNow, VASP and LTA) demonstrated that ticagrelor 60mg and ticagrelor 90mg twice daily had similar levels of platelet inhibition in DM patients as well as non-DM patients (p>0.05).

## Side Effects Specifically Related to Ticagrelor Treatment

### Dyspnoea

Patients taking ticagrelor often report shortness of breath that occurs at any time and is not usually related to exertion. Dyspnoea was initially reported in the DISPERSE 2 and ONSET/OFFSET studies (43) (44). In DISPERSE 2, dyspnoea was reported in 10.5% of patients treated with ticagrelor 90mg bd and 15.8% in the ticagrelor 180mg bd group compared to 6.4% in the clopidogrel group. The symptoms of dyspnoea resolved in 27% of these patients within 24 hours and in 25% after 24 hours. Of the remaining patients with symptoms after 24 hours, 48% had persistent symptoms of dyspnoea during ticagrelor treatment. In the ONSET/OFFSET study, dyspnoea was reported in 38.6% of patients in the ticagrelor group and 9.3% in the clopidogrel group (ticagrelor v clopidogrel p<0.001) (83). Three out of 57 patients stopped ticagrelor due to dyspnoea. In PLATO, patients randomised to receive ticagrelor also had a higher reported rate of dyspnoea compared to those receiving clopidogrel and this was again statistically significant (13.8% v 7.8%, p<0.001). Overall, however, there was a low rate of discontinuation of study medication due to dyspnoea (0.9% for ticagrelor and 0.1% for clopidogrel). In the ONSET/OFFSET and PLATO studies, no change in pulmonary function was demonstrated in those patients reporting dyspnoea (83) (84).

### Ventricular Pauses

Increased frequency of ventricular pauses has been noted with ticagrelor therapy and this phenomenon was first noted in the DISPERSE-2 trial. In PLATO, a substudy of 2908 patients had continuous seven-day Holter electrocardiogram monitoring at the time of randomisation and at one month (85). Ventricular pauses of 3 seconds or longer occurred in 5.8% of patients receiving ticagrelor compared to 3.6% of clopidogrel-treated patients (p=0.006) in the week following randomisation but in a similar proportion at one month (2.1% vs 1.7%, p=0.52). There were no significant differences between the treatment groups in relation to bradycardia-associated clinical events, including dizziness, syncope or permanent pacemaker implantation.

### Increase in Serum Creatinine

An increase in serum creatinine with ticagrelor treatment has been demonstrated. In PLATO, a greater than fifty per cent increase in serum creatinine levels was demonstrated in 7.4% of ticagrelor-treated patients compared to 5.9% of clopidogrel-treated patients (86). The increase in creatinine was not typically progressive despite on-going therapy and a fall in serum creatinine was frequently seen. Upon discontinuing ticagrelor treatment, mean creatinine levels fell to those observed in the clopidogrel group. There was no difference between treatment groups with respect to renal adverse events.

### Increase in Serum Uric Acid

DAPT with ticagrelor has been associated with an increase in serum uric acid levels that may contribute to endothelial dysfunction and a pro-thrombotic state (87).

In PLATO, there was an increase in serum uric acid levels of approximately 0.6 mg/dL from baseline in ticagrelor-treated patients and approximately 0.2 mg/dL in clopidogrel-treated patients. The incidence of reported gout did not differ between treatment groups (45). However in PEGASUS the incidence of gout was significantly more frequent with ticagrelor than with placebo (88).

A recent study looked at antiplatelet therapy and its effect on both uric acid levels and platelet reactivity (87). A total of 378 patients with either an ACS or undergoing elective PCI were enrolled; 233 were treated with aspirin and ticagrelor, the remainder with aspirin and clopidogrel. There was no difference in the baseline uric acid levels between the two groups but at 30-90 days a significant absolute and percentage increase in uric acid levels was found in the ticagrelor group (p=0.018). The variation in serum uric acid levels did not have an effect on platelet reactivity.

## Ticagrelor, Adenosine Plasma Concentration and Adenosine Uptake

The distinctive adverse effects seen with ticagrelor therapy may be due to ticagrelor’s interaction with adenosine metabolism. Adenosine and ticagrelor experiments have shown that ticagrelor inhibits adenosine uptake into erythrocytes through inhibition of equilibrative nucleoside transporter 1 (ENT 1), whereas other P2Y12 antagonists did not demonstrate any significant inhibition of this transporter (89) (90). Nylander et al investigated whether ticagrelor could inhibit platelet function via an increase in adenosine levels (91). When adenosine was added to whole blood (WB) in combination with dipyridamole, a potent inhibitor of adenosine reuptake, or the P2Y12 antagonists prasugrel or ticagrelor, adenosine was shown to contribute an additional antiplatelet effect. This antiplatelet effect was greater in association with dipyridamole and ticagrelor. In platelet-rich plasma, adenosine inhibited collagen-induced platelet aggregation and, when added in combination with ticagrelor, adenosine contributed an additional antiplatelet effect that was statistically significant. The study by Nylander *et al* looked at the *in-vitro* effects of ticagrelor on adenosine uptake using blood taken from healthy volunteers who had abstained from any antiplatelet treatment known to affect platelet function for at least ten days beforehand. In contrast, STEEL PCI involved *ex vivo* studies, using blood taken from patients with CAD undergoing elective PCI procedures who had received oral antiplatelet therapy prior to and after the procedure.

In addition to demonstrating inhibition of adenosine uptake, ticagrelor has also been shown to enhance adenosine-mediated hyperaemia in a canine model (90). In humans, a healthy volunteer study looked at coronary blood flow velocity in the left anterior descending artery prior to and during an infusion of adenosine (92). The volunteers were first randomised to a standard loading dose of ticagrelor or placebo. This study demonstrated a significant correlation between the coronary blood flow velocity response to intravenous adenosine infusion and circulating plasma ticagrelor concentration.

Ticagrelor’s ability to increase plasma adenosine concentration hypothetically leads to an improvement in coronary blood flow and is responsible for the unique side effect profile seen with ticagrelor therapy. It has also been suggested that the increased plasma uric acid levels seen with ticagrelor treatment are related to increased adenosine plasma concentration (APC).

## The Role Of Percutaneous Coronary Intervention in the Management of Coronary Artery Disease

### The History of Percutaneous Coronary Intervention

The first percutaneous transluminal coronary angioplasty (PTCA) was performed in September 1977 by radiologist Andreas Gruentzig, in Zurich, Switzerland (93). The patient, a 38 year-old man, underwent successful balloon angioplasty to his left coronary artery and had an uneventful recovery. After the success of this procedure, six patients were successfully treated with PTCA that same year. There were a number of limitations of the procedure due to the equipment available that was prone to traumatise the artery, a lack of guide wires, and angioplasty balloons that burst at low-pressure inflations. This really meant that the procedure was only suitable for patients with intractable angina with a single coronary artery lesion. During the decade spanning the mid-1970s to mid-1980s, the equipment utilised in PTCA was developed and improved and the procedure became more popular. However, PTCA continued to be hampered by two major issues, the risk of acute vessel closure and coronary artery re-stenosis at the site of the original plaque. Palmaz et al introduced the first balloon-mounted stent for use in peripheral arteries in 1985 and Schatz et al modified this stent for use in coronary disease, leading to the development of a commercially-available stent, the Palmaz-Schatz stent (94, 95). In 1986, Puel and Sigwart deployed the first coronary artery stent and, in 1987, Sigwart and colleagues were the first to report the use of a stent to prevent acute vessel closure during a PTCA procedure (96).

Early studies of patients with these stainless steel coronary artery stents demonstrated that vessel occlusion was still a significant problem despite aggressive anticoagulation, which led to prolonged hospital admission and an increased risk of serious or fatal bleeding. One study of 105 patients demonstrated that, although there was a significant increase in the minimal luminal diameter immediately after stent deployment, vessel occlusion occurred in nearly one-quarter of all patients (24%), usually within the first fourteen days. Follow-up angiography after an average of 5.7 months demonstrated a significant decrease in minimal lumen diameter and significant in-stent re-stenosis (97). Stent deployment, however, had a role as a bail-out procedure during acute or threatened vessel closure following PTCA and prevented the need for emergency CABG surgery (98).

In the early 1990s, the use of coronary artery stenting as standard treatment following balloon angioplasty was established after two randomised clinical trials compared the Palmaz-Schatz stent with balloon angioplasty alone. The BENESTENT and STRESS studies both demonstrated that the use of coronary artery stenting reduced the degree of restenosis when assessed angiographically. Clinical outcomes demonstrated that there was less frequent need for further angioplasty procedures in the stented groups (99) (100). As a result of these early follow-up studies, PCI using balloon angioplasty followed by coverage of the treated lesion with a stent became the normal practice. Although these bare-metal stents (BMS) prevented acute vessel recoil, they increased the risk of subacute thrombosis and presented the major problem of in-stent restenosis (ISR) due to neo-intimal hyperplasia.

The future developments in stent technology addressed the issue of neo-intimal hyperplasia whilst pharmacological adjuncts were developed to reduce the incidence of stent thrombosis. These developments heralded the introduction of DES, which, along with improved deliverability, have proved effective at reducing ISR and allowed the global use of PCI for high-risk patients and patients with complex coronary anatomy.

### First Generation Drug-Eluting Stents

DES are comprised of a platform or scaffold, similar to their BMS counterparts, a carrier polymer coating and an anti-proliferative drug. In April 2003, the Cypher sirolimus-eluting stent was launched, the first DES approved by the FDA. Sirolimus is naturally occurring in the Pacific yew tree and has potent antiproliferative, anti-inflammatory and immunosuppressive properties, which halt the cell cycle. This stent was evaluated in the RAVEL study that randomly assigned patients to a BMS or the Cypher DES. At 6 months, the degree of neointimal proliferation was 0% in the sirolimus-eluting stent group and there were no reports of stent thrombosis (29). These impressive results were supported by the SIRIUS trial (Sirolimus-Eluting Stent in De-Novo Native Coronary Lesions), a larger trial of 1058 patients with more complex lesions than those in RAVEL, which demonstrated superiority of Cypher sirolimus-eluting stent over BMS (101). Further multi-centre SIRIUS trials in Europe, Latin America and Canada confirmed the results of the original study (102) (103).

Paclitaxel is another potent antiproliferative drug used in DES. The TAXUS I study was the first to evaluate the safety of the paclitaxel-eluting TAXUS stent for the treatment of coronary artery lesions in humans. Sixty-one patients were randomised to receive the TAXUS stent or a BMS, the restenosis rate was 0% in the DES group and 10% in the BMS group (104). TAXUS II used the commercially available slow-release paclitaxel-eluting stent (TAXUS-SR) and an investigative moderate-release paclitaxel-eluting stent (TAXUS-MR) compared to a BMS. Both the slow-release and moderate-release TAXUS stents had the same dose of drug but drug release from the moderate-release stent was eight times higher in the first ten days. Both the TAXUS-SR and TAXUS-MR DES demonstrated superior outcomes compared to the control BMS group, which led to European approval of the slow-release stent (105). TAXUS IV evaluated long-term safety and efficacy of the TAXUS-MR DES compared to a BMS in patients with a single coronary artery lesion. Five-year follow up in 1230 patients showed the rate of target vessel revascularisation was significantly reduced in the TAXUS-MR group at 9 months and this benefit continued throughout the five year follow up period (106).

### Second Generation DES

The first of the second-generation DES was the zotarolimus-eluting Endeavor stent, which was studied in the ENDEAVOR I and II trials. The Endeavor stent has a phosphorylchlorine polymer coating and a cobalt alloy thin-strut scaffold. ENDEAVOR I demonstrated the safety of the stent whilst ENDEAVOR II compared the zotarolimus-eluting stent to a BMS in 1197 patients (107). The rate of ISR and target vessel revascularisation was significantly lower in the DES group (108). ENDEAVOR III compared late safety and efficacy outcomes with the zotarolimus-eluting and sirolimus-eluting stents. Although there was initially higher angiographic evidence of late lumen loss with the zotarolimus stent, there was similar late-term efficacy between the two stents. Five-year outcomes of the ENDEAVOR IV study comparing the Endeavor and Taxus stents demonstrated similar rates of target lesion revascularisation and led to FDA approval of the zotarolimus-eluting stent (109).

Everolimus is a sirolimus analogue and has the same antiproliferative and immunosuppressive properties; it is more lipophilic and consequently is rapidly absorbed into the vessel wall. The SPIRIT First study assessed the safety and efficacy of an everolimus-eluting stent made from a durable polymer on a cobalt chromium platform compared to BMS. Six-month results revealed significantly less late lumen loss in the everolimus group and similar clinical safety outcomes (110). SPIRIT II compared the Xience V everolimus-eluting stent with the Taxus stent and demonstrated superiority of the everolimus-eluting stent at six months in terms of ISR (111). The Xience V stent was further evaluated in the SPIRIT III study and demonstrated lower rates of target vessel revascularisation in the everolimus-eluting stent group compared with the paclitaxel-eluting stent (112). The SPIRIT IV trial was designed to study the clinical outcomes of the two DES used in SPIRIT II and III; rates of target lesion revascularisation were significantly lower for the everolimus-eluting stent at six months and two years (113). The COMPARE study also showed significantly reduced rates of target vessel revascularisation in the everolimus-eluting stent group compared to the paclitaxel-eluting stent group in 1800 unselected patients undergoing PCI.

### Bioabsorbable Stents

The stent polymers used for drug delivery can induce local vessel wall irritation, endothelial dysfunction and chronic inflammation at the site of stent deployment (114). This persistent inflammation and delayed arterial wall healing may have a role in precipitating late in-stent thrombosis and restenosis. In order to try and reduce this, there has recently been a focus on the development of bioabsorbable stents. The bioabsorbable stents have a poly-L-lactic acid scaffold that is degraded by hydrolysis of inter-lactic bonds of the long poly-L-lactic acid chains and results in particles that are phagocytosed by macrophages. The final product is lactic acid, which is metabolised into carbon dioxide and water via the Krebs cycle (115). After a period of months, the bioabsorbable stents leave behind the healed artery, allowing restoration of vaso-reactivity with the potential for vascular remodeling. However, biodegradable stents have several limitations: they are associated with local inflammation, just as their metal counterparts are, and their bioabsorption rate is slow and may still result in restenosis. The stent struts are thicker than the traditional metal stents and this can impact on their delivery, especially in small or tortuous vessels. The bioabsorbable everolimus-eluting stent with a poly-L-lactic acid scaffold (Absorb™ Bioresorbable Vascular Scaffold [BVS]) was assessed in the ABSORB study of thirty patients with a single coronary lesion. At twelve months, no patients had undergone target vessel revascularisation and no late stent thrombosis was reported (115). However, the two-year results of the ISAR-ABSORB registry have shown that target revascularisation was performed in 16% and definite stent thrombosis occurred in 3.8% (116). The AIDA study looked at 1845 patients undergoing PCI with 924 patients randomly assigned to receive a bioresorbable vascular scaffold and 921 patients assigned to receive a drug-eluting stent. The primary endpoint was a composite of cardiac death, target-vessel myocardial infarction, or target-vessel revascularization.This occurred in 105 patients in the bioresorbable group and in 94 patients in the stent group; 7% and 10.7%, respectively. Definite or probable stent thrombosis occurred in 31 patients in the bioresorbable group as compared with 8 patients in the stent group 3.5% vs. 0.9%, P<0.001 (117).

The development of DES has been the best method of reducing ISR secondary to neointimal hyperplasia. The issue of stent thrombosis, both acute and subacute thrombosis, is discussed later in this chapter.

### The Role of Percutaneous Coronary Intervention Today

Over 97,000 PCIs were carried out in the UK in 2014-15, fifty years after the first procedure was performed (118). The number of procedures had increased exponentially until the last couple of years when the number performed reached a plateau. Approximately 27% of all procedures in 2014 were primary PCIs for the emergency treatment of patients with STEMI. In the UK, there are 69 cardiology centres that receive patients with STEMI and perform primary PCI, urgent and elective cases. The other interventional cardiology centres perform a mix of elective procedures for patients with symptomatic but stable CAD and urgent coronary intervention for patients presenting with ACS.

### The Role of Percutaneous Coronary Intervention in Stable Coronary Artery Disease

Patients with symptoms of stable angina should be started on medical therapy in the first instance with the aim to alleviate their symptoms (119). However, if they have significant symptomatic angina, a change in severity or frequency of symptoms, or symptoms not relieved by optimal medical therapy then an interventional approach may be taken. In some cases, such as high-risk coronary anatomy demonstrated on diagnostic coronary angiography or worsening left ventricular function, then coronary intervention or, if appropriate, CABG is indicated. In the management of stable CAD, it is still unclear whether there is benefit of PCI over optimal medical therapy as an initial strategy (120). PCI procedures to treat stenoses that are not inducing ischaemia may, in some cases, be harmful (121). A number of large randomised studies have been designed to address the treatment of coronary stenoses in patients with stable disease. The COURAGE study randomised 2287 patients with objective evidence of myocardial ischaemia and significant coronary disease to PCI and OMT or OMT alone. The primary outcome of death from any cause and non-fatal MI was reported in 211 patients in the PCI group and 202 patients in the medical therapy group. PCI in addition to OMT did not reduce the risk of death or non-fatal MI (120). FAME I randomised 1005 patients to either angiography-guided PCI or fractional flow reserve (FFR) guided angioplasty. The study demonstrated that, in patients with multi-vessel CAD, measurement of FFR during PCI resulted in significantly reduced rates of the primary composite endpoint of death, MI and repeat revascularization at 1 year (121). The ORBITA study enrolled stable patients with severe single-vessel CAD. After a six-week period of medication optimisation, patients were randomised 1:1 to undergo PCI or a placebo procedure. Prior to randomisation, patients underwent cardiopulmonary exercise testing and stress echocardiography and, after 6 weeks of follow-up, the exercise test and echocardiogram were repeated. The primary endpoint of the study was difference in exercise time increment between groups.

Two hundred and thirty patients were recruited and 200 patients randomised, 105 patients to the PCI group and 95 to the placebo group. There was no significant difference in the primary endpoint of exercise time increment between the two groups (P=0·200). Serious adverse events included four pressure-wire related complications in the placebo group, which required PCI, and five major bleeding events, two in the PCI group and three in the placebo group (122).

### The Role of Percutaneous Coronary Intervention in the Management of Non-ST Elevation Myocardial Infarction

The National Institute for Health and Care Excellence (NICE) guidelines published in September 2014 and the European Society of Cardiology (ESC) guidelines published in 2015 recommend that, if clinically appropriate, coronary angiography should be performed within 72 hours once a diagnosis of NSTEMI has been made, with the ESC guidelines recommending more rapid angiography for higher-risk patients (123) (124). Invasive coronary angiography provides confirmation of the diagnosis of an ACS due to obstructive CAD and identification of the culprit lesion, thereby guiding further management. There are three possible outcomes following coronary angiography: i) medical therapy, including treatment with secondary prevention medications; ii) revascularisation by PCI; or iii) revascularisation by CABG.

The RITA 3 trial randomised 1810 patients with NSTEMI to an interventional strategy or conservative management; at four months, the rates of death, MI or refractory angina were significantly lower in the interventional group (125). At one year, the rates were similar and, at 5 years, it was shown that an invasive strategy led to long-term reduction in risk of death or non-fatal MI, particularly in high-risk patients (126). FRISC II compared an early invasive and early non-invasive strategy in unstable coronary disease. The early invasive group had significantly lower rates of death and MI and reduction in the rates of readmission (127).

### The Role of Primary Percutaneous Coronary Intervention in the Management of ST-Elevation Myocardial Infarction

Primary PCI (PPCI) is the preferred reperfusion strategy if it can be performed within guideline-mandated times. The ESC guidelines suggest PPCI should be performed within one hour after a diagnosis of STEMI is made in a PPCI centre, or within 90 minutes if the diagnosis is made at a non-PPCI hospital or by the ambulance service (128). The PPCI team is an experienced team that includes interventional cardiologists, skilled nursing staff, radiographers and physiology staff to facilitate safe and timely intervention.

Numerous studies have compared thrombolysis and PPCI for acute STEMI and have demonstrated the superiority of an interventional approach. A meta-analysis of 17 trials of patients with STEMI randomised to facilitated planned intervention or PPCI demonstrated that significantly greater numbers of patients in the thrombolysis group died and had higher rates of non-fatal reinfarction and urgent target vessel revascularisation. The drugs used to facilitate the procedure included high-dose heparin,GP IIb/IIIa inhibitors, full-dose and reduced-dose thrombolytic drugs and a combination of GP IIb/IIIa inhibitors with reduced-dose thrombolytic drugs (129).

### Arterial Access Route for Coronary Angiography and Angioplasty

Coronary angiography and angioplasty can be performed via the radial, brachial or femoral artery. A sheath with a haemostatic valve is inserted into the peripheral artery under local anaesthetic through which a diagnostic or guide catheter is passed. At the end of the procedure, the arterial puncture site is either closed by the use of manual pressure, a sealing device in the case of femoral access, or a compression device in radial access cases. The route of access used to facilitate PCI is of interest in terms of bleeding risk and overall risk of complications associated with the procedure. There is a reduction in major bleeding events when radial artery access is favoured over femoral artery access (130, 131).

The femoral artery had been the arterial access site of choice until recently. A femoral artery approach has several limitations, including the consensus that this route of access is relatively contraindicated in patients receiving anticoagulation therapy and those with severe peripheral vascular disease. Arterial complications during and post-procedure may be troublesome; the incidence of haematoma, pseudoaneurysm, arterio-venous fistula and surgical repair can be 2-8% (132). Patients are required to lay flat for at least one hour post-procedure, which may lead to discomfort due to other unrelated pre-existing medical problems. The increased morbidity and increased length of hospital stay associated with a trans-femoral approach have led to a change in practice and a move away from femoral access to radial access for angiography and PCI procedures.

There are several advantages to using the radial artery for procedural access. The radial artery is not an end artery, unlike the brachial and femoral arteries, since the hand receives dual arterial supply from the radial and ulnar arteries and so occlusion of the radial artery does not usually compromise blood supply to the hand. The arterial puncture site can be secured with a compression bracelet and the patient is therefore able to mobilise more quickly and, in an elective case, may be suitable for transfer to a ‘radial lounge’ day-case unit facilitating early discharge post-procedure.

The reported complication rate of radial access procedures appears lower than that of femoral procedures. The RIVAL study assessed if radial access was superior to femoral access in patients with ACS and looked at non-CABG-related bleeding rates. The rates of major bleeding were not significantly different between the two groups but the incidence of large haematoma formation and pseudoaneurysm was significantly higher in the femoral group (131). Another study of 8404 patients with NSTEMI and STEMI undergoing coronary angiography were randomised to trans-radial or trans-femoral access and BARC major bleeding events unrelated to CABG surgery were recorded. This study again showed a significant reduction in major bleeding in the trans-radial group. All-cause mortality was also significantly reduced in the radial cohort (133).

## Recommendations for duration of DAPT in Percutaneous Coronary Intervention

### DAPT in Elective Percutaneous Coronary Intervention

Aspirin-treated patients undergoing elective PCI should receive a 600-mg loading dose of clopidogrel before the procedure if the coronary anatomy is already known. This should be followed by clopidogrel 75mg od maintenance therapy in addition to low-dose aspirin. It has been suggested that the use of a 150-mg od maintenance dose in patients at high risk of thrombosis may be of benefit; however, no studies have demonstrated such a benefit in the short or long term (134). The GRAVITAS study evaluated standard- and high-dose clopidogrel treatment in patients with high on-treatment platelet reactivity (HTPR) following PCI. The primary endpoint of cardiovascular death, non-fatal MI and stent thrombosis at six months occurred at similar rates in the two groups (2.3% vs 2.3%, p=0.97) (135).

The duration of DAPT after elective PCI was two to three months for the sirolimus-eluting stent and six months for the paclitaxel-eluting stent. However, following a study of early and late stent thrombosis, the recommendation for DAPT following PCI using DES was increased to twelve months or longer (136). Current guidelines do not support the use of DAPT beyond twelve months following DES implantation and the ESC guidelines suggest six months of DAPT following PCI with newer generation DES is sufficient. A number of randomised trials have studied short-term (3-6 months) and long-term (12-24 months) DAPT and demonstrated no benefit in longer-term treatment with regard to ischaemic events (137) (138) (139).

### DAPT in NSTE-ACS Treated with Percutaneous Coronary Intervention

In patients undergoing PCI for NSTE-ACS, DAPT is recommended for 1 year. This extended duration of treatment with aspirin and a P2Y12 inhibitor reduces the risk of ST, recurrent MI and cardiovascular death (38). The ESC guidelines recommend 12 months of aspirin and a P2Y12 inhibitor unless there is a clear contraindication. Ticagrelor is recommended for all patients at moderate-to-high risk of ischaemic events regardless of the initial management strategy. Prasugrel is recommended for patients who are proceeding to PCI and clopidogrel for patients who have a contraindication to ticagrelor or prasugrel treatment or who require long-term oral anticoagulation (140).

### DAPT in STE-ACS Treated with Percutaneous Coronary Intervention

In patients presenting with STE-ACS undergoing primary PCI or following thrombolytic therapy, DAPT with aspirin and a P2Y12 inhibitor is recommended for up to twelve months. Some studies suggest there is no benefit in treatment with DAPT from 6 or 12 months after stenting with a DES to prevent ischaemic events or ST but these studies only included a limited number of STEMI patients. At present, ESC guidelines recommend treatment with DAPT for a minimum of one month after stenting with BMS and for a minimum of six months after DES implantation. In those patients who do not undergo reperfusion therapy, treatment should be for at least one month and for up to twelve months (141).

**1.9.4 Studies looking at Prolonged DAPT**

The PEGASUS-TIMI 54 trial studied two doses of ticagrelor, 90mg twice daily and 60mg twice daily, in patients with a history of MI between 1 and 3 years previously (1). The trial looked at long-term benefit of treatment with ticagrelor at these two different doses compared to placebo in patients with risk factors for recurrent atherothrombotic events. The 60mg dose had not been previously studied and the platelet sub-study of PEGASUS-TIMI 54 provided the first assessment of the pharmacokinetics and pharmacodynamics of this lower ticagrelor dose. The primary efficacy endpoint was the composite of cardiovascular death, myocardial infarction, or stroke whilst the primary safety endpoint was TIMI major bleeding. The two ticagrelor doses each reduced the rate of the primary efficacy endpoint (P=0.008). The rates of TIMI major bleeding were higher with ticagrelor 90mg and 60mg (2.60% and 2.30%, respectively) than with placebo (1.06%) (P<0.001 for each dose vs. placebo). The results demonstrated that both doses achieved high levels of platelet inhibition with no significant differences between the two ticagrelor groups (142).

The DAPT study enrolled 9961 patients after their PCI procedure for either stable CAD or ACS. After 12 months of treatment with DAPT using aspirin plus clopidogrel or prasugrel, patients were randomised to either continue receiving thienopyridine and aspirin or receive placebo and aspirin for a further 18 months. The two co-primary endpoints of the study were stent thrombosis and major adverse cardiovascular and cerebrovascular events (MACE, a composite of death, myocardial infarction, or stroke). The primary safety endpoint was moderate or severe bleeding. Prolonged treatment with DAPT reduced the rates of stent thrombosis (0.4% vs 1.4%, P<0.001) and MACE (4.3% vs 5.9%, P<0.001). The rate of myocardial infarction was also lower with continued DAPT (2.1% vs. 4.1%, P<0.001). As might be expected, the rate of moderate or severe bleeding was increased with continued aspirin and thienopyridine treatment (2.5% vs 1.6%, P=0.001). Overall, prolonged DAPT beyond 1 year after PCI significantly reduced the risks of stent thrombosis and MACE events but was associated with an increased risk of bleeding compared to aspirin monotherapy (143).

## Platelet Function Tests

### History of Platelet Function Testing

Currently, there is a wide variety of platelet function tests available that have different strengths and limitations, some of which are much longer-standing and well established (Table 1.1). The German anatomist Max Schultze described platelets for the first time in 1865 (144). By 1881, Bizzozero, an Italian pathologist, was studying platelets in vitro and correctly determined their role in haemostasis and thrombosis (145). William Osler, a physician, realised platelets contributed to atherosclerotic disease in 1886, after discovering their presence in white thrombi in aortic lesions and diseased heart valves. In 1906, the American pathologist James Homer Wright established that the megakaryocyte was the platelet precursor with the aid of a staining method he developed (146). Over the last century, the platelet has become the subject of increasing interest as its role in atherothrombotic disease has emerged (147). CVD remains the largest cause of mortality and morbidity in the world and the need for antiplatelet therapies and platelet testing has emerged (148).

The first platelet function test was performed over 100 years ago when William Duke introduced the concept of bleeding time (149).Bleeding time assesses the ability of platelets to form a plug and for many years was used in clinical practice to screen for acquired and congenital platelet disorders and to this day remains the only test of platelet function in vivo. By the 1960s, LTA using platelet rich plasma (PRP) was in use, with the capability of assessing platelet aggregation in response to the addition of various agonists (150). The 1980s heralded the introduction of WB platelet aggregometry, the study of platelets by flow cytometry and the quantification of substances secreted by platelets. Initially, PFTs were utilised in the diagnosis and management of patients with haemorrhage (151). Over the last twenty years, however, PFTs have been used in some centres for monitoring antiplatelet therapy, since a suboptimal response to the antiplatelet regimen may be associated with cardiovascular, cerebrovascular and peripheral arterial events (152). PFTs have also become important in the assessment of bleeding risk pre- and peri-operatively for patients undergoing surgical procedures or post-trauma (153) (154). Platelet function testing is also used in the assessment of platelet function in transfusion medicine pre- and post-transfusion (155). The increased application of PFTs led to bedside or ‘point-of-care’ testing and the development of simpler instruments that are easy to use and do not require the use of highly skilled laboratory staff.

### Light Transmittance Aggregometry

LTA was developed by Born in 1962 and has become the gold standard in platelet function testing (156). The original aggregometer consisted of a light source and an absorption meter and platelet function was assessed at room temperature. Designed to be performed on PRP, LTA uses a turbidimetric optical detection system to assess the pharmacodynamic response to various agonists. As platelet aggregation occurs in response to the addition of an agonist, the sample becomes more translucent and light transmission increases. Using different exogenous agonists, this method allows many of the platelet aggregation pathways to be assessed. Traditionally, the agonists used in LTA are ADP, epinephrine, collagen, AA and the antibiotic ristocetin. LTA allows *in vitro* quantification of the final common pathway of platelet aggregation via GP IIb/IIIa dependent bridging. The quantitative study of the aggregation curves (maximum amplitude of aggregation, amplitude at defined time points, slope of the initial increase, shape change) allows identification of the physiological and biochemical mechanisms that control platelet aggregation (157).

The commercially-available aggregometers are user-friendly and record the rate and percentage increase from 0% (the optical density of the PRP) to 100% (the optical density of platelet-poor plasma (PPP)). This data is presented graphically and documents the increase in light transmission over time during platelet aggregation, commonly recorded over 4 to 6 minutes. The quantitative study of the aggregation curves (maximum amplitude of aggregation, amplitude at defined time points, slope of the initial increase, shape change) identifies the physiological and biochemical mechanisms that control platelet aggregation (157).

LTA has, in a number of studies, demonstrated the relationship between HTPR and the risk of future atherothrombotic events in patients treated with antiplatelet drugs. Gum et al studied 376 patients with stable CVD on aspirin monotherapy using LTA with ADP and AA. Five per cent (n=17) of the population were deemed to be ‘aspirin resistant’ and this was associated with increased risk of death, MI or CVA (24% vs 10%, HR 3.12 95% CI 1.1-8.9, p=0.03) (158). Another study of aspirin therapy in 125 patients with CAD looked at platelet response after treatment and found that estimation of aspirin ‘resistance’ was highly assay-dependent. LTA using AA indicated lower rates of aspirin ‘resistance’ than other methods using different agonists (157). As a consequence of the fact that the therapeutic effects of aspirin are highly reliable and predictable, PFTs are only recommended for monitoring the effects of aspirin when information about compliance is likely to aid management.

Using LTA, Matetzky et al were among the first to demonstrate the relationship between HTPR and higher rates of ischaemic events following PCI for STEMI in sixty patients treated with aspirin and clopidogrel. Seven recurrent cardiovascular events (88%) occurred in patients with P2Y12-mediated HTPR, formerly referred to as clopidogrel ‘resistance’ (159). Gurbel et al also measured platelet reactivity using LTA in 192 patients undergoing PCI on DAPT in the Prepare Post Stenting Study. Patients who went on to have ischaemic events had higher rates of ADP-induced platelet aggregation (p=0.02) and those in the highest quartile of platelet aggregation had the greatest frequency of ischaemic events (160). The relationship between HTPR and the risk of further atherothrombotic events was again shown in the POPULAR (Do Platelet Function Assays Predict Clinical Outcomes in Clopidogrel-Pretreated Patients Undergoing Elective PCI) study, which compared platelet reactivity using multiple PFTs(161). A large study population (n=1069) of patients on clopidogrel undergoing elective PCI had LTA performed using ADP at 5µM and 20 µM. The primary composite endpoint of all-cause mortality, non-fatal acute MI, stent thrombosis and ischaemic stroke occurred more frequently in patients with HTPR (11.7% vs 6% p<0.01 using 5 µM ADP and 12% vs 6.2% p<0.001 using 20 µM ADP).

Overall, LTA is the oldest established method for assessing platelet reactivity and may provide prognostic information, particularly in clopidogrel-treated patients, where response to treatment is so widely variable. However, the lack of standardization between different laboratories, issues related to the effects of centrifugation on platelet reactivity and the inability of LTA to function as a point-of-care system has hampered its adoption into routine clinical practice, other than in the assessment of patients with potential inherited bleeding disorders.

### VerifyNow

VerifyNow (previously Ultegra Rapid Platelet Function Analyzer; Accumetrics Inc., San Diego, CA, USA) utilises WB for turbidimetric optical detection of platelet aggregation. The test comprises a single-use cartridge with four wells, each of which contains a chrome-plated mixing ball, fibrinogen-coated beads and a platelet agonist. The activated receptors on the platelets bind to nearby platelets via the fibrinogen-coated beads, aggregation of the platelets and beads reduces the turbidity of the sample and light transmittance increases. The VerifyNow system is a closed one and does not require any additional blood handling beyond the initial process of adding blood to the anticoagulant tube. There are three VerifyNow assays available for the assessment and evaluation of platelet inhibition in response to aspirin, the thienopyridines and GP IIb/IIIa inhibitors. The agonists used in each assay are AA in the aspirin assay, ADP (including prostaglandin E1 to suppress intracellular free calcium) in the P2Y12 assay and thrombin-receptor-activating peptide (TRAP) in the GPIIb/IIIa assay. VerifyNow results are reported as Aspirin Reaction Units (ARU), P2Y12 Reaction Units (PRU) and Platelet Aggregation Units (PAU) for each of the individual assays. In addition, the VerifyNow P2Y12 assay has an additional channel, known as the BASE channel, utilizing TRAP to provide an estimate of what the PRU level would be in the absence of any P2Y12 inhibition and this is used to calculate VerifyNow percent inhibition although most PFT studies have used PRU as the primary measure.

Defined thresholds of the reaction unit values have been used to discriminate between adequate responders and non-responders to antiplatelet medications. In 2008, Price et al reported the relationship between HTPR and clinical outcome in 307 patients using the P2Y12 VerifyNow assay with a cut-off of PRU ≥ 235: HTPR on clopidogrel was predictive of outcomes after DES implantation, including ST (162). Marcucci et al used PRU ≥ 240 as a cut-off to define HTPR in patients (n=683) undergoing PCI with BMS or DES and demonstrated this was a significant and independent predictor of the primary endpoint of cardiovascular death and non-fatal MI (HR 2.55, 95% CI 1.08-6.07 p=0.034; HR 3.36, 95% CI 1.49-7.58 p=0.004) (163). The ARMYDA-PRO group assessed 30-day rates of major adverse cardiovascular events (MACE) in each quartile distribution of PRU, demonstrating that primary endpoints occurred more frequently in patients with PRU levels in the upper quartile compared to those in the lower quartile (20% vs. 3% p=0.034), which was exclusively due to peri-procedural MI. Multivariable analysis showed that pre-PCI PRU levels in the fourth quartile were associated with a six-fold increased risk of 30-day MACE, suggesting that pre-PCI HTPR may predict 30-day events (odds ratio 6.1; 95% CI 1.1-18.3 p=0.033) (164).

GRAVITAS (Standard-vs-high dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomised trial) was a large-scale trial that recruited patients undergoing PCI for stable CAD or ACS (n=5429) and assessed the value of tailoring clopidogrel dose in patients with HTPR as determined by the VerifyNow P2Y12 assay (135). HTPR was defined as PRU ≥ 230 although a second cut-off of PRU 208 was also identified *post hoc* as a predictor of death, MI and stroke, confirmed at one year after elective PCI in the 3T/2R study (165). VerifyNow PRU < 208 was associated with a significantly lower risk of reaching the primary endpoint at 60 days (HR 0.23; CI 0.05-0.98 p=0.047).

A more recent and larger observational study, the ADAPT-DES study (Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents: a prospective multicenter registry study) (n=8665), also used PRU cutoffs of 208 and 230 with endpoints of definite or probable ST, all-cause mortality, MI and clinically relevant bleeding (166).HTPR was an independent predictor of stent thrombosis or MI in the first 12 months but not a predictor of death. The VerifyNow P2Y12 assay has established itself as the simplest and most reliable way of assessing response to P2Y12 inhibitors and has been used extensively both in research studies and, to a lesser extent, in clinical practice.

### The Multiplate Analyser

Impedance platelet aggregometry was developed in the 1980s by Cardinal and Flower and a point-of-care test that implements this principle using WB has recently been developed (167). Multiplate (Roche, Switzerland previously Dynabyte, Munich, Germany) measures increases in electrical resistance, or impedance, caused when activated platelets attach to electrodes (168). The Multiplate analyser, often referred to as multiple electrode platelet aggregometry (MEA), has five channels and utilises disposable test cells containing two independent sensor units, each housing two silver-coated copper electrodes measuring 3.2mm in length. The analyser measures the change in impedance for each of the two sensors simultaneously and presents this data as arbitrary aggregation units (AU). The AU are plotted against time to calculate the area under the curve expressed as AU.min. Multiplate allows quick and comprehensive platelet function testing with the manual addition of various platelet agonists that can be prepared at differing concentrations. The use of different agonists has made Multiplate a valuable diagnostic tool in the assessment of bleeding and for monitoring antiplatelet therapy.

Sibbing et al investigated 1608 patients with CAD undergoing PCI and assessed ADP-induced platelet aggregation after a clopidogrel loading dose (169).The primary endpoint was definite stent thrombosis at 30 days. Patients in the upper quintile of response (n=323), represented by a post-treatment threshold of 416 AU.min, were defined as low responders. The primary endpoint occurred in 10 patients, of whom 7 were low responders, demonstrating a significantly higher risk of stent thrombosis in that group (OR: 9.4; 95% CI 3.1-28.4; p<0.0001). The optimal cut-off value to predict stent thrombosis, according to receiver operating characteristic (ROC) analysis, was 468 AU.min. The Multiplate assay had 70% sensitivity, 84% specificity and area-under-the-ROC-curve of 0.78 (95% CI; 0.6-0.96, p=0.001). This was the first study to use stent thrombosis as a primary endpoint and demonstrated the strong predictive value of MEA.

In 2010, Eshtehardi et al assessed 219 patients on DAPT (aspirin and clopidogrel) listed for PCI (170). Early, definite stent thrombosis occurred in 1.4% of the cohort but occurred in 10.5% of those that were low responders to both aspirin and clopidogrel as demonstrated by HTPR in response to both AA (as a measure of aspirin response) and ADP (as a measure of clopidogrel response). Peri-procedural MI occurred in 19 (9%) with the highest incidence (26.3%) in the dual low responder group (p<0.001). Using MEA, dual low responders were independently associated with the composite ischaemic endpoints of MI and stent thrombosis (OR 7.35 95% CI 2.21-24.43, p<0.001). However, interpretation of such studies of ‘dual response’ is complicated by the fact that the platelet P2Y12 receptor is involved in amplifying the response to AA, as a consequence of AA-induced dense granule release of ADP, and so the so-called ‘aspirin’ assay is additionally affected by P2Y12 inhibitors.

Overall, the Multiplate ADP test has shown good prognostic value in clopidogrel-treated patients undergoing PCI. Although still significant, the consumable costs of Multiplate are less than those of the VerifyNow system but slightly more skill and training are required in its use and it is slightly less suited to a point-of-care setting. Like VerifyNow, the uptake into clinical practice has been impaired by lack of evidence supporting improved clinical outcomes when the system is used for guiding therapy.

### Platelet Function Analyser-100

The Platelet Function Analyser-100 (PFA-100®; Siemens Healthcare Diagnostics, Inc., Deerfield, IL, USA) uses a cartridge-based system that contains a capillary, sample reservoir and an aperture contained within a membrane coated with either collagen and epinephrine or collagen and ADP. Citrated blood is aspirated at high shear rates through the disposable cartridge. When platelets in WB come into contact with the membrane they are activated and aggregate at the aperture site, leading to its occlusion. The PFA-100 measures the duration (in seconds) from the start of the test to the closure time of the aperture. The PFA-100 is a rapid point-of-care test that can be performed using small quantities of citrated WB.

Gianetti et al hypothesized that platelet reactivity measured by PFA-100 could predict recurrent coronary events after PCI and they did demonstrate that increased platelet activation is an independent predictor of recurrent ischaemic events (171). However, other groups have demonstrated lack of sensitivity and specificity of the PFA-100 assays for the effects of either aspirin or P2Y12 inhibitors and so this system is not recommended for monitoring these drugs. This is in distinction to the value of the PFA-100 in detecting moderate-to-severe von Willebrand disease since it is sensitive to levels of functional VWF (172).

### Plateletworks

### 

Plateletworks(Helena Laboratories; Beaumont, TX, USA) is a point-of-care test using WB that rapidly measures the platelet count before and after platelet aggregation. An EDTA and a citrated sample are required and the test must be performed within ten minutes following blood collection. The EDTA tube acts as the control and the citrate tube, with the addition of agonists (AA, collagen or ADP), as the test sample. The Plateletworks test measures platelet aggregation by measuring and comparing the number of free platelets in the two tubes. The results are presented as percent platelet aggregation (or alternatively as percent platelet inhibition if pre-treatment and post-treatment samples are available)(173).Plateletworks kits are compatible with any impedance cell counter and thereby provide quantification of the number of platelets present within the sample and an assessment of their function.

In the POPULAR study, Breet et al showed that the 1-year primary endpoint occurred more frequently in patients with HTPR, compared to those without, including when assessed with the Plateletworks assay (12.6% vs 6.1% p=0.005) (161). They suggested that the assay had the largest increase in predictive value of all evaluated tests (LTA, VerifyNow, PFA-100 and Plateletworks).

The Plateletworks assay is limited by lack of standardisation and the requirement usually to transfer samples for platelet counting so it is less established and evidence-based than VerifyNow or Multiplate.

### VASP Phosphorylation

Vasodilator-stimulated phoshoprotein (VASP) is an intracellular protein that is not phosphorylated under normal circumstances; its phosphorylation is regulated by the cAMP cascade and is stimulated by prostaglandin E1 (PGE1). Dephosphorylation occurs through stimulation of the P2Y12 receptor by ADP. Persistent VASP phosphorylation correlates with P2Y12 receptor inhibition and therapeutic thienopyridine treatment. The VASP phosphorylation assay is specific for the P2Y12 receptor so can be used to assess receptor inhibition even in the presence of GP IIb/IIIa antagonists. The VASP assay is performed using WB with PGE1 or with PGE1 and ADP incubated at room temperature and then fixed with a paraformaldehyde solution. The VASP phosphorylation test is performed using flow cytometry utilizing polyclonal mouse antibodies. A platelet reactivity index (PRI) or VASP index is calculated using the corrected mean fluorescence intensities in the presence of PGE1 alone or PGE1 and ADP. The PRI or VASP index is expressed as a mean percentage and is inversely correlated with platelet reactivity (174). Of note, the assay is insensitive to low levels of P2Y12 receptor inhibition and this can lead to an inappropriately high number of patients being classified as HTPR (175) (176). A PRI result > 50% has previously been proposed as the optimal cut-off to define clopidogrel non-responsiveness; however, some investigators believe that this overestimates the frequency of HTPR and suggest that PRI values >60% should be used.

Bonello et al studied 144 patients having PCI after a loading dose of clopidogrel (176). Flow cytometric assessment of VASP phosphorylation was performed at 25 ± 3 hours. The mean PRI was 66 ± 20%. Forty-eight percent of patients had platelet reactivity lower than the normal range after a loading dose of clopidogrel and considerable inter-individual variability was observed (PRI 9-98%). Twenty-one patients had a major cardiovascular event during the 6-month follow-up period. VASP phosphorylation analysis demonstrated that clopidogrel resistance is associated with an increased risk of MACE and PRI result has a high negative predictive value for MACE. A further study of 16 patients with subacute stent thrombosis (SAT), identified from 1684 consecutive patients undergoing PCI on DAPT, compared PRI values with those of a group of 30 PCI patients without SAT. There was a significant difference in PRI between the group with SAT (63 ± 10%) and the control group (40 ± 11%; p<0.0001) (177).

Overall, the VASP phosphorylation assay has added to clinical knowledge about the relationship between HTPR and adverse clinical outcomes but the requirement of specialist equipment and skilled personnel, combined with some variability in the assay results, limits its usefulness for monitoring the effects of P2Y12 inhibitors in clinical practice.

### Cone and Plate(let) Analyser

The Cone and Plate(let) Analyzer (CPA; Image Analysis Monitoring Platelet Adhesion Cone and Plate Technology) is a point-of-care test that mimics physiological blood flow conditions to assess platelet function in WB. The original CPA test assessed platelet aggregation on a plate coated with extra-cellular matrix. The CPA concept has been incorporated into the automated and computerized IMPACT system (DiaMed, Cressier, Switzerland), which uses polystyrene rather than extra-cellular matrix. The IMPACT analyser has the facility to stain and microscopically image platelets adhering and aggregating on the plate at an applied shear rate produced by the spinning of a cone on the polystyrene plate. The analyser measures the surface area of the well covered by platelet aggregates and the average size of the aggregates. The addition of agonists in this test has allowed the instrument to be used in the monitoring of antiplatelet therapy but it has not yet gained widespread acceptance (178).

### Thromoboelastography

Hartert first described thromboelastography in 1948 as a method to comprehensively examine all aspects of haemostatic function using a single WB sample (179). Thromboelastograph, thromboelastography and TEG had been used as generic terms in the literature up until 1996 when thromboelastograph and TEG became trademarks of Haemoscope Corporation (Niles, IL). ROTEM, a laboratory instrument utilizing rotational thromboelastometry, is marketed by Pentapharm GmbH (Munich, Germany). Both TEG and ROTEMare used to assess clot formation, clot stabilisation and dissolution under conditions of shear stress reflecting in vivo conditions. They provide a convenient and highly effective method for monitoring WB coagulation.

TEG principally consists of a disposable cup that oscillates around a pin suspended from a torsion wire. The pin remains motionless until clotting begins but the subsequent development of fibrin strands unite the cup and pin. The union of the cup and pin is directly proportional to the strength of the clot. As the clot lyses, the bond naturally decreases. An electromagnetic transducer detects the tension in the wire and it is this signal that is amplified and graphically displayed as a TEG tracing. The ROTEM system uses different technology: the motion of the pin is detected by an optical system and the movement is initiated from the pin, not the cup.

These instruments quickly produce a graphical representation of clot formation and lysis after the addition of different reagents for each test and for this reason have become a point-of-care test in clinical settings where the assessment of bleeding risk is required. Although best established for assessing coagulation function, particularly in patients following CABG surgery, the adaptation of these tests by adding platelet agonists (so-called ‘platelet mapping’) has also provided useful data on their potential role in assessing both coagulation and platelet function (180).

Curzen et al designed a study to establish whether TEG could be modified and utilised as a 15 minute test, the short-thromboelastography (s-TEG), to allow rapid assessment of individual response to antiplatelet therapy (181). The s-TEG area under the curve (AUC15) and maximum amplitude (MA) includes a novel modification producing results in 15 minutes so can be utilised as a point-of-care test. A study of volunteers taking no medication, volunteers pre and post 300mg aspirin and patients pre and post 600mg clopidogrel demonstrated that there was minimal intra-and inter-individual variability in MA and AUC15 in the AA, ADP and thrombin channels. When s-TEG was compared to VerifyNow, there was reasonable correlation between AA AUC15 and VN ARU and between ADP AUC15 and VN PRU (182).

**Table 1‑1**: Methods for Assessing Platelet Function

|  |  |  |
| --- | --- | --- |
| Light transmittance aggregometry | Platelet rich plasma | Optical detection system to assess increase in light transmittance in response to addition of platelet agonists |
| VerifyNow analyser | Whole blood | Optical detection of platelet aggregation using cartridges with fibrinogen-coated beads and different platelet agonists |
| Impedance aggregometry | Whole blood | Measurement of increase in electrical impedance due to formation of platelet aggregates on electrodes |
| PFA-100 analyser | Whole blood | Measurement of time to closure of an aperture by platelet aggregates |
| Plateletworks system | Whole blood | Single-platelet count before and after addition of platelet agonist |
| VASP phosphorylation assay | Whole blood | Blood mixed with PGE1 or PGE1 and ADP is fixed with paraformaldehyde solution. Samples are then mixed with a fluorescently labelled antibody to phosphorylated VASP before measurement of fluorescence using flow cytometry |
| Cone and plate analyser | Whole blood | Measurement of the surface area covered by platelet aggregates and the average size of platelet aggregates |
| Thromboelastography | Whole blood | Assessment of thrombus formation, stabilisation and dissolution under conditions of low shear stress |

## Platelet Markers

### Markers Of Platelet Activation

A clinically valuable and relevant marker of platelet activation must be specific and accurately measurable. There are three groups of substances that can be measured and quantified: those released from the platelet granules, those expressed on the surface of the platelet and those that are synthesized and secreted. Alpha-granules in unactivated platelets contain a number of growth factors, clotting proteins and the cell adhesion molecule P-selectin, which is rapidly transported from its granular location to the plasma membrane when platelet activation occurs. P-selectin is subsequently shed into plasma in its soluble form. P-selectin expressed on the surface of platelets binds to P-selectin glycoprotein ligand-1, which is expressed on almost all leucocytes and mediates platelet tethering and adhesion to circulating monocytes. There is evidence that P-selectin is also important for inter-platelet aggregation, stabilizing the initial GP IIb/IIIa–fibrinogen interactions and allowing the formation of large, stable platelet aggregates (183).

P-selectin expressed on the platelet surface and platelet monocyte aggregates are measured by flow cytometry, whilst plasma soluble P-selectin levels are measured using monoclonal antibody based ELISA (enzyme-linked immunosorbent assay) tests. Raised levels of platelet-monocyte aggregates and soluble P-selectin are found in a spectrum of cardiovascular disorders, including atrial fibrillation,ischaemic heart diseaseand congestive cardiac failure, but not in renal failure (184)-(185).Platelet-leucocyte aggregates may be a more sensitive marker of platelet activation than surface P-selectin expression as degranulated platelets rapidly shed surface P-selectin *in vivo* (186), (187). Circulating aggregates have been shown to be a sensitive marker of platelet activation and an early marker of acute MI (188).

There are two major disadvantages with the measurement of WB or plasma markers of *in vivo* platelet activation: the first is the high possibility of platelet activation during venipuncture, which may lead to inaccurate, spurious results, and the second is the lack of clinical and laboratory data to support its use. On the other hand, *in vitro* measurement of ADP-induced platelet P-selectin expression correlates well with platelet P2Y12 inhibition and there is continuing interest in this method for monitoring the effects of P2Y12 inhibitors (175).

Measurement of urinary metabolites of TXA2 has also been assessed as a marker of *in vivo* platelet activation (189).TXA2 is synthesized, via platelet COX 1 (the target of aspirin), and released upon platelet activation, serving as a positive feedback loop by activating other platelets via TP receptors (190). The stable urinary metabolite, 11-dehydro-thromboxane B2, can be measured and has shown correlation with clinical outcomes (189).However, interpretation is limited by the fact that around 30% of this metabolite is derived from non-platelet sources and so the assay does not specifically assess platelet COX-1 inhibition by aspirin (191).A more specific and gold standard assessment of aspirin effect is serum thromboxane B2 measurement. Thrombin generated during serum isolation *ex vivo* leads to platelet activation and the release of TXA2 that is rapidly converted into its stable metabolite, thromboxane B2, which can be measured by ELISA (192). However, this methodology does not lend itself to point-of-care testing and is predominantly a research tool.

### Markers Of Platelet Turnover

Platelets in the circulation are in equilibrium, balanced by platelet production and platelet consumption. Individuals with increased platelet turnover have a population of larger immature reticulated circulating platelets released from the bone marrow that can be measured in the peripheral blood by automated flow cytometry (187).

Platelet turnover, although not a measure of platelet function, correlates with platelet aggregationand it has been shown that larger platelets are enzymatically and metabolically more active and have a higher thrombotic potential than smaller platelets (193-196).

Using automated flow cytometry, platelet turnover can be analysed using thiazole orange stain, which crosses the cell membrane and binds to intracellular RNA (ribonucleic acid). This technique can be used to derive the relative fraction or provide the absolute number of immature large platelets present in the sample. The absolute number of immature platelets has the strongest correlation with platelet aggregation(197).Several studies have shown that ACS patients have higher levels of immature reticulated platelets compared to healthy individuals (198, 199).

COX-2 catalyses the conversion of AA to prostaglandin (PG)H2 which leads to the synthesis of several platelet products, including TXA2, prostaglandin E and prostaglandin I2. COX-2 is present in less than 10% of normal platelets but is present in megakaryocytes and is expressed by young platelets and can therefore be detected in patients with high rates of platelet turnover (200). COX-2 is not inhibited by standard low-dose aspirin regimens, allowing significant COX-2-dependent TXA2 production.

## Conclusion

Chapter 1 reviewed the antiplatelet therapy commonly used in the management and treatment of CAD and the evidence to support its use in stable CAD and in ACS patients. The history of PCI has been outlined documenting the evolution of the stent technology and the changing requirement for DAPT with stent development. The PFTs available for the monitoring of antiplatelet therapy have been described and the evidence supporting their ability to predict future ischaemic events presented. All of these areas are of interest and relevant to the STEEL PCI study.

STEEL PCI is a study of two antiplatetet agents; clopidogel at the standard dose of 75mg od and ticagrelor at both the 90mg twice-daily dose and the 60mg twice-daily dose regimen. The primary objective of the STEEL PCI study was to assess the effects of a single loading regimen and two different maintenance regimens of ticagrelor on whole blood adenosine reuptake compared to a standard regimen of clopidogrel. As discussed in chapter 1, there are limited data on adenosine reuptake and plasma adenosine concentrations in patients taking antiplatelet therapy and no data in this particular cohort of patients undergoing elective PCI. In this study, platelet inhibition will be assessed by the VerifyNow and LTA platelet function tests; as discussed, these have been well validated in multiple studies. Although STEEL PCI is a relatively small study, we will be looking at the influence of genetic variability in *CYP2C19* on the relative antiplatelet efficacy of a standard regimen of clopidogrel in comparison with the two maintenance regimens of ticagrelor.

# CHAPTER 2 METHODS AND MATERIALS



## Introduction

STEEL PCI is a study of clopidogrel and two different doses of ticagrelor in patients with stable CAD undergoing elective PCI. Clopidogrel is routinely used for patients undergoing elective PCI in this setting whilst ticagrelor is used for those patients presenting with ACS. Whilst ST is unusual in elective, urgent and emergency PCI, it does however remain a complication of the procedure. Ticagrelor provides higher and more reliable levels of platelet inhibition compared to clopidogrel but this has only been assessed in the ACS setting. Clopidogrel has been extensively studied in this setting of stable CAD treated by PCI whilst ticagrelor at both the 60mg and 90mg twice-daily dose regimens had not, hence there is a lack of platelet function data in this particular cohort of patients. There are also limited data concerning the effect of ticagrelor 90 mg twice-daily on cellular adenosine uptake in different patient populations and no data on the impact of the ticagrelor 60mg twice-daily dose on this. The assessment of cellular adenosine uptake in ticagrelor-treated patients is important since it has been previously demonstrated that ticagrelor-treated patients have higher levels of circulating plasma adenosine, which may contribute to the pleiotropic effects of ticagrelor therapy. STEEL PCI is a clinical study and a real-world study so it was imperative that the study was conducted in patients undergoing elective PCI.

The secondary objectives of STEEL PCI include the assessment of peri-procedural high sensitivity-troponin (hs-TnT) release and peri-procedural MI. The mechanisms for peri-procedural MI are unclear and, furthermore, peri-procedural MI in this setting with ticagrelor treatment has not been assessed. Obvious causes of peri-procedural MI, such as side branch occlusion and distal embolization of atheroma, will be noted. However, it is unclear if microthromboembolic phenomena have a part to play in peri-procedural troponin release. STEEL PCI will assess if peri-procedural hs-TnT release is affected by the degree of platelet inhibition.

The study objectives and study design are described in detail in this chapter.

## STEEL PCI Study Objectives

### Primary Objective

The primary objective of the STEEL PCI study was to assess the effects of a single loading regimen and two different maintenance regimens of ticagrelor on whole blood adenosine reuptake compared to a standard regimen of clopidogrel.

### Secondary Objective

The secondary objectives of the study were:

1. Estimate the incidence of peri-procedural MI following elective PCI in patients pre-treated with either ticagrelor or clopidogrel
2. Assess the effects of two regimens of ticagrelor compared to clopidogrel on platelet aggregation in stable CAD patients managed with PCI
3. Determine the influence of genetic variability in *CYP2C19* on the relative antiplatelet efficacy of clopidogrel and two regimens of ticagrelor in stable CAD patients managed with PCI

### Safety Objective

The primary safety objective was to estimate the incidence of PLATO-defined major and minor bleeding at one month in patients treated with one of the two regimens of ticagrelor or clopidogrel. All adverse events were recorded including the incidence of dyspnoea at one month.

### Exploratory Objective

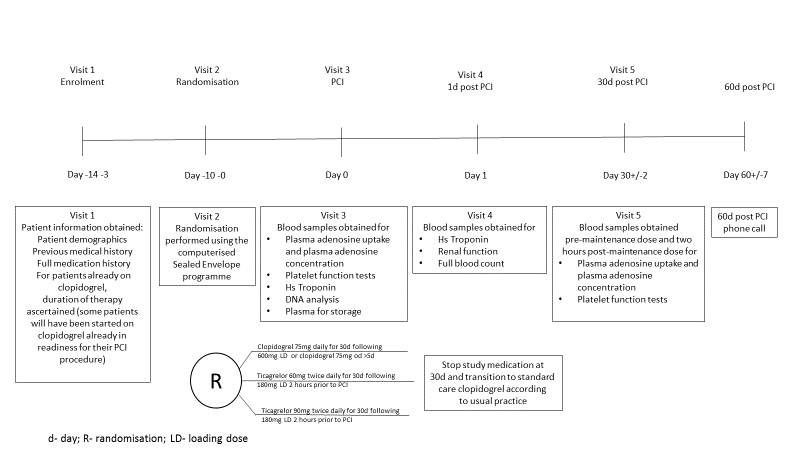
An exploratory objective of the study was to determine the relationship between adenosine reuptake inhibition and plasma levels of ticagrelor and its active metabolite AR-C124910XX.

## STEEL PCI Study Design

One hundred and eighty patients undergoing elective PCI for stable CAD were recruited to the study and provided written informed consent. All patients were concurrently prescribed aspirin and in many cases were commenced on aspirin monotherapy prior to or following their diagnostic coronary angiogram. Patients were randomised, using a web-based randomisation system (sealedenvelope.com), to one of three antiplatelet regimens in a 1:1:1 fashion in an open-label design. The study was designed to run for one month (30+/-2 days) to allow blood samples to be obtained for analysis of plasma adenosine levels and PFTs when medication levels are at steady state and when platelet inhibition is likely to be consistent. There was no justification to continue any longer than one month because of the very low risk of stent thrombosis after this timepoint and patients were then transitioned onto clopidogrel therapy for the duration of their DAPT.

1. Clopidogrel *either* 600mg loading dose at least four hours prior to the PCI procedure *or* 75mg daily for at least 5 days prior to the procedure, followed by clopidogrel 75mg daily for 1 month.
2. Ticagrelor 180mg loading dose two hours prior to the PCI procedure followed by 60mg twice daily for 1 month.
3. Ticagrelor 180mg loading dose two hours prior to the PCI procedure followed by 90mg twice daily for 1 month.

**Figure 2‑1**: Study Flow Chart



### Patient Selection

All 180 patients were recruited from the Department of Cardiology at the Northern General Hospital part of the Sheffield Teaching Hospitals Foundation Trust, Sheffield, South Yorkshire, UK. Recruitment of the 180 aspirin-treated patients started in July 2015 and was completed by January 2017. Using the cardiac catheter laboratory scheduling lists, patients that had been listed by an Interventional Cardiologist for a PCI procedure were identified. The patient’s notes were reviewed at the pre-assessment clinic and, if there were no obvious exclusion criteria, then the written information leaflet about the study was provided. Patients were mostly recruited from the cardiology pre-assessment clinic and occasionally recruited on the day of their PCI procedure. Only patients with stable CAD that had had a diagnostic coronary angiogram, either at the Northern General or at their local district general hospital, which demonstrated CAD suitable for treatment by PCI were approached. An electronic screening log of all patients approached about the study was kept.

## STEEL PCI Study Inclusion and Exclusion Criteria

### Inclusion Criteria

The inclusion criteria for patients suitable for recruitment into the study were as follows:

1. Provision of informed consent prior to any study specific procedures.
2. Male or female aged greater than 18 years.
3. Previous invasive coronary angiography with plan for PCI with coronary stent implantation for stable CAD.

### Exclusion Criteria

Patients were not recruited if any of the following exclusion criteria were fulfilled:

1. Requirement for a chronic total occlusion to be crossed in order for *any* stent implantation to proceed.
2. Plan for coronary angiography with a view to PCI if appropriate (i.e. current coronary anatomy not known)
3. Intention to use platelet function tests or genotyping to guide antiplatelet therapy.
4. Known allergy to or intolerance of aspirin, clopidogrel or ticagrelor.
5. Treatment with antiplatelet medication apart from aspirin or clopidogrel that cannot be stopped 10 days prior to PCI (e.g. ticagrelor, prasugrel, dipyridamole, ticlodipine, abciximab, tirofiban) for example because of continuing indication.
6. Planned treatment or consideration of treatment with oral antiplatelet medication other than aspirin or clopidogrel following PCI.
7. Planned use of a GP IIb/IIIa antagonist for the PCI procedure
8. Myocardial infarction within the past 12 months.
9. Current or planned use of an oral anticoagulant (e.g. warfarin, dabigatran, rivaroxaban, apixaban).
10. Previous history of intracranial haemorrhage or other intracranial pathology associated with increased bleeding risk.
11. Haemoglobin <100g/L or other evidence of active bleeding
12. Peptic ulceration documented by endoscopy within the last 3 months unless healing proven by repeat endoscopy.
13. History of acute or chronic liver disease (eg cirrhosis).
14. Treatment in the last 10 days or requirement for on-going treatment with a strong CYP3A4 inhibitor or inducer.
15. Requirement for ongoing treatment with simvastatin or lovastatin at a dose greater than 40mg per day.
16. Treatment with a CYP3A4 substrate with a narrow therapeutic index (e.g. cyclosporine, quinidine).
17. Requirement for ongoing treatment with a moderate or strong CYP2C19 inhibitor that is known or predicted to impair the response to clopidogrel (omeprazole, esomeprazole, fluconazole, fluvoxamine, fluoxetine, moclobemide, voriconazole, ciprofloxacin, cimetidine, carbamazepine, oxcarbamazepine or chloramphenicol).
18. End-stage renal failure requiring dialysis.
19. History of alcohol or drug abuse in the last year.
20. Co-morbidity associated with life expectancy less than one year.
21. Females of child-bearing potential unless negative pregnancy test at screening and willing to use effective contraception (i.e. established use of oral, injected or implanted hormonal methods of contraception *or* placement of an intrauterine device or intrauterine system *or* barrier methods of contraception with spermicide *or* sole male partner with prior vasectomy and confirmed absence of sperm in ejaculate for the duration of treatment with study medication.
22. Any other condition deemed by the investigator to place the patient at excessive risk of bleeding with ticagrelor.

At the time of enrollment and after informed consent was obtained, patient demographics were recorded. The height, weight, previous medical history and, in particular, history of hypertension, dyslipidaemia, DM, smoking, previous MI, PCI, CABG, cerebrovascular accident or peripheral vascular disease was documented. Care was taken to ensure there was no history of intracranial bleeding. The dose of aspirin was established and, in those patients already on clopidogrel, the duration of therapy was ascertained and whether or not a loading dose had been administered was recorded. A full history of other medication was also obtained.

## Blood Samples Obtained During The STEEL PCI Study

Blood samples for whole blood adenosine re-uptake, estimation of plasma adenosine concentration and PFTs were taken on the day of PCI prior to heparinisation (visit 3), at one month pre-dose of study medication and two hours post-dose of study medication (visit 5). A blood sample for DNA extraction was taken at the time of the PCI procedure. A blood sample for hs-TnT measurement was taken at the time of the PCI and at 18-24 hours post PCI (visits 3 and 4). All study patients had full blood count (FBC) and urea and electrolyte (U&E) samples taken as part of their standard care prior to the PCI procedure. These samples were routinely obtained at the pre-assessment clinic visit by a member of nursing or nursing auxiliary staff or by the patient’s General Practice surgery if a telephone-call pre-procedure assessment had been carried out. Full details of blood sample scheduling and blood tests obtained are shown in Table 2.1.

### Visit 3 Blood Samples

Blood samples were collected by members of the research team either prior to the PCI procedure or at the time of the procedure. Samples collected beforehand were obtained from a large calibre ante-cubital vein using a 21G butterfly needle and the use of a tourniquet was kept to a minimum. Venous blood samples were collected using the S-Monovette system for the pharmacodynamic measurements of adenosine uptake and APC and by syringe for the other study blood tests. The S-Monovette tubes contained a precise volume of stop solution. The blood collected by syringe was gently transferred to the other Vacutainer and VerifyNowbottles as outlined in the table below.

In patients without a suitable calibre ante-cubital vein or where blood collection time coincided with their procedure, blood samples were collected from the arterial sheath. Following insertion of the arterial sheath into either the radial, brachial or femoral artery, the operator obtained the blood sample before administration of heparin. Five millilitres of blood was aspirated from the sheath and discarded before aspiration of blood into a 2ml syringe (for pharmacodynamic measurements) and a further 35.5ml aspirated into another syringe. The 2ml blood sample was immediately transferred into the S-Monovette tube containing stop solution. The remaining blood was transferred to the Vacutainer and VerifyNow bottles as outlined before. All blood tubes were gently inverted five times and then immediately transported to the Cardiovascular Research Unit laboratory at the Northern General Hospital. Care was taken to ensure that the blood samples were taken avoiding unnecessary trauma and agitation of the samples, both at the time of collection and after collection.

### Visit 4 Blood Samples

Visit 4 blood samples were obtained 18-24 hours after the procedure. Venepuncture was performed by a member of the research team using the Vacutainer system. Samples were taken for the determination of hs-TnT , U&E and FBC. A citrated sample was also taken for plasma storage.

### Visit 5 Blood Samples

Study patients were advised to withhold their morning dose of study medication on the day of this visit. Blood samples were collected by a member of the research team and obtained from a large calibre ante-cubital vein using a 21G butterfly needle and again the use of a tourniquet was kept to a minimum. Venous blood samples were collected using the S-Monovettesystem for the pharmacodynamic measurements of adenosine uptake and adenosine plasma concentration and by syringe for the other study blood tests. Following the initial pre-dose blood test, patients took their last dose of study medication and repeat blood samples were obtained two hours later.

### Blood Test Analysis

The whole blood adenosine re-uptake and PFTs were all processed at the Cardiovascular Research Unit (CVRU), Northern General Hospital, Sheffield by members of the CVRU. The plasma adenosine concentration samples were centrifuged at the CVRU and then stored at -80°C prior to analysis by staff at the University of Sheffield Chemistry Department.

The FBC, U&E and hs-TnT tests were all analysed at the central hospital laboratory at the Northern General Hospital.

**Table 2‑1**: Volume of blood drawn from each patient according to measurement

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Assessment |  | Sample volume (ml) | No. of samples | Total volume (ml) |
|  |  |  |  |  |
| Safety bloods | U&Es | 5 | 4 | 20 |
|  | FBC | 4 | 4 | 16 |
| Pharmacodynamic | Adenosine uptake | 2 | 3 | 6 |
| measurement | & plasma |  |  |  |
|  | concentration |  |  |  |
|  | VerifyNow | 2 | 3 | 6 |
|  | LTA | 4 | 3 | 12 |
| Pharmacogenomic | | 8 | 1 | 8 |
| Plasma Storage |  | 8 | 5 | 40 |
| Pharmacokinetic |  | 2 | 4 | 8 |
| measurement |  |  |  |  |
| Total |  |  | 27 | 116 |

## Visit 4 The Day Of The Percutaneous Coronary Intervention Procedure

### The Percutaneous Coronary Intervention Procedure

All procedures were performed in the Cardiac Catheter Suite at the Northern General Hospital, Sheffield. All cases were elective PCI procedures and patients had all had diagnostic coronary angiography performed beforehand. Patients undergoing coronary angiography +/- coronary angioplasty or pressure wire assessment of coronary stenoses were excluded from the study. The PCI procedure information was collected retrospectively from the patients’ clinical records and from the InfoFlex(Chameleon Information Management Services Ltd) reporting system. The arterial access site, coronary artery or arteries treated, number of coronary stenoses treated and reference vessel diameter were recorded. The type of stent, diameter and total length of stent used during the procedure was also recorded. It was noted if the PCI involved a bifurcation procedure or coverage of a side branch greater than 2mm in diameter. Any adverse event that occurred during the PCI procedure was documented.

### Assay of Cellular Adenosine Uptake in Whole Blood

A sample of WB (6ml) was collected into a standard EDTA tube. The handling of the WB samples for cellular adenosine uptake was performed by Heather Judge (Post-doctoral Research Associate) and Jessica Hanson and Hannah Stokes (students from Sheffield Hallam University on placement as research technicians within the CVRU). Samples of EDTA WB were pipetted into tubes containing adenosine (1 µmol/L final concentration); cellular adenosine uptake by erythrocytes and potentially other blood cells was halted either by including cold stop solution in the tube (0 seconds, T0) or by the addition of cold stop solution at 15, 30 or 60 seconds (T15, T30 and T60). The stop solution consisted of 40μmol/L dipyridamole, 13.2mmol/L disodium ethylenediaminetetraacetic acid, 50μmol/L erythro-9-(2-hydroxy-3-nonyl)adenine, 200μmol/L αβ methylene adenosine-5’- diphosphate, 50μmol/L iodotubercidin, 40μmol/L p-nitrobenzylthioinosine and 0.9% w/v sodium chloride. The sample was centrifuged at 1500g for 10 minutes at 4°C then deproteinised by the addition of 60µl ice-cold 70% perchloric acid before undergoing a further centrifugation at 13000g for 5 minutes at 4°C. Residual plasma adenosine concentration was then analysed by high performance liquid chromatography (HPLC).

Analysis of cellular adenosine uptake and plasma adenosine concentration were the primary objectives of STEEL PCI. A previous study by Bonello *et al*, which was discussed in more detail elsewhere in this chapter, suggested ticagrelor led to higher circulating levels of plasma adenosine in ACS patients. STEEL PCI was therefore designed to assess plasma adenosine levels in patients undergoing elective PCI treated with ticagrelor at two different doses.

### Plasma Adenosine Levels

The circulating APC in venous whole blood was assessed using the method previously reported by Bonello et al (201). Blood samples were collected into Monovette tubes containing 0.9ml of stop solution (as described in section 2.6.2) allowing rapid mixing of the blood and stop solution to prevent adenosine uptake and degradation of adenosine. The volume of whole blood added was 2.1ml. These samples were handled by Heather Judge, Jessica Hanson and Hannah Stokes at the CVRU. The sample was centrifuged at 1500g for 10 minutes at 4°C then deproteinised by the addition of 60µl ice-cold 70% perchloric acid before undergoing a further centrifugation at 13000g for 5 minutes at 4°C. APC was then analysed by high performance liquid chromatography (HPLC) by staff at the University of Sheffield Chemistry Department.

### VerifyNow

The VerifyNow assay was performed by staff at the CVRU. VerifyNow cartridges were purchased from Accumetrics (SanDiego, USA) and stored at room temperature. The quality control procedure was performed prior to sample analysis on the days of visit 3 and 5. The VerifyNow blood sample was left at room temperature for 20 minutes before testing and all samples were analysed within one hour. The VerifyNow P2Y12 assay cartridge was removed from the foil packaging with care and the needle protective sheath removed. When prompted by the analyser, the cartridge was inserted. The 2ml Greiner Bio-One Vacuette citrate tube was inverted gently five times before being firmly placed onto the needle and the sample was automatically processed. The PRU value and percentage inhibition were recorded.

### Light Transmittance Aggregometry

LTA was used to assess ADP-induced platelet aggregation using the eight-channel platelet aggregation profiler (PAP 8, Biodata Corporation). LTA was performed by staff at the CVRU. WB samples collected in citrated tubes were centrifuged at 200RCF at room temperature for 10 minutes to produce PRP. An aliquot of the PRP was pipetted into a clean 10ml tube and centrifuged at 1500 RCF for a further 10 minutes. The resulting PPP sample was diluted with 10µL saline and added to two cuvettes and incubated for 1 minute at 37°C. Using ADP at a final concentration of 20 mol/L as an agonist, platelet aggregation was recorded for 6 minutes. All samples were analysed within 2 hours of collection.

The secondary objectives of the study were to assess the effects of two regimens of ticagrelor compared to clopidogrel on platelet aggregation. Both VerifyNow and LTA, as previously described, have been used in multiple studies to assess the effect of antiplatelet therapy and are well validated.

### High Sensitivity Troponin-T

Blood was collected for hs-TnT measurement before PCI was performed and 18-24 hours post-procedure. In a very small number of patients, the blood sample for hs-TnT was collected on the same day as the procedure due to logistical issues. In this case, the hs-TnT blood sample was collected at least six hours post-procedure. The samples were immediately transported to the Northern General hospital laboratory for analysis (Cobas, Modular Analytics E170).

### Pharmacokinetics

Venous blood samples were collected along with the other samples at visit 3 and 5. Blood was collected into Vacutainer lithium heparin tubes and placed on ice prior to centrifugation at 4°c and then stored at -80°c prior to analysis. Plasma concentrations of ticagrelor and its active metabolite AR-C124910XX were determined using liquid chromatography and tandem mass spectrometry by York Bioanalytical Solutions (Upper Poppleton, York, UK)

### Pharmacogenetics

Venous blood samples were collected as described. The WB sample was stored at -80°C prior to DNA extraction. DNA was analysed for *CYP2C19* LOF (\*2-\*8) and GOF (\*17) alleles, and for the relevant single-nucleotide polymorphisms in CY3A43, UGT2B7 and SLC01B1 that have been reported to influence plasma levels of ticagrelor and/or its active metabolite. These analyses were conducted by the University of Sheffield core facility for genotyping.

## STEEL PCI Statistical Analysis Plan

### Sample size and statistical analysis

The primary endpoint of the study was in vitro adenosine uptake post-maintenance dose at 1 month, measured as residual adenosine concentration at 15 seconds after ex-vivo addition of adenosine. The sample size was based on (1) our preliminary in-vitro studies of adenosine uptake indicating 15 seconds as the optimal time for assessing residual adenosine concentration and previous data indicating an estimated residual adenosine concentration at

15 seconds post-mixing in the adenosine uptake assay of 0.80 ± 0.051 μmol/L for the ticagrelor 90 mg group and 0.45 ± 0.068 μmol/L for clopidogrel (201) and (2) the assumption that the effects of ticagrelor 60 mg would yield levels between those with ticagrelor 90mg and clopidogrel: data on forty-two patients per group were required in each group to provide >90% power to detect a 0.05 μmol/L higher mean residual adenosine level in the ticagrelor 60mg group compared with the clopidogrel group, with a significance threshold of 0.05 and assuming a common SD of 0.06 μmol/L, and >99% power to detect a similar difference between the ticagrelor 90mg and clopidogrel groups to that previously reported. 60 patients were, therefore, required in each group to allow for 30% drop-out or sample failure at 1 month.

Secondary endpoints were the effects of two different regimens of ticagrelor compared to clopidogrel on plasma adenosine concentration, platelet function measurements and the PCI-induced troponin release (determined as increase from pre-PCI to post-PCI). Since both ticagrelor group patients received the same treatment of a 180-mg loading dose of ticagrelor at the time of PCI, additional analyses were performed by pooling the two ticagrelor dose groups for analysis of phamacodynamic endpoints at this timepoint. The proposed sample size provided >90% power to detect differences in platelet aggregation between each ticagrelor group and the clopidogrel group, with an alpha of 0.05. However, to allow for multiple group comparisons, statistical significance was attached to P values <0.01 for the secondary outcome measures.

Data were analysed using SAS version 9.3 (SAS Institute, Cary, North Carolina) and expressed as mean and SD for normally-distributed data or median and interquartile range for non-parametric data. Continuous data for the three treatment groups were compared using Kruskal-Wallis test. Categorical variables were compared using the Fisher exact test. High platelet reactivity was defined as VerifyNow PRU > 208 or LTA response > 59% (202). Myocardial infarction was defined according to the 3rd Universal Definition (203). Bleeding events were defined according to the PLATO study criteria (204).

The STEEL PCI study was supported by Statistician Kathleen Baster from the University of Sheffield Statistics Services Unit, who advised on the statistical analyses to be used and reviewed the final analyses.

# 2.8. Ethics and Trial Registration

STEEL PCI was granted research ethics approval on 23 December 2014 (reference number 14/YH/1274; IRAS project ID 167117) with approval of a substantial amendment on 25 March 2015. The study was carried out in accordance with the Declaration of Helsinki. STEEL PCI was registered with clinicaltrials.gov and the identifier is NCT02327624. Staff of the Clinical Research Office of Sheffield Teaching Hospitals monitored the study. A data monitoring committee periodically reviewed the conduct of the study and clinical outcomes. The data and safety monitoring board was led by Professor Robert Wilcox, University of Nottingham, and the board reviewed reports on AEs (adverse events) and SAEs (significant adverse events) throughout the study.

|  |  |
| --- | --- |
|  |  |

# 2.9 Conclusion

This chapter has detailed the design of STEEL PCI, explaining the background of the study and its primary and secondary objectives. This forms the background for this thesis. This is the first time that ticagrelor at two different maintenance doses has been used in the setting of stable CAD and it was expected that ticagrelor at both the standard 90mg twice-daily dose and the lower 60mg twice-daily dose would provide significantly higher levels of platelet inhibition compared to clopidogrel. The effect of ticagrelor on cellular adenosine uptake and APC in stable CAD has not been previously assessed and therefore it was uncertain whether findings of other groups would be reproduced. In addition, STEEL PCI is the first study to explore the effect of ticagrelor on the rate of peri-procedural MI at the time of elective PCI in this group of patients with stable symptoms.

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| --- |
|  |

# CHAPTER 3: CLINICAL CHARACTERISTICS AND OUTCOMES IN THE STEEL PCI STUDY



## Introduction

The following chapters present the results of the STEEL PCI study. Some of the results and data within this chapter and chapters 4, 5, 6 and 7 are presented in an original research article entitled ‘Study of Two Dose Regimens of Ticagrelor Compared With Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention for Stable Coronary Artery Disease’ (205). However, the results and discussion surrounding these results presented in the following chapters are more in-depth and comprehensive.

## Study population

One hundred and eighty patients were recruited to the study (Figure 3.1). Sixty patients in the clopidogrel group, 56 in the ticagrelor 60mg bd group and 58 in the ticagrelor 90mg bd group underwent an invasive procedure. Some patients did not proceed to PCI for several reasons including significant disease progression requiring surgical management or non-flow limiting coronary stenoses on updated angiography.

One hundred and fifty-five patients completed the study period of maintenance therapy with either clopidogrel 75mg od (n=53), ticagrelor 60mg bd (n=54) or ticagrelor 90mg bd (n=48). One patient in the ticagrelor 60mg bd group was subsequently found to have been taking an excluded medication (a strong CYP3A inducer) and was excluded from the main analysis.

The demographic characteristics, cardiovascular risk factors and concomitant medications were well matched between the groups at randomization and subsequent timepoints, as were the procedural characteristics for those proceeding with PCI (Table 3.1-3.3). At the time of their procedure, 100% patients were receiving aspirin 75 mg daily and continued on this for the duration of the study.

**Figure 3‑1**: Study CONSORT flow diagram. Number of patients in each of the three treatment groups (clopidogrel, ticagrelor 60mg bid and ticagrelor 90mg bid) at each stage of the study.



**Table 3‑1**: Baseline demographics and medications for patients at enrolment

|  |  |  |  |
| --- | --- | --- | --- |
|  | Clopidogrel | Ticagrelor 60mg | Ticagrelor 90mg |
|  | n=60 | n=60 | n=60 |
| Age, years, mean (SD) | 63.7 (11.9) | 67 (8.6) | 64.9 (8.3) |
| Male sex, n (%) | 47 (78.3%) | 50 (85.2%) | 50 (85.2%) |
| Body weight, kgs, median (interquartile range) | 85.5 (77-102) | 87.5 (73-96) | 85 (79-98) |
| Body mass index, mean (SD) | 30.3 (5.7) | 28.8 (3.7) | 30 (4.9) |
| Race, n (%) |  |  |  |
| White | 59 (98.3%) | 59 (98.3%) | 58 (96.7%) |
| Black | 1 (1.7%) | 0 (0%) | 1 (1.7%) |
| Asian | 0 (0%) | 1 (1.7%) | 1 (1.7%) |
| Cardiovascular risk factors, n (%) | |  |  |
| Current smoker | 7 (11.7%) | 3 (5%) | 7 (11.7%) |
| Hypertension | 42 (70%) | 37 (61.7%) | 41 (68.3%) |
| Dyslipidemia | 54 (90%) | 54 (90%) | 58 (96.6%) |
| Diabetes mellitus | 13 (21.7%) | 8 (13.3%) | 13 (21.7%) |
| Medical history, n (%) |  |  |  |
| Myocardial infarction | 9 (15%) | 7 (11.7%) | 4 (6.7%) |
| PCI | 6 (10%) | 7 (11.7%) | 8 (13.3%) |
| CABG | 3 (5%) | 5 (8.3%) | 3 (5%) |
| Cardiac failure | 5 (8.3%) | 2 (3.3%) | 2 (3.3%) |
| Transient ischaemic attack | 3 (5%) | 3 (5%) | 3 (5%) |
| Non-haemorrhagic stroke | 1 (1.7%) | 0 (0%) | 2 (3.3%) |
| Peripheral arterial disease | 7 (11.6%) | 5 (8.3%) | 3 (5%) |
| COPD | 6 (10%) | 6 (10%) | 3 (5%) |
| Concomitant medication, n (%) |  |  |  |
| Aspirin 75mg daily | 60 (100%) | 60 (100%) | 60 (100%) |
| Beta-blocker | 53 (88.3%) | 45 (75%) | 41 (68.3%)\* |
| ACE inhibitor | 16 (26.7%) | 19 (31.7%) | 18 (30%) |
| Statin | 54 (90%) | 54 (90%) | 52 (86.7%) |

\*All comparisons between the groups are not significant other than treatment with beta-blocker (p=0.03). SD: standard deviation.

**Table 3‑2**: Demographic, procedural characteristics and medications for patients proceeding with percutaneous coronary intervention

|  |  |  |  |
| --- | --- | --- | --- |
|  | Clopidogrel | Ticagrelor 60mg | Ticagrelor 90mg |
|  | n=57 | n=54 | n=51 |
| Age, years, mean (SD) | 64.6 (8.5) | 66.9 (8.6) | 66.0 (7.73) |
| Male sex, n (%) | 44 (77%) | 46 (85%) | 42 (82%) |
| Body weight, kgs, median (IQR) | 85.5 (77-102) | 88.0 (73-97) | 85.0 (80-98) |
| Body mass index, mean (SD) | 30.3 (5.7) | 28.8 (3.7) | 30.0 (4.6) |
| Race, n (%) |  |  |  |
| White | 56 (98%) | 53 (98%) | 49 (96%) |
| Black | 1 (2%) | 0 (0%) | 1 (2%) |
| Asian | 0 (0%) | 1 (2%) | 1 (12%) |
| Cardiovascular risk factors, n (%) | |  |  |
| Current smoker | 7 (12%) | 6 (11%) | 6 (12%) |
| Hypertension | 39 (68%) | 37 (69%) | 34 (67%) |
| Dyslipidemia | 51 (90%) | 47 (87%) | 49 (96%) |
| Diabetes mellitus | 12 (21%) | 11 (20%) | 12 (24%) |
| Medical history, n (%) |  |  |  |
| Myocardial infarction | 9 (16%) | 9 (17%) | 4 (8%) |
| PCI | 5 (9%) | 5 (9%) | 7 (14%) |
| Coronary artery bypass graft | 3 (5%) | 3 (6%) | 1 (2%) |
| Cardiac failure | 5 (9%) | 5 (9%) | 2 (4%) |
| Transient ischaemic attack | 3 (5.3%) | 3 (5.6%) | 2 (4%) |
| Non-haemorrhagic stroke | 1 (1.8%) | 1 (1.9%) | 2 (4%) |
| Peripheral arterial disease | 6 (11%) | 5 (9%) | 3 (6%) |
| COPD | 5 (9%) | 5 (9%) | 3 (6%) |
| Concomitant medication, n (%) |  |  |  |
| Aspirin 75mg daily | 57 (100%) | 54 (100%) | 51 (100%) |
| Beta-blocker | 50 (88%) | 40 (74%) | 33 (65%) |
| ACE inhibitor | 15 (26%) | 18 (33%) | 13 (26%) |
| Statin | 51 (90%) | 48 (89%) | 44 (86%) |
| CYP2C19 LOF carrier, n (%) | 18 (32%) | 20 (37%) | 12 (24%) |
| Procedural characteristics |  |  |  |
| Number of vessels treated, mean (SD) | 1.2 (0.5) | 1.2 (0.4) | 1.1 (0.6) |
| Number of lesions treated, mean (SD) | 1.5 (0.8) | 1.5 (0.7) | 1.4 (0.7) |
| Total stent length, mm, mean (SD) | 39 (27) | 39 (23) | 37 (24) |
| Minimum stent diameter, mm, mean (SD) | 3.0 (0.6) | 3.0 (0.5) | 3.0 (0.5) |
| Bifurcation treated, n (%) | 1 (2%) | 4 (7%) | 2 (4%) |
| Left main stem treated, n (%) | 1 (2%) | 3 (6%) | 2 (4%) |
| Arterial Access, n (%) |  |  |  |
| Radial | 45 (79%) | 41 (76%) | 38 (75%) |
| Femoral | 10 (18%) | 13 (24%) | 12 (24%) |
| Radial-to-femoral\* | 2 (4%) | 0 (0%) | 0 (0%) |
| Brachial | 0 (0%) | 0 (0%) | 1 (2%) |

SD: standard deviation. COPD: chronic obstructive pulmonary disease. ACE: angiotensin-converting enzyme. CYP2C19 LOF: loss-of-function allele carrier for cytochrome P450 2C19.

Groups were compared using Kruskal-Wallis or Chi-square tests, as appropriate: all P values > 0.1 except for beta-blockers (p = 0.02)

\*In a small number of patients it was not possible to complete the procedure via the radial approach and so the operator then changed to the femoral approach

**Table 3‑3**: Demographic and procedural characteristics and medications at 1 month

|  |  |  |  |
| --- | --- | --- | --- |
|  | Clopidogrel | Ticagrelor 60mg | Ticagrelor 90mg |
|  | n=53 | n=53 | n=48 |
| Age, years, mean (SD) | 65.0 (8.4) | 66.6(8.4) | 66(7.747) |
| Male sex, n (%) | 43 (81.1%) | 45 (84.9%) | 40 (83.3%) |
| Body weight, kgs, median (IQR) | 87.0 (77-102) | 68 (61-72) | 85 (79-98) |
| Body mass index, mean (SD) | 30.5 (5.8) | 28.9 (3.7) | 30.1 (4.7) |
| Race, n (%) |  |  |  |
| White | 52 (98.1%) | 52 (98.1%) | 46 (95.8%) |
| Black | 1 (1.9%) | 0 (0%) | 1 (2.1%) |
| Asian | 0 (0%) | 1 (1.9%) | 1 (2.1%) |
| Cardiovascular risk factors, n (%) | |  |  |
| Current smoker | 6 (11.3%) | 2 (3.8%) | 4 (8.3%) |
| Hypertension | 37 (69.8%) | 31 (58.5%) | 31 (64.6%) |
| Dyslipidemia | 37 (69.8%) | 47 (88.7%) | 46 (95.8%) |
| Diabetes mellitus | 11 (20.8%) | 7 (13.2%) | 11 (22.9%) |
| Medical history, n (%) |  |  |  |
| Myocardial infarction | 8 (15.1%) | 7 (13.2%) | 4 (8.3%) |
| PCI | 5 (9.4%) | 7 (13.2%) | 7 (14.6%) |
| CABG | 3 (5.7%) | 4 (7.5%) | 1 (1.9%) |
| Cardiac failure | 5 (9.4%) | 1 (1.9%) | 0 (0%) |
| Transient ischemic attack | 3 (5.7%) | 3 (5.7%) | 2 (3.8%) |
| Non-hemorrhagic stroke | 1 (1.9%) | 0 (0%) | 1 (1.9%) |
| Peripheral arterial disease | 5 (9.4%) | 3 (5.7%) | 3 (6.25%) |
| COPD | 4 (7.6%) | 1 (1.9%) | 2 (4.2%) |
| Concomitant medication, n (%) |  |  |  |
| Aspirin 75mg daily | 53 (100%) | 53 (100%) | 47 (97.9%) |
| Beta-blocker | 46 (86.8%) | 40 (75.5%) | 31 (64.6%) |
| ACE inhibitor | 15 (28.3%) | 18 (34.0%) | 11 (22.9%) |
| Statin | 47 (88.7%) | 47 (88.7%) | 41 (85.4%) |
| CYP2C19 LOF carrier, n (%) | 17 (32.1%) | 18 (34.0%) | 9 (18.8%) |
| Procedural characteristics |  |  |  |
| Number of vessels treated, mean (SD) | 1.2 (0.45) | 1.23 (0.42) | 1.25 (0.48) |
| Number of lesions treated, mean (SD) | 1.51 (0.78) | 1.53 (0.7) | 1.44 (0.68) |
| Total stent length, mm, mean (SD) | 37.5 (25.4) | 39.2 (23.4) | 36.7 (24.4) |
| Minimum stent diameter, mm, mean (SD) | 3.03 (0.53) | 2.98 (0.49) | 3.03 (0.48) |
| Bifurcation treated, n (%) | 1 (1.9%) | 4 (7.5%) | 2 (4.2%) |
| Left main stem treated, n (%) | 1 (1.9%) | 3 (5.7%) | 2 (4.2%) |
| Chronic total occlusion treated, n (%) |  |  |  |
| Arterial Access, n (%) |  |  |  |
| Radial | 41 (77.4%) | 40 (75.5%) | 35 (72.9%) |
| Femoral | 10 (18.9%) | 13 (24.5%) | 12 (25%) |
| Radial-to-femoral\* | 2 (3.8%) | 0 (0%) | 0 (0%) |
| Brachial | 0 (0%) | 0 (0%) | 0 (0%) |

SD: standard deviation. PCI: percutaneous coronary intervention. CABG: coronary artery bypass graft surgery. COPD: chronic obstructive pulmonary disease. ACE: angiotensin-converting enzyme. CYP2C19 LOF: loss-of-function allele carrier for cytochrome P450 2C19.

\*In a small number of patients it was not possible to complete the procedure via the radial approach and so the operator then changed to the femoral approach

## Percutaneous Coronary Intervention Procedures

One hundred and sixty-two study participants underwent a PCI procedure at study visit 3. In keeping with standard practice, the majority of procedures were performed via the radial artery. In three cases, the initial access route was via the radial artery but converted to a femoral approach during the procedure. A number of patients (n=11) did not have a coronary artery stent deployed for a number of reasons. In a number of patients (n=6), the coronary artery stenosis did not appear as significant on the updated images obtained using a coronary artery guide catheter. In these cases, the decision was made not to proceed to PCI or the operator performed fractional flow reserve assessment of the coronary lesion and demonstrated the lesion was not flow-limiting and did not require stenting. In three patients, the coronary artery disease had progressed significantly since the diagnostic angiogram had been performed and the operator deemed coronary artery by-pass grafting was a more appropriate method of revascularisation. In two study patients, it was not possible to cross the coronary lesion and therefore no coronary stent was deployed.

In total, 274 stents were deployed in 200 coronary arteries; the length, diameter and number of stents according to treatment group is shown in table 3.2. The choice of stent used was at the discretion of the operator, however 273 out of the 274 stents used in the STEEL PCI study patients were drug-eluting. The average diameter and length of stent was 2.9mm and 38.5mm, respectively. Seven (3.9%) of the PCI procedures utilised a dedicated bifurcation technique in the treatment of the coronary artery disease.

## Results Of Safety Blood Tests

### Creatinine

The mean serum creatinine levels for all treatment groups at all study visits and the mean change in serum creatinine from visit 3 (at the time of PCI) to each subsequent measurement are shown in Tables 3-4 and 3-5. The Mann-Whitney test was used to compare the absolute change in creatinine following a standard loading dose of either clopidogrel or ticagrelor and no statistical difference was seen between the groups (p=0.328). No statistical difference was seen in creatinine between the day of the PCI procedure and the following day, or between the day of PCI and at one month.

**Table 3-4**: Serum Creatinine at Each Study Visit

|  |  |  |  |
| --- | --- | --- | --- |
| Creatinine (mmol/l)  Mean **(**SD) | Clopidogrel | Ticagrelor  60mg | Ticagrelor  90mg |
| Baseline | 90.2 (23.3) | 88.6 (23.7) | 83.3 (15.5) |
| Visit 3 | 84.5 (22.6) | 81.9 (24.8) | 75.8 (12.2) |
| Visit 4 | 89.3 (21.3) | 89 (26.9) | 85.6 (14.3) |
| Visit 5 | 89.5 (22.5) | 86.3 (20.3) | 82.3 (14.6) |

**Table 3-5**: Mean Change in Serum Creatinine From the Day of PCI to Each Subsequent Study Visit

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Creatinine (mmol/l) | Clopidogrel | Ticagrelor | Ticagrelor | p |
| Mean **(**SD) | 60mg | 90mg |  |
| Visit 3-4 | 9.3 (7.95) | 5.39 (11.2) | 9.30 (7.9) | 0.11 |
| Visit 3-5 | 5.51 (7.14) | 4.12 (13.2) | 5.87 (7.3) | 0.98 |

### Haemoglobin

### 

The mean haemoglobin levels for all treatment groups at all study visits and the mean change in haemoglobin from visit 3 at the time of PCI to each subsequent measurement are shown in Tables 3-6 and 3-7. There was no statistical difference when absolute change in haemoglobin following a standard loading dose of clopidogrel or ticagrelor was compared (p=0.325). No difference in haemoglobin was seen between the study visit at the time of PCI and at one month for each treatment group (p=0.97, 0.73, 0.69 for clopidogrel, ticagrelor 60mg and ticagrelor 90mg respectively).

**Table 3‑6**: Haemoglobin at Each Study Visit

|  |  |  |  |
| --- | --- | --- | --- |
| Haemoglobin (g/L) | Clopidogrel | Ticagrelor | Ticagrelor |
| Mean (SD) |  | 60mg | 90mg |
| Baseline | 140.5 (14.4) | 140.4 (13.6) | 142.7 (14.1) |
| Visit 3 | 138.5 (13.8) | 136.9 (14.4) | 139.6 (13.3) |
| Visit 4 | 138.4 (13.4) | 139.1 (14.6) | 138.1 (14.2) |
| Visit 5 | 138.2 (14.3) | 136.6 (16.6) | 137.6 (12.8) |

**Table 3-7**: Mean Change in Haemoglobin From the Day of PCI to Each Subsequent Study Visit

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Haemoglobin (g/L) | Clopidogrel | Ticagrelor | Ticagrelor | p |
| Mean (SD) |  | 60mg | 90mg |  |
|  |  |  |  |  |
| Visit 3-4 | 0.02 (6.4) | 0.83 (6.4) | 0.42 (6.7) | 0.5982 |
| Visit 3-5 | 0.09 (7.6) | 0.31 (7.3) | 0.02 (6.0) | 0.8939 |

### Platelets

The mean platelet count for all treatment groups at all study visits and the mean change in platelet count from visit 3 at the time of PCI to each subsequent measurement are shown in Tables 3-8 and 3-9. No difference in platelet count was seen between study visit 3 and at one month for each treatment group (p=0.87, 0.51, 0.48 for clopidogrel, ticagrelor 60mg and ticagrelor 90mg respectively).

**Table 3-8**: Platelet Count at Each Study Visit

|  |  |  |  |
| --- | --- | --- | --- |
| Platelet Count (x109/L) | Clopidogrel | Ticagrelor | Ticagrelor |
| Mean (SD) |  | 60mg | 90mg |
| Baseline | 253.7 (56.3) | 245.6 (67.3) | 240.9 (62.9) |
| Visit 3 | 246.3 (29.9) | 223.9 (48.3) | 230.3 (57.5) |
| Visit 4 | 244.3 (55.9) | 228.6 (52.5) | 246 (64.1) |
| Visit 5 | 243.1 (50.8) | 244.1 (59.3) | 241.4 (64.5) |

**Table 3-9**: Mean Change in Platelet Count From the Day of PCI and Subsequent Study Visits

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Platelet Count (x109/L) | Clopidogrel | Ticagrelor | Ticagrelor | p |
| Mean (SD) |  | 60mg | 90mg |  |
| V3-V4 | -4 (24.1) | 6.9 (25.6) | 7.4 (19.1) | 0.0178 |
| V3-V5 | -5.9 (31.3) | 15.3 (40.6) | 6 (29) | 0.7168 |

### High Sensitivity Troponin T

The results of hs-TnT on the day of PCI and one day post-PCI are shown in Table 3-10. The mean change in hs-TnT from visit 3 (prior to PCI) to visit 4 (one-day post-PCI) was 50.5 ng/l for the clopidogrel group and 66.1 ng/l for the combined ticagrelor groups. The mean change was 83.2 ng/l for the ticagrelor 60mg group and 47.8ng/l for the ticagrelor 90mg group. The median (IQR) change in hs-TnT the morning after PCI were 16.9 (6.5 – 46.9) ng/l for the clopidogrel group, 22.4ng/l (5.5-53.8) ng/L for the ticagrelor 60mg group and 17.7 (8.1-46.9) for the ticagrelor 90mg (p=0.95, Kruskal-Wallis test). Therefore treatment with a standard loading dose of ticagrelor pre-procedure did not have any impact on troponin release or the rates of peri-procedural myocardial necrosis compared with a standard loading regimen of clopidogrel.

The proportion of patients in the clopidogrel group and combined ticagrelor groups with hs-TnT values more than the upper reference limit of 14 ng/l post-procedure were compared using Fisher’s exact test. There was no significant difference between the clopidogrel and ticagrelor groups (74.1% vs. 80%, p=0.843). The number of patients having a more than 20-fold rise in hs-TnT from baseline at visit 3 to post-procedure at visit 4, either symptomatic or asymptomatic, was determined for each of the three treatment groups. Eight (15.1%) patients in the clopidogrel group and 14 (15.4%) patients in the combined ticagrelor groups had more than a 20-fold rise in hs-TnT (p>0.999). Two (3.7%) clopidogrel-treated patients and 6 (6.6%) ticagrelor-treated patients had hs-TnT measurements >20 times the upper reference limit (p=0.71).

Reassuringly, there was no significant difference between the treatment groups in the mean change in hs-TnT measured before and after the PCI procedure. STEEL PCI assessed the incidence of type 4a peri-procedural MI following elective PCI in patients treated by either a standard loading regimen of clopidogrel or ticagrelor 180mg loading dose. There were almost equal numbers of patients in the combined ticagrelor groups with more than 20-fold rise in hs-TnT compared to the clopidogrel group. There were also numerically higher numbers of patients with hs-TnT measurements >20 times the upper reference limit in the ticagrelor groups. This implies that hs-TnT release is not related to the degree of platelet inhibition, which will be discussed further in chapter 4.

**Table 3-10**: High sensitivity-Troponin T on the Day of PCI and One Day Post-PCI

|  |  |  |  |
| --- | --- | --- | --- |
| Troponin (ng/l) | Visit 3 | Visit 4 | Change |
|  |  |  |  |
| Clopidogrel |  |  |  |
| Mean (SD) | 8.9 (7.3) | 59.43 (73.6) | 50.5 (72.2) |
| Median (IQR) | 7.0 (4.6 - 10.9) | 26.2 (13.2 - 67.0) | 16.9 (6.5 - 53.9) |
|  |  |  |  |
| Ticagrelor |  |  |  |
| Mean (SD) | 8.8 (10.4) | 81.4 (139.9) | 66.1 (126.5) |
| Median (IQR) | 5.9 (4.2 - 9.2) | 30.2 (14.4 – 60.5) | 22.3 (6.2 - 50.6) |

## Adverse Events and Serious Adverse Events Reported in the STEEL PCI Study

The tolerability of the ticagrelor 60mg bd dose appeared slightly better than the 90mg bd dose due to less frequently reported episodes of dyspnoea in the ticagrelor 60mg group (7.1% vs 19.0%, P=0.09). Two patients (3.6%) in the ticagrelor 60mg group and 3 patients (5.2%) in the ticagrelor 90mg group stopped study medication prematurely due to adverse effects. No patients reported shortness of breath in the clopidogrel group and no patients stopped clopidogrel prematurely due to adverse effects. The proportion of patients reporting dyspnoea as a side effect is consistent with other studies of ticagrelor.

A number of other adverse events and serious adverse events occurred during the one month study period (Table 3.11). Reported adverse events included bruising, epistaxis, gastro-oesophageal reflux, nausea, diarrhoea, gout and shingles. Two patients (3.7%) in the ticagrelor 60mg group and 3 patients (6.3%) in the ticagrelor 90mg group stopped study medication prematurely due to adverse side effects. One patient (1.9%) randomised to clopidogrel treatment had their medication stopped by the treating physician after oral anticoagulation was started for treatment of a pulmonary embolus.

There were no incidences of peri-procedural or spontaneous myocardial infarctions according to the third universal definition of myocardial infarction (203). There was no report of stroke. There were no PLATO-defined major or minor bleeding events and there was no stent thrombosis event in any of the treatment groups.

**Table 3-11**: Adverse events

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Clopidogrel  N= 60 | Ticagrelor 60mg  N = 56 | Ticagrelor 90mg  N = 58 |
| Serious Adverse Events | | N (%) | N (%) | N (%) |
| Procedural |  |  |  |  |
| Arterial access site bleeding | | 2 (3.3) | 2 (3.6) | 0 (0) |
| Arterial access site haematoma | | 0 (0) | 1 (1.8) | 2 (4) |
| Pericardial effusion | | 0 (0) | 1 (1.8) | 0 (0) |
| Radial artery dissection | | 0 (0) | 0 (0) | 1 (1.7) |
| Non-procedural |  |  |  |  |
| Chest pain |  | 2 (3.3) | 2 (3.6) | 0 (0) |
| Palpitations |  | 0 (0) | 0 (0) | 1 (1.7) |
| Vasovagal syncope | | 0 (0) | 1 (1.8) | 1 (1.7) |
| Systemic thromboembolism | | 0 (0) | 0 (0) | 1 (1.7) |
| Venous thromboembolism | | 1 (1.7) | 0 (0) | 0 (0) |
|  |  |  |  |  |
| Adverse Events | | N (%) | N (%) | N (%) |
| Procedural |  |  |  |  |
| Coronary artery haematoma | | 0 (0) | 1 (1.8) | 0 (0) |
| Non-procedural |  |  |  |  |
| Hypertension | | 0 (0) | 0 (0) | 1 (1.7) |
| Palpitations |  | 0 (0) | 1 (1.8) | 0 (0) |
| Oedema | | 0 (0) | 1 (1.8) | 0 (0) |
| Pre-syncope or syncope |  | 0 (0) | 2 (3.6) | 1 (2) |
| Dyspnoea |  | 0 (0) | 4 (7.1) | 11 (19.0)\*\* |
| Anaemia |  | 0 (0) | 1 (1.8) | 0 (0) |
| Bruising |  | 2 (3.3) | 1 (1.8) | 1 (1.7) |
| Epistaxis |  | 0 (0) | 0 (0) | 1 (1.7) |
| Fatigue |  | 0 (0) | 2 (3.6) | 0 (0) |
| Gastrointestinal symptoms | | 3 (5.0) | 3 (5.4) | 2 (3.4) |
| Gout |  | 1 (1.7) | 1 (1.8) | 0 (0) |
| Haematospermia/haematuria | | 0 (0) | 0 (0) | 2 (3.4) |
| Non-cardiac chest pain | | 2 (3.3) | 3 (5.4) | 6 (10.3) |
| Rash |  | 0 (0) | 1 (1.8) | 1 (1.7) |
| Shingles |  | 1 (1.7) | 0 (0) | 1 (1.7) |

\*\* P < 0.001 vs clopidogrel. All other P = non-significant.

## Compliance with study medication

At the end of the study at Visit 5, study participants’ compliance with medication was calculated. Patients returned any study medication they had left over and it was therefore possible to identify if any doses had been missed. The level of compliance in the clopidogrel group was 100%, 98.2% in the ticagrelor 60mg twice daily group and 97.8% in the ticagrelor 90mg twice daily group.

## Discussion

One hundred and fifty-four patients (85.6%) completed one month of study medication post-PCI. A numerically smaller group of patients in the ticagrelor 90mg group completed the study compared to the other two groups. This was due to a combination of factors including withdrawal of consent prior to the day of PCI, procedural failure, non-significant coronary artery disease, progression of coronary artery disease and one case of non-cardiac-related death.

Overall, however, the three treatment groups were well matched in terms of their demographics, patient characteristics and concomitant medications although significantly more patients in the clopidogrel group were treated with oral beta-blockers. The PCI procedures were predominantly performed via the radial artery and the large majority of stents deployed were DES in keeping with current guidelines.

The results of safety blood tests (serum creatinine and full blood count) were reassuring. The mean increase in serum creatinine levels for the three treatment groups from the day of PCI to the following day was statistically non-significant. In this study, no significant rise in serum creatinine was seen following one month of anti-platelet therapy, either standard treatment with clopidogrel or the two different doses of ticagrelor. In contrast, PLATO demonstrated a significant rise in creatinine from baseline to one month in the ticagrelor-treated patients compared to the clopidogrel group. No difference in haemoglobin was seen for the three treatment groups at any time point and there was no PLATO-defined minor or major bleeding event. The platelet count was not statistically different at any study visit regardless of treatment regimen. These results provide some preliminary reassurance about the safety of using ticagrelor for PCI in stable CAD patients but a much larger study would be required that was adequately powered for bleeding events in order to better assess its safety compared with clopidogrel.

The incidence of peri-procedural MI in patients with stable CAD undergoing elective PCI has not been previously studied. In STEEL PCI, the mean increase in hs-TnT after PCI was not significantly different following a standard clopidogrel loading regimen or a standard loading dose of 180mg ticagrelor. There was no peri-procedural MI event according to the third universal definition of myocardial infarction. There were substantial numbers of patients with asymptomatic rises in troponin after PCI but no evidence that ticagrelor was more effective than clopidogrel in attenuating troponin release, suggesting that the extent of myocardial injury induced by PCI is not usually sensitive to levels of platelet P2Y12 inhibition in a low-risk population. One potential explanation for this is that the myocardial injury is often not mediated by platelet-rich thrombus, for example occurring as a result of embolization of atherosclerotic plaque material. These findings suggest that asymptomatic increases in hs-TnT do not represent a suitable surrogate indicator of thrombotic events in patients undergoing PCI for stable CAD.

# CHAPTER 4: RESULTS OF THE WHOLE-BLOOD ADENOSINE UPTAKE ASSAY AND ADENOSINE PLASMA CONCENTRATION



## Introduction

The effect of two different regimens of ticagrelor on adenosine uptake and plasma concentration following a standard loading dose and after one month of maintenance therapy has not been assessed in the setting of stable CAD treated by elective PCI. Additionally, the effect of the lower dose of ticagrelor (60 mg twice daily) on adenosine uptake and plasma concentration has not been previously studied in any population. STEEL PCI is, therefore, the first study of both available maintenance doses of ticagrelor on adenosine whole-blood uptake in this elective PCI setting. Based on a previous study of patients with ACS treated with ticagrelor 90mg bd, both the 60mg and 90mg ticagrelor doses used in the study were expected to inhibit adenosine uptake in whole blood and provide significantly higher APC compared to patients receiving clopidogrel. APC measurement at the time of PCI following a standard loading dose of ticagrelor and after one month of maintenance therapy is important since the PLATO study raised the hypothesis that some of the clinical benefit seen with ticagrelor therapy may be due to pleiotropic effects unrelated to high and consistent levels of platelet inhibition. More specifically, it has been hypothesised that ticagrelor’s interaction with adenosine metabolism may be responsible for some of the significant clinical benefit seen in ACS patients treated with ticagrelor, as part of DAPT, compared with clopidogrel.

## Whole-Blood Adenosine Uptake Measurement

The primary objective of the STEEL PCI study was to assess the effect of ticagrelor on whole-blood adenosine reuptake by comparing the rate of uptake in ticagrelor-treated patients with that in clopidogrel-treated patients since clopidogrel is not expected and has not been demonstrated to affect adenosine metabolism. The blood samples for assessment of whole-blood adenosine uptake were collected and analysed as described in Chapter 2. Notably, adenosine uptake was halted at 0, 15, 30 and 60 seconds by the addition of a pharmacological stop solution and the residual plasma adenosine concentration was measured by HPLC in order to reflect the rate of cellular uptake of adenosine.

Following a standard loading regimen of clopidogrel or a 180-mg loading dose of ticagrelor, results were available for 160 study patients on the day of the PCI procedure. After one month of maintenance therapy with either clopidogrel 75mg od, ticagrelor 60mg bd or ticagrelor 90mg bd, results were available for 137 patients pre-dose and 139 patients post-dose at one month. Several patients had already taken study medication on the day of the one-month study visit so only the post-dose samples were obtained.

## Results of the Whole-Blood Adenosine Uptake Assay At The Time of PCI

Following either a standard loading regimen of clopidogrel or a 180-mg loading dose of ticagrelor on the day of PCI, the results of the whole-blood adenosine uptake assay demonstrate no significant difference in residual plasma adenosine concentration at any time point between the treatment groups (Figure 4-1). The residual plasma adenosine concentration was lower at each subsequent time point after addition of adenosine and the greatest reduction in residual adenosine occurred between 0 and 15 seconds, as expected since circulating adenosine is rapidly taken up by erythrocytes and other cells in vivo. Since patients in the two ticagrelor groups received the same loading dose of ticagrelor prior to blood sampling at the time of PCI, pooled analysis of the ticagrelor group data was also performed and again showed no significant difference between clopidogrel- and ticagrelor-treated patients (Table 4-1) The results demonstrate no effect of a standard loading dose of ticagrelor on whole-blood adenosine uptake.

**Figure 4‑1**: Whole blood in vitro adenosine uptake

Residual adenosine levels at 0-60 seconds after mixing adenosine 1 μmol/L with blood samples obtained following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor according to assigned treatment group. **Data are** mean ± SD. Kruskal-Wallis test used to calculate P value.



**Table 4‑1**: Mean Residual Plasma Adenosine Concentration According to Time after In Vitro Addition of Adenosine to Whole Blood Samples Collected At The Time of PCI

|  |  |  |  |
| --- | --- | --- | --- |
| Time (s) | Clopidogrel  n = 54 | Ticagrelor  n = 104 | P value |
|  | mol/L | mol/L |  |
| 0 | 1.037 ± 0.25 | 1.051 ± 0.212 | 0.3496 |
| 15 | 0.274 ± 0.133 | 0.283 ± 0.127 | 0.2842 |
| 30 | 0.151 ± 0.119 | 0.146 ± 0.1 | 0.8961 |
| 60 | 0.077 ± 0.075 | 0.067 ± 0.059 | 0.7958 |

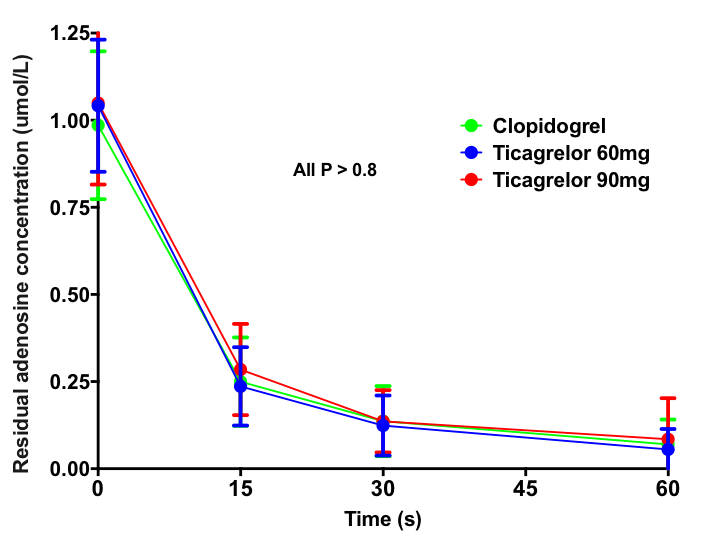
Data are mean ± SD

## Results of the Whole-Blood Adenosine Uptake Assay after One Month of Maintenance Therapy

Whole-blood adenosine uptake was assessed at one month both pre- and post-maintenance dose. No significant difference in adenosine uptake was seen at one month between the three different treatment regimens, either pre- or post-maintenance dose of study medication (Figure 4-2 and 4-3). Again, adenosine was rapidly taken up from the plasma in whole blood with the most significant decrease in residual plasma adenosine concentration occurring between the 0 and 15 second time points. These results indicate no significant effect of maintenance therapy with either ticagrelor 90mg twice-daily or ticagrelor 60mg twice-daily on adenosine uptake in whole blood.

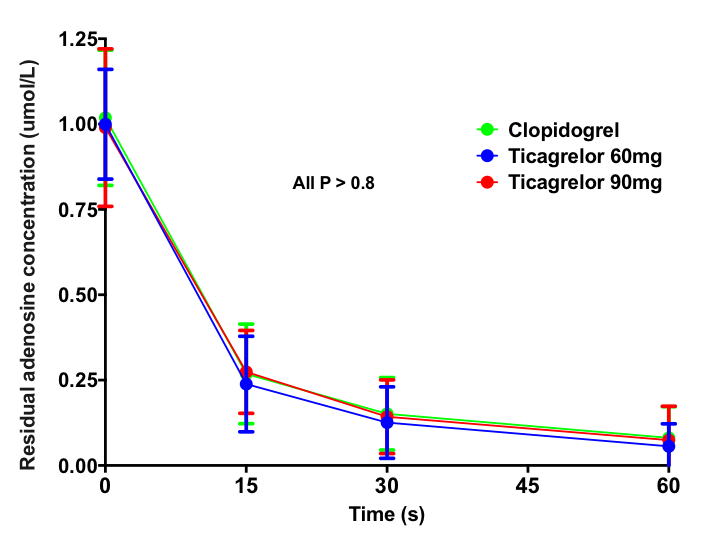
**Figure 4‑2**: Whole blood in vitro adenosine uptake

Residual adenosine levels at 0-60 seconds after mixing adenosine 1 μmol/L with blood samples obtained after one month of treatment pre-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bid). **Data are** mean ± SD.



**Figure 4‑3**: Whole blood in vitro adenosine uptake

Residual adenosine levels at 0-60 seconds after mixing adenosine 1 μmol/L with blood samples obtained at one month of treatment post-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bd). **Data are** mean ± SD.



## Adenosine Plasma Concentration Measurement

## 

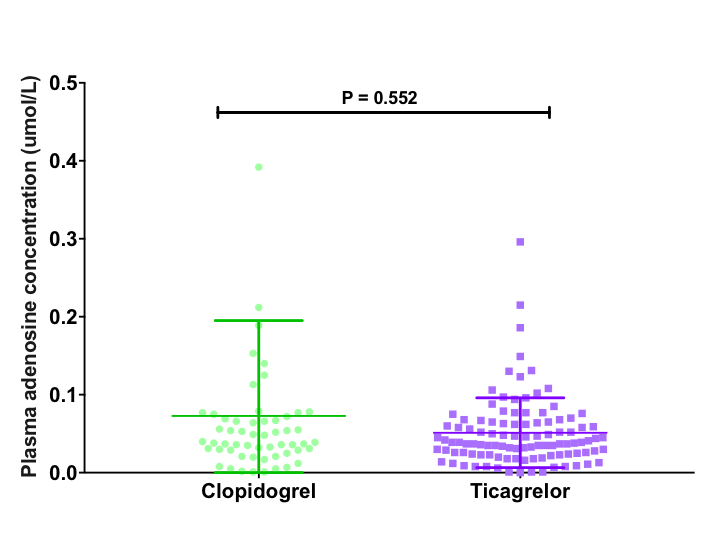
Blood samples for APC analysis were collected and analysed by HPLC as described in Chapter 2. Following either a standard loading regimen of clopidogrel or a 180-mg loading dose of ticagrelor, results of APC were available for 160 study patients on the day of the PCI procedure. After one month of maintenance therapy with either clopidogrel 75mg od, ticagrelor 60mg bd or ticagrelor 90mg bd, APC results were available for 136 patients pre-dose and 139 patients post-dose.

## Results of Adenosine Plasma Concentration Measurement

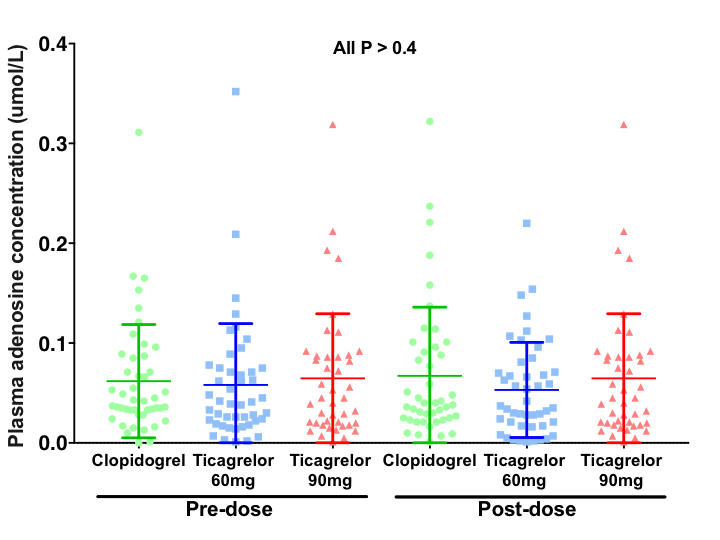
Following a standard loading dose of clopidogrel or ticagrelor on the day of PCI, the APC results were not significantly different between the treatment groups (p= 0.7655). The results of individual APC measurements according to treatment group are illustrated in Figure 4-4. No difference in the mean (SD) APC in either group was seen; mean (SD) in the clopidogrel group 0.053 ± 0.044 mol/L and 0.051 ± 0.044 mol/L in the combined ticagrelor group, median (IQ range) 0.039 (0.028-0.068) mol/L and 0.039 (0.024–0.065) mol/L respectively.

Similarly no difference in APC between the three treatment groups, clopidogrel, ticagrelor 60mg bd and ticagrelor 90mg bd, was seen after maintenance therapy at one month, either pre-dose or post-dose. At one month, prior to maintenance dose the mean (SD) APC was 0.062 ± 0.057 mol/L in the clopidogrel group, 0.057 ± 0.061 mol/L in the ticagrelor 60mg and 0.065 ± 0.065mol/L in the ticagrelor 90mg groups. The median (IQ range) APC was 0.042 (0.031-0.086) mol/L, 0.039 (0.019-0.075) mol/L and 0.045 (0.02-0.087) mol/L in the clopidogrel, ticagrelor 60mg and ticagrelor 90mg groups, respectively (Figure 4-5).

**Figure 4‑4**: Plasma adenosine levels following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor. **Data are** mean ± SD.



**Figure 4‑5**: Plasma adenosine levels after one month of treatment, pre-maintenance dose and post-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bd). **Data are** mean ± SD.



## Discussion

STEEL PCI is the first study of its kind to investigate ticagrelor at 60mg and 90mg versus clopidogrel in this elective setting in patients with stable CAD undergoing PCI. It is also the first study to assess the effects of the three different anti-platelet regimens on adenosine plasma concentration following standard loading regimens and after one month of maintenance therapy. The study succeeded in its primary objective to assess the effect of ticagrelor on whole blood adenosine reuptake. Following a standard loading regimen of either clopidogrel or ticagrelor, results of the whole-blood adenosine uptake assay were available for 160 study patients on the day of the PCI procedure and 137 patients pre-dose and 139 patients post-dose at one month. A similar number of patients had APC determined as a reflection of circulating plasma adenosine levels. After a standard loading regimen of clopidogrel or ticagrelor, STEEL PCI conclusively demonstrated no significant difference in either whole blood adenosine uptake or APC between the treatment groups at the time of PCI. These results, following standard loading regimens prior to PCI, are not in keeping with those of a previous study by Bonello et al (201). In their study of ACS patients, residual adenosine concentrations after in vitro addition of adenosine to whole blood as well as APC in ticagrelor-treated patients were significantly higher than in those treated with clopidogrel.

One hundred and eighty patients were recruited to STEEL PCI whereas, in contrast, Bonello at al studied 60 patients randomised to ticagrelor or clopidogrel. The results of whole-blood adenosine uptake and APC in STEEL PCI following clopidogrel or ticagrelor loading are consistent within the two treatment groups implying that a loading dose of ticagrelor does not have an effect on adenosine metabolism since clopidogrel is not known to, or expected to, influence adenosine metabolism. These results were substantiated by the observations at one month either pre-dose or post-dose when, again, no differences were seen between the three treatment groups (clopidogrel, ticagrelor 60mg bd and ticagrelor 90mg bd).

The greatest reduction in residual adenosine concentration occurred between 0 and 15 seconds, as expected since circulating adenosine is rapidly taken up by erythrocytes and other cells in whole blood. Reassuringly the residual adenosine concentration in the whole-blood uptake assay at 0 seconds was consistently around 1μmol/L, reflecting accurate measurement of the added concentration of adenosine in addition to the much lower concentrations found in circulating blood. This also suggests that the stop solution was highly effective in preventing metabolism of the added adenosine. The expected profiles of adenosine uptake, with maximum uptake between 0 and 15 seconds, also indicate success of the assay in accurately reflecting cellular adenosine uptake. The almost overlapping curves of adenosine uptake in the clopidogrel and ticagrelor groups provides confidence in lack of relevant effect of ticagrelor and differs markedly from the profiles demonstrated by Bonello et al.

The reasons for these apparently negative results are unclear. The data clearly show that adenosine uptake over 1 minute in whole blood samples was assessed accurately as the expected baseline levels of adenosine after in vitro addition of 1 μmol/L indicated the efficacy of the stop solution in preventing further adenosine uptake. The results also demonstrated almost complete adenosine uptake at 1 minute, which indicates efficacy of the stop solution in preventing further adenosine generation. One possible hypothesis for the difference in results seen in the two studies may be due to the different composition of pharmacological stop solution utilized, with Bonello et al potentially using less effective stop solution although the details are unclear in their paper. The stop solution used in the assay in STEEL PCI for adenosine metabolism included additional inhibitors than those used by Bonello *et al*. The stop solution also contained p-nitrobenzylthioinosine as an additional inhibitor of adenosine uptake and iodotubercidin as a potent adenosine kinase inhibitor and therefore may have been more effective. A recent study was also in agreement with our findings: a healthy volunteer study found no impact of ticagrelor on plasma adenosine levels (206).

Another study using the same methodology also demonstrated no impact of ticagrelor on plasma adenosine concentration in patients with acute coronary syndrome who were awaiting coronary artery bypass graft surgery (207). This

suggests that the results of the plasma adenosine levels are not due to the particular cohort of patients studied in STEEL PCI.

Further work would be required to explore whether these differences in stop solution may provide adequate explanation for the different study findings.

The results of the whole-blood adenosine uptake assay and adenosine plasma concentration measurement at all three time points (post loading regimen and pre- and post-maintenance dose at one month) have consistently shown no difference in effect of standard loading and maintenance regimens of clopidogrel or ticagrelor on adenosine metabolism. Given that ticagrelor is known to inhibit ENT-1, these results indicate that the therapeutic concentrations of ticagrelor and its active metabolite are insufficient to provide the level of ENT-1 blockade required to impact on either the ex vivo measurement of adenosine uptake or circulating plasma levels of adenosine. Whether more subtle effects of ticagrelor on adenosine metabolism could be detected by other assays remains to be explored.

# CHAPTER 5: RESULTS OF THE PLATELET FUNCTION TESTS



## Introduction

The VerifyNow and LTA platelet function tests were employed in the STEEL PCI study to assess platelet inhibition at each of the study visits. These are two reliable platelet function methods that have been used previously in large studies to assess the effects of anti-platelet therapy. The point-of-care VerifyNow P2Y12 assay was performed as outlined previously and the results were presented as percentage inhibition and P2Y12 Reaction Units (PRU).

Defined thresholds of PRU values have been used to discriminate between responders and non-responders to antiplatelet medications. In STEEL PCI, a threshold of PRU <208 was utilized as this has previously been shown in GRAVITAS (Standard-vs-high dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the GRAVITAS randomised trial) to be a predictor of death, MI and stroke. These outcomes were confirmed in the 3T/2R study one year after elective PCI (165).

The ADAPT-DES study (Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents: a prospective multicenter registry study) (n=8665), also validated a PRU cutoff of 208 with endpoints of definite or probable ST, all-cause mortality, MI and clinically relevant bleeding (166).

Light transmittance aggregometry was performed as previously described using adenosine diphosphate 20μM as the agonist and percentage maximal and final aggregation were recorded. A cut-off of 59% platelet aggregation response was used in STEEL PCI. In a number of studies, LTA has demonstrated the relationship between HTPR, defined as platelet aggregation response >59%, and the risk of future atherothrombotic events in patients treated with antiplatelet drugs (159),(208).

In STEEL PCI, the PFTs chosen to assess platelet inhibition were the VerifyNow system and LTA. The VerifyNow is a well-utilised point-of-care test for assessing platelet function. There is no sample processing involved and the test itself is quick and easy to perform with very little staff training required. There are three separate cartridges available to assess response to aspirin, the P2Y12 inhibitors and GPIIb/IIIa inhibitors, respectively. However the major disadvantage of the VerifyNow system is the high cost of the single-use cartridges. LTA is historically the gold standard in terms of platelet function testing. The ability to use various exogenous agonists means that different platelet aggregation pathways can be investigated and this is a major advantage, although in STEEL PCI we used ADP as the agonist as we were primarily interested in the differential effects of clopidogrel and ticagrelor on P2Y12-mediated platelet aggregation. However, the LTA system does require considerable sample handling and processing and so there is a requirement for staff training, meaning that the LTA analysis is more time-consuming and requires greater expertise. Furthermore, LTA is susceptible to changes in plasma turbidity that may arise as a result of dietary fat intake. Overall, VerifyNow seems to be a more reliable means of assessing P2Y12 inhibition than LTA but is more costly in terms of consumable costs.

## Results of the VerifyNow P2Y12 Assay at the Time of PCI

Following a standard loading regimen of clopidogrel or ticagrelor, patients treated with ticagrelor achieved greater levels of platelet inhibition assessed by the VerifyNow P2Y12 assay. Percentage inhibition results were available for 58 patients randomised to clopidogrel and 111 patients randomised to ticagrelor treatment. Mean (SD) percentage inhibition was 27.2±25.2 in the clopidogrel group and 84.3±.21.3 and 88.3±11.4 in the ticagrelor 60mg and ticagrelor 90mg groups (Figure 5-1). VerifyNow PRU results were available for 60 patients randomised to clopidogrel and 120 patients randomised to ticagrelor groups. Mean (SD) PRU following a standard loading dose was 176.1±69 in the clopidogrel group and 37.1±47.9 and 27.4±24.9 in the ticagrelor 60mg and ticagrelor 90mg groups respectively (Figure 5-2). Since both ticagrelor groups received the same loading dose prior to PCI, we also assessed pooled data for the two ticagrelor groups: Mean (SD) percentage inhibition following a standard loading dose was 27.2±25.2 in the clopidogrel group and 85±19.5 in the combined ticagrelor group (p=0.0026). Mean (SD) PRU following a standard loading dose was 176.1±69 in the clopidogrel group and 33.5±41.7 in the combined ticagrelor group. The figures for the clopidogrel and pooled ticagrelor groups are included in the appendix (Figures 1 and 2).

Patients randomised to a standard loading dose of clopidogrel ideally received this at least four hours prior to PCI. Some patients had PCI before four hours had elapsed. However the levels of P2Y12 platelet inhibition did not appear to be significantly different between those patients that had PCI less than or greater than four hours following a standard loading dose. Reassuringly both groups contained a similar number of patients with very low levels of P2Y12 platelet inhibition (Figures 5.3 and 5.4).

**Figure 5‑1**: Individual VerifyNow P2Y12 assay results expressed as VerifyNow percentage inhibition following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor.

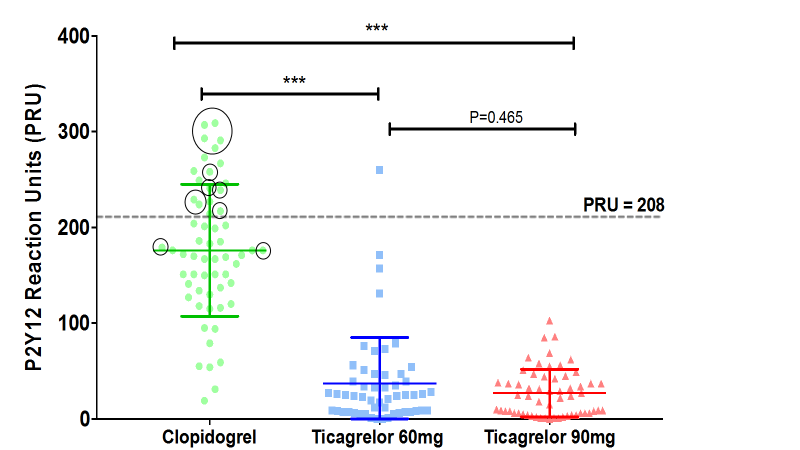


**Figure 5‑2**: Individual VerifyNow P2Y12 assay results expressed as P2Y12 reaction units following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor.



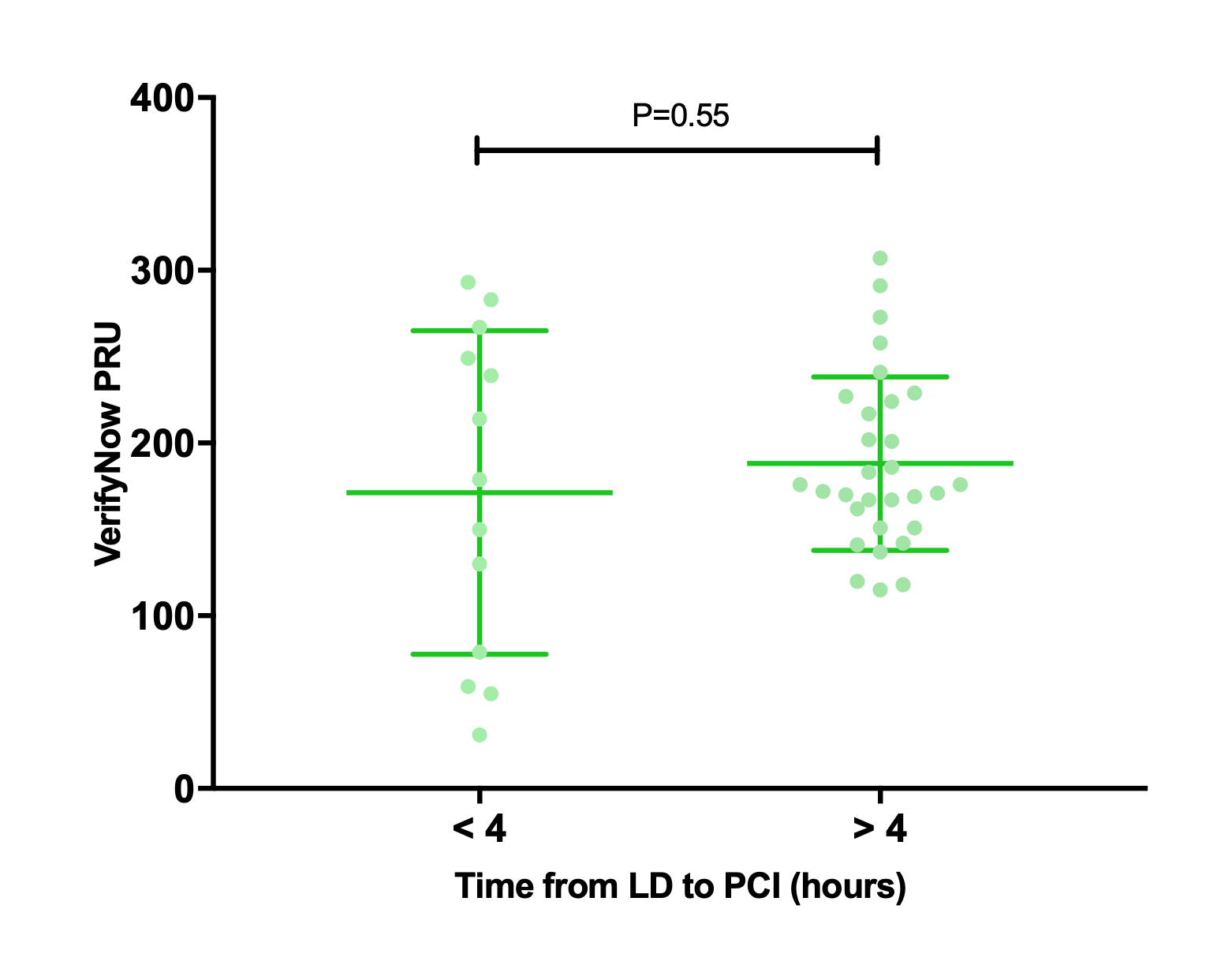
The **dashed line**indicates a level of 208 PRU as a previously-established threshold for high platelet reactivity. **Solid lines with error bars**indicate mean ± SD. \* P < 0.01; \*\*\* P < 0.0001.

**Figure 5‑3**: Individual VerifyNow P2Y12 assay results expressed as P2Y12 reaction units following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor identifying patients with VerifyNow zero percentage inhibition



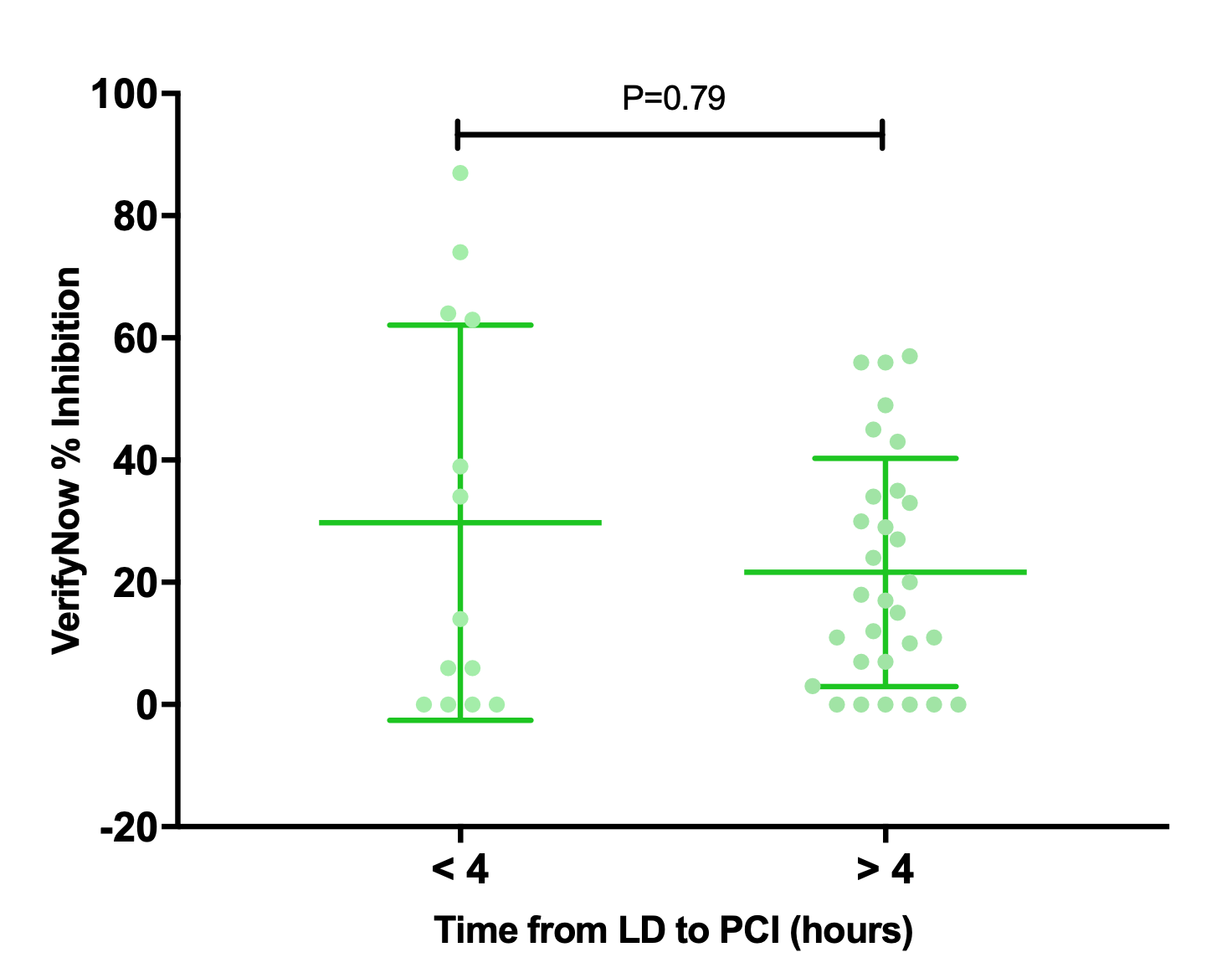
The **dashed line**indicates a level of 208 PRU as a previously-established threshold for high platelet reactivity. **Solid lines with error bars**indicate mean ± SD. \* P < 0.01; \*\*\* P < 0.0001. **Black circles** indicate those patients with VerifyNow zero percentage inhibition.

**Figure 5‑4**: Individual VerifyNow P2Y12 assay results expressed as P2Y12 reaction units following a standard loading regimen of clopidogrel



**Solid lines with error bars**indicate mean ± SD.

**Figure 5‑5**: Individual VerifyNow P2Y12 assay results expressed as VerifyNow percentage inhibition following a standard loading regimen of clopidogrel



**Solid lines with error bars**indicate mean ± SD.

Thirteen patients in the clopidogrel group had 0% platelet inhibition after a standard loading regimen of clopidogrel whereas no patients in either ticagrelor group had 0% inhibition following a standard 180-mg loading dose. Of the thirteen patients in the clopidogrel group with 0% inhibition at visit 3, details of the time of loading dose and start of the PCI procedure are available for 12 patients. Eight patients (66.7%) had their loading dose of clopidogrel more than 4 hours prior to the start of the procedure or had more than 5 days of pre-treatment. In four patients, the study bloods were taken less than four hours post loading dose, three of these patients however had 0% inhibition at visit 5 both pre and post-maintenance dose. This implies that timing of the loading dose was not the main factor in the lack of response to clopidogrel treatment. VerifyNow P2Y12 percent inhibition results are available for 12 of these patients at one month (Table 5-1). Five of these clopidogrel-treated patients with 0% platelet inhibition after a standard loading dose had 0% platelet inhibition pre and post maintenance dose after one month of clopiodgrel therapy. Three patients had very low levels (<10%) of platelet inhibition pre-dose with no increase after a maintenance dose.

A small number of ticagrelor-treated patients (n=5) had lower levels of platelet inhibition compared to the whole ticagrelor group at visit 3. All of these patients received a standard loading dose of ticagrelor 180mg two or more hours prior to their PCI procedure. Results at one month are available for four patients and one withdrew consent following PCI so data is not available for this patient. The four patients were all randomised to the ticagrelor 60mg group, the results of the VerifyNow percent inhibition are shown in Table 5-2. All patients with lower levels of platelet inhibition at the time of PCI achieved high levels of platelet inhibition during maintenance therapy with ticagrelor 60mg.

High platelet reactivity, as assessed by the VerifyNow P2Y12 assay, was seen infrequently in the ticagrelor group (n=1) at the time of PCI (Table 5-4). This patient also had high platelet reactivity when assessed by LTA.

This patient with high platelet reactivity at the time of PCI, with a PRU value of 260, had PRU 19 and PRU 31 pre- and post-dose, respectively, on ticagrelor 60mg at one month. In comparison, 18 patients (25%) in the clopidogrel group had high platelet reactivity at the time of PCI when this was assessed using VerifyNow PRU. PRU results at one month are available for 16 of these patients and 10 patients had persistently-high platelet reactivity defined by a PRU>208 post maintenance dose.

**Table 5‑1**: VerifyNow Percentage Inhibition at One Month for Clopidogrel Treated Patients with 0% Inhibition at time of PCI

|  |  |  |
| --- | --- | --- |
| Enrolment Number | Pre-dose | Post-dose |
| 4 | 16 | - |
| 13 | 0 | 0 |
| 17 | 2 | 0 |
| 23 | 0 | 0 |
| 30 | 0 | 0 |
| 58 | 34 | 32 |
| 76 | 0 | 8 |
| 105 | 0 | 0 |
| 126 | 8 | 0 |
| 160 | 6 | 5 |
| 176 | 22 | 14 |
| 181 | 0 | 0 |

**Table 5‑2**: VerifyNow Percentage Inhibition at One Month for Ticagrelor Treated Patients with the Lowest Levels of Inhibition at Time of PCI

|  |  |  |  |
| --- | --- | --- | --- |
| Enrolment Number | Day of PCI | Pre-dose | Post-dose |
| 86 | 11 | 94 | 87 |
| 109 | 13 | 95 | 96 |
| 158 | 39 | 75 | 72 |
| 169 | 7 | 69 | 79 |

## Results of the VerifyNow P2Y12 Assay At One Month

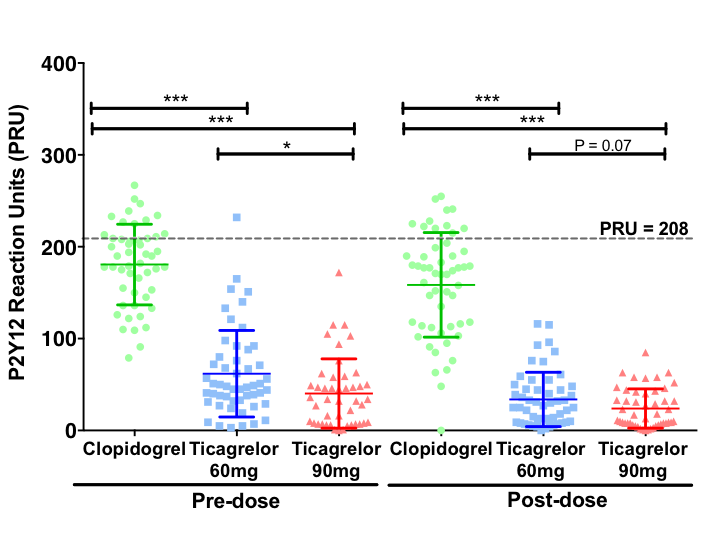
VerifyNow results were available for 146 study patients pre-dose and 152 patients post-dose at the one month study visit (Table 5-6). Both the ticagrelor 60mg and ticagrelor 90mg maintenance doses achieved greater platelet inhibition than clopidogrel at one month (Figures 5-6 and 5-7). The mean (SD) pre-dose percentage inhibition values were 21±17, 72±22and 82±17 for the clopidogrel, ticagrelor 60mg and ticagrelor 90mg groups, respectively. The post-dose percentage inhibition was 32.2±22.3 for the clopidogrel group, 84.5±15.8 and 90±9 for the ticagrelor 60mg and 90mg groups.

The mean (SD) pre-dose PRU values were 181 ± 44, 62 ± 47 and 40 ± 38 for the clopidogrel, ticagrelor 60mg and ticagrelor 90mg groups and post-dose PRU values were 159 ± 57, 34 ± 30 and 24 ± 21, respectively. No patients in the ticagrelor 90mg bd group had high platelet reactivity (PRU>208) at one month compared to one patient in the ticagrelor 60mg bd group (Table 5.4). This patient had PRU value of 232 at one month pre-dose and 39 post-dose with PRU of 1 at the time of PCI; their drug compliance at one month was calculated at 100%. High platelet reactivity was more common in the clopidogrel group at all the timepoints compared to both ticagrelor groups (Table 5-4).

**Table 5‑3**: Number of Study Patients with VerifyNow Results at One Month

|  |  |  |
| --- | --- | --- |
|  | Pre-dose | Post-dose |
| Clopidogrel | 50 | 52 |
| Ticagrelor 60mg | 51 | 52 |
| Ticagrelor 90mg | 45 | 48 |

**Figure 5‑6**: Individual VerifyNow P2Y12 assay results expressed as P2Y12 reaction units (PRU) after one month of treatment, pre-maintenance dose and post-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bd).



The **dashed lines**indicate a level of 208 PRU as a threshold for high platelet reactivity. **Solid lines with error bars**indicate mean ± SD. \* P < 0.01; \*\*\* P < 0.0001.

**Figure 5‑7**: Individual VerifyNow percentage inhibition after one month of treatment, pre-maintenance dose and post-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bd).



**Solid lines with error bars**indicate mean ± SD. \* P < 0.01; \*\*\* P < 0.0001.

**Table 5‑4**: Proportions of patients with high platelet reactivity according to predefined threshold values

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Clopidogrel | | Ticagrelor 60mg | | Ticagrelor 90mg | |
| Threshold of high platelet reactivity | n | N (%) | n | N (%) | n | N (%) |
| VerifyNow PRU >208 |  |  |  |  |  |  |
| Post-loading dose | 59 | 18 (31) | 53 | 1 (2) | 57 | 0 (0) |
| 1 month, pre-dose | 50 | 14 (28) | 51 | 1 (2) | 45 | 0 (0) |
| 1 month, post-dose | 52 | 11 (21) | 52 | 0 (0) | 48 | 0 (0) |
|  |  |  |  |  |  |  |
| LTA 20μM ADP >59% |  |  |  |  |  |  |
| Post-loading dose | 59 | 18 (31) | 54 | 4 (7) | 56 | 1 (2) |
| 1 month, pre-dose | 50 | 30 (60) | 51 | 6 (12) | 45 | 2 (4) |
| 1 month, post-dose | 53 | 22 (42) | 52 | 2 (4) | 48 | 1 (2) |

LTA: light transmittance aggregometry. n = number of patients with available data in each treatment group, N = number of patients with values above the given threshold value. % = (N/n) x100.

## Results of Light Transmittance Aggregometry

LTA maximum platelet aggregation results were available for 59 patients in the clopidogrel group and 109 patients in the combined ticagrelor group at the time of PCI. Mean (SD) percent aggregation was 52.4±15 in the clopidogrel group and 33.7±13.8 and 31.5±14.4 in the ticagrelor 60mg and ticagrelor 90mg groups (Figure 5-8). Overall the mean LTA responses were lower in the ticagrelor group compared to the clopidogrel group at the time of PCI. The mean (SD) percent aggregation for the pooled ticagrelor data was 52.4±15 in the clopidogrel group and 32.3±14.3 in the combined ticagrelor group (p <0.0001). The figure for the clopidogrel and pooled ticagrelor groups are included in the appendix (Figure 3).

Five patients (4.6%) in the ticagrelor group had high platelet reactivity when assessed by LTA with platelet aggregation >59% compared to 18 patients (31%) in the clopidogrel group (p=0.0004). The five patients randomised to ticagrelor treatment all had a standard loading dose greater than 2 hours before their PCI procedure. Results at one month were available for four of these patients, all had <59% platelet aggregation at one month.

The mean (SD) percentage aggregation results at one-month pre maintenance dose were 60.1±13.3, 42.8±12.8 and 36.8±12 for the clopidogrel, ticagrelor 60mg and ticagrelor 90mg groups respectively. Post-dose percent aggregation was 54.8±13, 38.2±12.5 and 33.9±10.5 for the three treatment groups (Figure 5-9). High platelet reactivity with maximal platelet aggregation >59% was seen infrequently in the ticagrelor 60mg group at one month (n=6) and in even smaller numbers of patients randomised to the ticagrelor 90mg regimen (n=2).

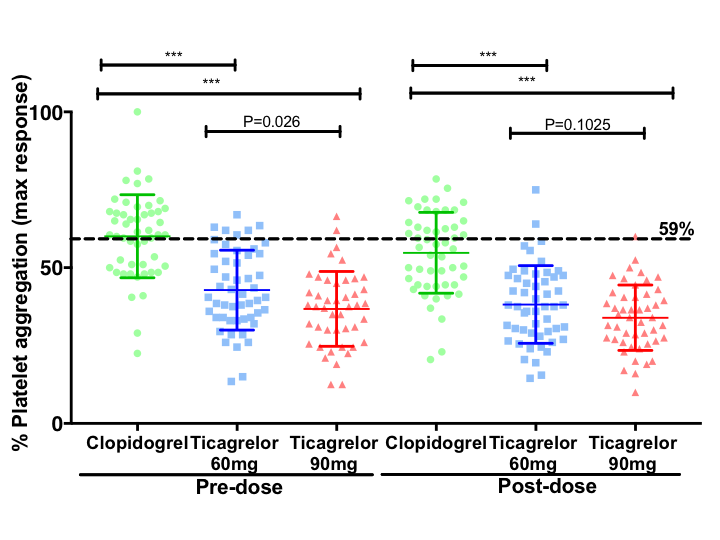
The LTA and VerifyNow results demonstrate that a standard loading dose of ticagrelor followed by maintenance therapy at either 60mg bd or 90mg bd provides significantly greater levels of platelet inhibition in the elective PCI setting.

**Figure 5‑8**: Individual results for the platelet aggregation measured by light transmittance aggregometry in response to ADP 20 μmol/L following a standard loading regimen of clopidogrel or 180mg loading dose of ticagrelor.



The dashed lines indicate a level of 59% as a threshold value for high platelet reactivity. Solid lines with error bars indicate mean ± SD. \*\*\* P < 0.0001.

**Figure 5‑9**: Individual results for the platelet aggregation measured by light transmittance aggregometry in response to ADP 20 μmol/L after one month, pre-maintenance dose and post-maintenance dose for each of the three treatment groups (clopidogrel 75mg od, ticagrelor 60mg bd and ticagrelor 90mg bd).



The dashed lines indicate a level of 59% as a threshold value for high platelet reactivity. Solid lines with error bars indicate mean ± SD. \*\*\* P < 0.0001.

## Discussion

In the STEEL PCI study, following a standard loading regimen of either clopidogrel or ticagrelor, ticagrelor-treated patients had significantly lower levels of platelet reactivity when this was assessed by the VerifyNow P2Y12 assay and measured as either percent inhibition or PRU. The results of mean percentage aggregation measured by LTA also demonstrated lower levels of platelet reactivity following a ticagrelor-loading dose. The results of this study are in keeping with those of other studies of antiplatelet therapy comparing clopidogrel and ticagrelor (43) (44), (45).

Thirteen clopidogrel-treated patients (22%) had 0% inhibition measured by the VerifyNow P2Y12 assay at the time of PCI, which is similar to the proportion seen in other studies and highlights the issue of clopidogrel non-responsiveness. Sixty-seven percent of these patients had low levels of platelet inhibition at one month. Eighteen patients in the clopidogrel group had PRU>208 at the time of PCI compared to only one ticagrelor-treated patient, illustrating the much greater reliability of ticagrelor. Ten clopidogrel-treated patients continued to have PRU>208 at one month post maintenance dose. This level of PRU was chosen as a cut-off after it was identified in the elective PCI 3T/2R study as a predictor of death, MI and stroke with a PRU < 208 associated with a significantly lower risk of reaching the primary endpoint at 60 days (209). This level of PRU was also used in the ADAPT-DES study which used endpoints of definite or probable ST, all-cause mortality, MI and clinically relevant bleeding.In this study high platelet reactivity was an independent predictor of ST or MI in the first 12 months but not a predictor of death (166). In agreement with the VerifyNow results, there were a statistically significant number of clopidogrel-treated patients with high platelet reactivity demonstrated by LTA platelet aggregation >59% following a standard loading dose. Eighteen clopidogrel-treated patients (31%) had platelet aggregation >59% at the time of PCI and 16 (89%) pre-dose and 15 (83%) post dose at one month had platelet aggregation >59%.

The results of the platelet function tests in STEEL PCI are in keeping with those from the PLATO Platelet sub-study. In the PLATO sub-study, following a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor, the results of the VerifyNow and LTA assays demonstrated that clopidogrel achieved lower levels of inhibition with marked inter-individual variability compared to ticagrelor. As demonstrated in STEEL PCI, patients enrolled in the PLATO sub-study had lower VerifyNow P2Y12 PRU measurements in the ticagrelor 90mg bd group pre and post maintenance therapy compared to the clopidogrel group. Similarly, the LTA results showed higher levels of platelet aggregation in the clopidogrel group. The PEGASUS platelet sub-study also studied ticagrelor at both the 90mg and 60mg doses. The VerifyNow results obtained from STEEL PCI are broadly concordant with those reported in the PEGASUS platelet sub-study, higher mean levels of platelet inhibition was seen in both ticagrelor groups, but a greater SD was seen with the ticagrelor 60mg regimen. In the PEGASUS sub-study the mean pre-dose PRU values were 59 ± 63 and 47 ± 43 for the 60 mg and 90 mg ticagrelor doses respectively and the post-dose values were 29 ± 39 and 20 ± 19 respectively. Similar mean LTA responses were also seen in the two studies.

The results of the VerifyNow P2Y12 assay and LTA are not always in agreement. Patients with high platelet reactivity defined by LTA platelet aggregation >59% may not have VerifyNow PRU>208 and vice versa. This is not uncommon and previous studies have also documented this discordance between the two different platelet function tests. Although there is a relationship between high platelet reactivity and clinical outcome, there is no consensus as to which platelet function test is the most appropriate or reliable method to ascertain platelet reactivity, highlighting the importance of employing two different platelet function tests in the study.

The STEEL PCI platelet function test results have demonstrated that both regimens of ticagrelor achieve greater platelet inhibition than clopidogrel both after a standard loading dose and after one month of maintenance therapy in patients undergoing elective PCI for stable CAD.

# Chapter 6: PHARMACOKINETICS OF TWO REGIMENS OF TICAGRELOR IN PATIENTS UNDERGOING ELECTIVE PCI



## Introduction

Clopidogrel is a thienopyridine pro-drug that requires hepatic metabolism to produce an active metabolite, which binds irreversibly to the P2Y12 receptor and in turn blocks the binding of ADP to this receptor (210). The interindividual variability in efficiency of clopidogrel active metabolite generation has been extensively reported in the literature and so no further work was performed in STEEL PCI to characterise this. Ticagrelor is not a pro-drug but does have an active metabolite, AR-C124910XX, that is equipotent to ticagrelor and contributes approximately 30% of the total inhibitory effect (202, 210, 211). In the PEGASUS TIMI-54 platelet function substudy, pharmacokinetics and pharmacodynamics of ticagrelor maintenance therapy with either 90 mg or 60 mg twice daily were described in stable patients with prior myocardial infarction but the pharmacokinetics of a ticagrelor loading dose and either regimen of ticagrelor have not been previously reported either in patients undergoing PCI or in patients with stable CAD without the requirement for prior MI within 1 to 3 years.Additionally, the pharmacokinetics of the 60mg twice-daily maintenance dose of ticagrelor have only been studied to a limited extent in patients with prior MI. In STEEL PCI, we therefore assessed pharmacokinetics of the two studied regimens of ticagrelor in stable CAD patients undergoing elective PCI in order to provide more detailed information in a distinct population to those assessed previously with either dose regimen of ticagrelor.

## Results

Following a 180-mg loading dose of ticagrelor, the mean (SD) plasma levels of ticagrelor were 1087 ± 686 ng/mL and 1129 ± 382 ng/mL, respectively, for the ticagrelor 60mg and ticagrelor 90mg groups; corresponding mean (SD) levels of AR-C124910XX were 225 ± 131 ng/mL and 220 ± 113 ng/mL, respectively (Figure 6-1). Since the two ticagrelor groups received the same regimen of ticagrelor prior to PCI, the two groups were also considered together: pooled data for plasma ticagrelor and AR-C124910XX levels following a loading dose were 1109 ± 549 ng/mL and 223 ± 121 ng/mL, respectively (Figure 4 in appendix). After 1-month maintenance therapy with either ticagrelor 60mg bd or ticagrelor 90mg bd, pre-dose mean (SD) levels of ticagrelor were 278 ± 217 ng/mL and 365 ± 189 ng/mL, respectively. Pre-dose mean (SD) levels of AR-C124910XX were 97 ± 55 ng/mL and 127 ± 73 ng/mL, respectively. After 1-month of maintenance therapy with ticagrelor, the results show that pre-dose levels of ticagrelor were significantly different between the 60mg and 90mg groups whereas pre-dose levels of its active metabolite were not significantly different between the groups. The post-dose mean (SD) levels of ticagrelor were 510 ± 281 ng/mL and 776 ± 347 ng/mL and mean (SD) levels of AR-C124910XX were 135 ± 69 ng/mL and 199 ± 96 ng/mL, respectively (Figure 6-2). The results at 1-month following maintenance ticagrelor therapy demonstrate that the levels of ticagrelor and its active metabolite were significantly different with higher levels achieved in the ticagrelor 90mg group.

**Figure 6‑1**: Ticagrelor and Active Metabolite AR-C124910XX Plasma Concentration

Individual results for plasma concentrations of ticagrelor and active metabolite AR-C124910XX following a standard loading dose of ticagrelor. Solid lines with error bars indicate mean ± SD. Mann-Whitney test used to calculate P value.



**Figure 6‑2**: Ticagrelor and Active Metabolite AR-C124910XX Plasma Concentration

Individual results for plasma concentrations of ticagrelor and active metabolite AR-C124910XX after one month, pre-maintenance dose and post-maintenance dose with either ticagrelor 60 mg or ticagrelor 90 mg twice daily. Solid lines with error bars indicate mean ± SD. Mann-Whitney test used to calculate P value.



## Excluded patient on strong CYP3A inducer

One patient randomized to ticagrelor 60mg bd was subsequently found to have been taking a strong CYP3A inducer throughout the study and was, therefore, included in error. Their pharmacodynamic and pharmacokinetic data were excluded from the main analyses to avoid misleading comparison of the groups. It was confirmed that no other patients in the study received excluded medication. The patient was informed of this error and agreed for their individual data to be presented anonymously in view of the scientific interest. After ticagrelor 180-mg loading dose, their VerifyNow P2Y12 assay showed a PRU value of 220 and percentage inhibition of 21%. At one month pre- and post-maintenance dose, these values were 223 PRU and 4% pre-dose and 208 PRU and 21% post-dose, respectively. LTA results were consistent with these values, 61% following the standard loading dose, 73.5% and 60% pre- and post-maintenance dose, respectively, at one month. Corresponding to these low levels of platelet P2Y12 inhibition, plasma levels of ticagrelor and AR-C124910XX were also low, indicating ultra-rapid metabolism of ticagrelor and its active metabolite: Following the ticagrelor loading dose, levels were 55 ng/mL and 105 ng/mL, respectively; pre-maintenance dose at 1 month, the levels were 7.5 ng/mL and 36.4 ng/mL, respectively, and post-maintenance dose the levels were 18.6 ng/mL and 60 ng/mL, respectively. Consequently, it was demonstrated that the plasma levels of ticagrelor and AR-C124910XX were considerably lower than the mean levels seen in the remainder of the corresponding ticagrelor group cohorts.

## Discussion

In STEEL PCI, we compared the pharmacodynamic effects of ticagrelor and clopidogrel and obtained data on the 60mg bd dose of ticagrelor for the first time in stable CAD patients undergoing PCI. Following a standard loading dose of ticagrelor, as expected there were comparable levels of plasma ticagrelor and AR-C124910XX between the two ticagrelor groups. At 1 month, the levels of plasma ticagrelor and AR-C124910XX between the ticagrelor 60mg and 90mg groups were not statistically different pre-maintenance dose, no doubt reflecting a statistical limitation of the sample sizes. However, post-maintenance dose, the plasma ticagrelor and AR-C124910XX levels were significantly higher in the ticagrelor 90mg group, as expected. Interestingly, the lower plasma levels of ticagrelor and AR-C124910XX seen in the ticagrelor 60mg group at one month following a maintenance dose did not translate into lower levels of platelet inhibition when this was assessed by the VerifyNow assay and LTA (Chapter 5) (Figure 5 in appendix). This likely reflects the potency of ticagrelor, such that plasma levels achieved by ticagrelor 60mg twice daily are probably able to saturate the platelet P2Y12 receptors leading to a ceiling effect in the level of inhibition of platelet reactivity.

The patient who was excluded from the main study analyses as a result of incorrect inclusion, due to co-medication with a strong CYP3A inducer, provided important insights into the potential hazard of combining ticagrelor with such a medication. The post loading dose ticagrelor level was 55 ng/mL compared to a mean level of 1109 ng/mL in the remainder of the ticagrelor-treated patients. This demonstrates the key role of CYP3A in metabolising ticagrelor and shows how rapidly ticagrelor is metabolized under the influence of a strong CYP3A inducer. On the other hand, the post loading dose AR-C124910XX level was less dramatically affected, being 105 ng/mL vs a mean level of 223 ng/mL in the remainder of the ticagrelor-treated patients. This likely reflects the timing of the sampling early after absorption of the ticagrelor loading dose: both ticagrelor and AR-C124910XX are metabolized by CYP3A enzymes but the first step is for ticagrelor to be converted to AR-C124910XX so sampling early after absorption of the ticagrelor might show a greater impact of CYP3A induction on ticagrelor level compared to AR-C124910XX. On the other hand, levels of ticagrelor and AR-C124910XX pre- and post-maintenance dose at 1 month also showed more dramatic impact on the ticagrelor levels in this patient, raising the question as to whether ticagrelor metabolism might be more dependent on CYP3A than AR-C124910XX metabolism. In any case, these findings illustrate the importance of careful medication review and checking relevant CYP3A-mediated drug interactions when using ticagrelor and avoiding the use of ticagrelor in patients receiving strong CYP3A inducers, particularly since we also documented high platelet reactivity in this patient, which might have exposed the patient to higher risk of stent thrombosis in a higher-risk setting than elective PCI for stable CAD.

# CHAPTER 7: PHARMACOGENOMIC STUDIES OF CLOPIDOGREL AND TICAGRELOR IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE UNDERGOING ELECTIVE PERCUTANEOUS CORONARY INTERVENTION



## Introduction

Clopidogrel is a second-generation thienopyridine that, when converted to its active metabolite, binds irreversibly to the platelet P2Y12 receptor. The pharmacodynamic efficacy of clopidogrel is limited in some individuals due to poor efficiency of active metabolite formation. This is partly due to genetic variation in the activity of key cytochrome P450 (CYP) enzymes mediating this (71, 212). The CYP2C19 enzyme plays a major role in the two-step metabolic process in the liver that converts clopidogrel, via an intermediate metabolite, to its active form and this has been demonstrated by the significant reduction of clopidogrel’s pharmacodynamic effect in the presence of CYP2C19 inhibitors (213). Poor pharmacodynamic response or loss-of-function (LOF) alleles in *CYP2C19* (designated \*2 to \*8) have been associated with increased risk of stent thrombosis and other thrombotic events in clopidogrel-treated patients (69, 214). Some studies have suggested that a gain-of-function (GOF) allele in *CYP2C19* (designated \*17) increases the pharmacodynamic response to clopidogrel, associated with increased bleeding risk, but these findings have not been supported in other studies.

Since ticagrelor is not a pro-drug, it is not dependent on metabolism for its antiplatelet activity but its metabolite AR-C124910XX has similar potency of inhibition of the P2Y12 receptor. Ticagrelor is metabolised in the liver via CYP3A4 and CYP3A5 to produce active and inactive metabolites, explaining why co-administration of strong CYP3A4/5 inhibitors should be avoided. Genetic variants affecting ticagrelor and AR-C124910XX levels were assessed in a genome-wide association study in ACS patients participating in the PLATO study (75). This study identified three genetic loci that were associated with plasma ticagrelor and/or AR-C124910XX levels, namely *SLCO1B1*, *UGT2B7*, and *CYP3A4*. A single nucleotide polymorphism in *SLCO1B1* that was significantly associated with ticagrelor and AR-C124910XX levels, designated rs4149056, had a minor allele frequency of 17-18% whereas the variants in the other genes were uncommon. However, these genetic variants that significantly affected ticagrelor pharmacokinetics had only weak effects on ticagrelor and AR-C124910XX levels as well as no detectable impact on efficacy or safety of ticagrelor in ACS patients (75).

In STEEL PCI, genetic analyses were performed in stable CAD patients with the aims of, firstly, comparing the pharmacodynamic effects of clopidogrel and ticagrelor in those with and without genetically-predicted impairment of CYP2C19 activity and, secondly, confirming the results of previous pharmacogenomic analyses of ticagrelor and AR-C124910XX. DNA was extracted from whole blood and analysed for the relevant genetic variants of *CYP2C19*, *CY3A43*, *UGT2B7* and *SLC01B1*.

## Genetic analysis methods

DNA was extracted from whole blood samples using Chemagen Chemagic 10k kits (Perkin Elmer, Baesweiler, Germany) followed by elution in Tris-EDTA buffer. The DNA was quantified using Quantifluor® dsDNA System (Promega, Madison, WI, USA). Genotyping was done using Taqman assays (Applied Biosystems, Life Technologies, Pleasanton, CA, USA) using an Applied Biosystems 7900HT Real-Time PCR System. The alleles genotyped included: *CYP2C19* loss-of-function alleles \*2 (rs4244285), \*3 (rs4986893), \*4 (rs28399504), \*5 (rs56337013), \*6 (rs72552267) \*7 (rs72558186) and \*8 (rs41291556); *CYP2C19* gain-of-function allele \*17 (rs12248560); *CYP3A43* (rs62471956); *UGT2B7* (glucuronosyl transferase family 2 member B7) (rs61361928); and *SLC01B1* (solute carrier organic anion transporter family member 1B1 (rs4149056).

## Genetic analyses of *CYP2C19*

After a standard loading regimen of clopidogrel or 180-mg loading dose of ticagrelor, the VerifyNow results showed numerically higher platelet reactivity in clopidogrel-treated patients carrying a LOF allele for *CYP2C19* (200 ± 72 PRU) versus those without a LOF allele (166 ± 66 PRU) (Table 1), consistent with previous studies, but this study was not powered to assess statistical significance for this comparison and sought only to assess any effects of *CYP2C19* variants on the comparisons between clopidogrel and ticagrelor. The presence or absence of a LOF *CYP2C19* allele did not influence the relationship between clopidogrel and ticagrelor: even in those patients without a LOF allele, the pharmacodynamic response was greater, with more consistent platelet inhibition, with ticagrelor compared to clopidogrel loading regimens (all P< 0.001; Table 7-1).

There were similar findings following one month of maintenance therapy with numerical trends consistent with higher platelet reactivity in clopidogrel-treated patients carrying a LOF *CYP2C19* allele, versus those without, but no relevant impact on the comparison between clopidogrel and either dose of ticagrelor: each maintenance dose of ticagrelor was associated with significantly greater platelet inhibition, both pre- and post-dose, than clopidogrel in patients with or without a LOF *CYP2C19* allele (Table 7-2).

There were weak numerical trends suggesting greater platelet inhibition by clopidogrel in carriers of a GOF *CYP2C19* allele, consistent with some previous reports, but this had no relevant impact on the comparison between the effects of either loading regimens or maintenance therapy of clopidogrel compared with ticagrelor: a loading dose of ticagrelor and each maintenance regimen of ticagrelor achieved significantly greater and more consistent platelet inhibition than clopidogrel loading and maintenance regimens regardless of GOF *CYP2C19* allele status (Tables 7-3 and 7-4).

**Table 7‑1**: VerifyNow P2Y12 results following standard loading regimens of clopidogrel or ticagrelor at the time of PCI according to CYP2C19 loss-of-function allele carrier status

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *CYP2C19* genotype status | Clopidogrel | |  | Ticagrelor | | P value |
|  | n | Mean ± SD |  | n | Mean ± SD |  |
| LOF |  |  |  |  |  |  |
| % inhibition | 18 | 17 ± 24 |  | 31 | 83 ± 24 | <0.001 |
| PRU | 18 | 200 ± 72 |  | 31 | 35 ± 49 | <0.001 |
|  |  |  |  |  |  |  |
| No LOF |  |  |  |  |  |  |
| % inhibition | 41 | 32 ± 25 |  | 79 | 87 ± 16 | <0.001 |
| PRU | 41 | 166 ± 66 |  | 79 | 30 ± 33 | <0.001 |

LOF: *CYP2C19* loss-of-function allele carrier. PRU: P2Y12 reaction units.

**Table 7‑2**: VerifyNow P2Y12 results following one month of clopidogrel or ticagrelor according to CYP2C19 carrier status

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| VerifyNow P2Y12 assay | Clopidogrel | | Ticagrelor 60mg | | Ticagrelor 90mg | | P value Clop vs | P value Clop vs | P value T60mg vs |
|  | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | T60mg | T90mg | T90mg |
| LOF |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 16 | 187 ± 47 | 16 | 77 ± 67 | 8 | 22 ±18 | 0.0003 | 0.0001 | 0.023 |
| PRU Post dose | 16 | 176 ± 60 | 16 | 35 ± 25 | 8 | 8 ± 3 | <0.0001 | <0.0001 | 0.003 |
| % inhibition  Pre dose | 16 | 19 ± 19 | 16 | 67 ± 27 | 8 | 90 ± 9 | 0.0002 | <0.0001 | 0.038 |
| % inhibition  Post dose | 16 | 22 ± 24 | 16 | 86 ± 10 | 8 | 97 ±1 | <0.0001 | <0.0001 | 0.003 |
|  |  |  |  |  |  |  |  |  |  |
| No LOF |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 34 | 178 ± 43 | 34 | 55 ± 34 | 36 | 42 ± 39 | <0.0001 | <0.0001 | 0.11 |
| PRU Post dose | 36 | 155 ± 49 | 35 | 32 ± 32 | 39 | 27 ± 22 | <0.0001 | <0.0001 | 0.64 |
| % inhibition  Pre dose | 34 | 22 ± 17 | 34 | 75 ± 15 | 36 | 82 ± 17 | <0.0001 | <0.0001 | 0.03 |
| % inhibition  Post dose | 36 | 37 ± 20 | 35 | 86 ± 15 | 39 | 89 ± 10 | <0.0001 | <0.0001 | 0.56 |

LOF: CYP2C19 loss-of-function allele carrier. PRU: P2Y12 reaction units.

**Table 7‑3**: VerifyNow P2Y12 results following standard loading regimens of

clopidogrel or ticagrelor at the time of PCI according to CYP2C19 gain of function allele carrier status

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| VerifyNow results according to CYP2C19 genotype | Clopidogrel | |  | Ticagrelor | | p value |
|  | n | Mean ± SD |  | n | Mean ± SD |  |
| GOF |  |  |  |  |  |  |
| % inhibition | 18 | 30 ± 23 |  | 34 | 86 ± 19 | <0.0001 |
| PRU | 18 | 164 ± 60 |  | 34 | 29 ± 36 | <0.0001 |
|  |  |  |  |  |  |  |
| No GOF |  |  |  |  |  |  |
| % inhibition | 41 | 26 ± 26 |  | 76 | 85 ± 19 | <0.0001 |
| PRU | 41 | 181 ± 73 |  | 76 | 33 ± 39 | <0.0001 |

**Table 7‑4**: VerifyNow P2Y12assay results following one month of clopidogrel or ticagrelor according to CYP2C19 gain-of-function carrier status

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| VerifyNow P2Y12 assay | Clopidogrel | | Ticagrelor 60mg | | Ticagrelor 90mg | | P value Clop vs T60mg | P value Clop vs T90mg | P value T60mg vs T90mg |
|  | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD |  |  |  |
| GOF |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 15 | 170 ± 50 | 12 | 61 ± 39 | 16 | 47 ± 47 | <0.0001 | <0.0001 | 0.3067 |
| PRU Post dose | 16 | 148 ± 58 | 12 | 35 ± 41 | 17 | 28 ± 26 | <0.0001 | <0.0001 | 0.7027 |
| % inhibition | 15 | 19 ± 19 | 12 | 67 ± 27 | 16 | 90 ± 9 | <0.0001 | <0.0001 | 0.4419 |
| Pre dose |
| % inhibition | 16 | 37 ± 23 | 12 | 85 ± 19 | 17 | 88 ± 11 | <0.0001 | <0.0001 | 0.8525 |
| Post dose |
|  |  |  |  |  |  |  |  |  |  |
| No GOF |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 35 | 185 ± 41 | 38 | 62 ± 51 | 28 | 34 ± 29 | <0.0001 | <0.0001 | 0.0119 |
| PRU Post dose | 37 | 163 ± 57 | 39 | 33 ± 26 | 30 | 21 ± 19 | <0.0001 | <0.0001 | 0.0659 |
| % inhibition | 35 | 21 ± 17 | 38 | 73 ± 21 | 28 | 85 ± 13 | <0.0001 | <0.0001 | 0.0026 |
| Pre dose |
| % inhibition | 37 | 30 ± 22 | 39 | 86 ± 11 | 30 | 91 ± 18 | <0.0001 | <0.0001 | 0.04 |
| Post dose |

## Analyses of genetic variants associated with ticagrelor pharmacokinetics

Out of the ticagrelor-treated patients with available genetic data, there were 26 carriers of the minor *SLC01B1* allele rs4149056 versus 84 patients with the major allele in the ticagrelor groups combined. There was no consistent effect of this *SLC01B1* variant on plasma ticagrelor or AR-C124910XX levels after a 180-mg ticagrelor loading dose (Table7- 5) or after 1 month treatment with ticagrelor 90mg or 60mg bd (Table 7-6). Corresponding to this lack of significant effect on ticagrelor pharmacokinetics, there was no effect of the rs4149056 variant on platelet reactivity either following the ticagrelor loading dose (Table 7-7) or after 1 month of maintenance therapy with either ticagrelor 90mg or 60mg bd (Table 7-8). None of the patients in STEEL PCI carried the rare alleles for *CYP3A43* (rs62471956) or *UGT2B7* (rs61361928).

**Table 7‑5**: Plasma ticagrelor and AR-C124910XX results at time of PCI according to SLC01B1 carrier status

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Carrier | | Non-carrier | | P |
|  | n | Mean ± SD | n | Mean ± SD | value |
|  |  |  |  |  |  |
| Ticagrelor (ng/mL) | 26 | 1126 ± 658 | 84 | 1097 ± 516 | 0.976 |
| AR-C124910XX (ng/mL) | 26 | 223 ± 153 | 84 | 218 ± 117 | 0.687 |

**Table 7‑6**: Plasma ticagrelor and AR-C124910XX results at one month according to SLC01B1 carrier status

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Carrier | | Non-carrier | | P |
|  | n | Mean ± SD | n | Mean ± SD | value |
| Ticagrelor (ng/mL) |  |  |  |  |  |
| T60 pre dose | 14 | 277 ± 152 | 36 | 280 ± 242 | 0.627 |
| T60 post dose | 14 | 521 ± 318 | 34 | 498 ± 272 | 0.912 |
| T90 pre dose | 10 | 342 ± 204 | 37 | 375 ± 187 | 0.626 |
| T90 post dose | 10 | 813 ± 433 | 37 | 764 ± 332 | 0.715 |
|  |  |  |  |  |  |
| AR-C124910XX (ng/mL) |  |  |  |  |  |
| T60 pre dose | 14 | 94 ± 47 | 36 | 95 ± 60 | 0.737 |
| T60 post dose | 14 | 129 ± 59 | 34 | 130 ± 80 | 0.665 |
| T90 pre dose | 10 | 126 ± 51 | 37 | 132 ± 73 | 0.576 |
| T90 post dose | 10 | 208 ± 84 | 37 | 198 ± 101 | 0.461 |

T60: Ticagrelor 60mg group; T90: Ticagrelor 90mg group

**Table 7‑7**: VerifyNow P2Y12 assay results following standard loading regimens of clopidogrel or ticagrelor at the time of PCI according to SLC01B1 genotype carrier status

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| VerifyNow results according to | Clopidogrel | |  | Ticagrelor | | p value |
| SLC01B1 genotype |  |  |  |  |  |  |
|  | n | Mean ± SD |  | n | Mean ± SD |  |
| Carrier |  |  |  |  |  |  |
| % inhibition | 15 | 22 ± 22 |  | 25 | 86 ± 20 | <0.0001 |
| PRU | 15 | 191 ± 53 |  | 25 | 37 ± 56 | <0.0001 |
|  |  |  |  |  |  |  |
| Non-carrier |  |  |  |  |  |  |
| % inhibition | 44 | 29 ± 26 |  | 84 | 96 ± 16 | <0.0001 |
| PRU | 44 | 171 ± 74 |  | 84 | 30 ± 31 | <0.0001 |

**Table 7‑8**: VerifyNow P2Y12 assay results following one month of clopidogrel or ticagrelor according to SLC01B1 genotype carrier status

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SLC01B1 genotype | Clopidogrel | | Ticagrelor 60mg | | Ticagrelor 90mg | | P value  Clop vs T60mg | P Value Clop vs T90mg | P value T60mg vs T90mg |
|  | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD |  |  |  |
| Carrier |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 14 | 193 ± 31 | 14 | 39 ± 21 | 10 | 45 ± 54 | <0.0001 | <0.0001 | 0.594 |
| PRU Post dose | 10 | 172 ± 37 | 14 | 23 ± 19 | 10 | 22 ± 23 | <0.0001 | <0.0001 | 0.965 |
| % inhibition | 14 | 20 ± 17 | 14 | 83 ± 9 | 10 | 79 ± 26 | <0.0001 | <0.0001 | 0.635 |
| Pre dose |
| % inhibition | 10 | 32 ± 18 | 14 | 91 ± 8 | 10 | 90 ± 10 | <0.0001 | <0.0001 | 0.784 |
| Post dose |
|  |  |  |  |  |  |  |  |  |  |
| Non-carrier |  |  |  |  |  |  |  |  |  |
| PRU Pre dose | 36 | 176 ± 48 | 36 | 71 ± 52 | 34 | 37 ± 30 | <0.0001 | <0.0001 | 0.0023 |
| PRU Post dose | 39 | 159 ± 58 | 37 | 37 ± 32 | 37 | 24 ± 22 | <0.0001 | <0.0001 | 0.0622 |
| % inhibition | 36 | 22 ± 18 | 36 | 69 ± 22 | 34 | 85 ± 13 | <0.0001 | <0.0001 | 0.0006 |
| Pre dose |
| % inhibition | 39 | 33 ± 24 | 37 | 84 ± 15 | 37 | 90 ± 9 | <0.0001 | <0.0001 | 0.0681 |
| Post dose |

## Discussion

Genetic variation in the activity of key CYP enzymes partly explains limited efficacy of clopidogrel in some individuals, including loss-of-function alleles in *CYP2C19* that have been associated with reduced clopidogrel active metabolite formation and increased risk of stent thrombosis (69, 71, 212, 214, 215). In STEEL PCI, we compared the effects of relevant genetic polymorphisms in *CYP2C19* on ticagrelor and clopidogrel pharmacodynamic efficacy. Following standard loading regimens or maintenance doses of clopidogrel or ticagrelor, the presence or absence of LOF or GOF alleles had no effect on the comparison between the ticagrelor and clopidogrel groups in terms of platelet inhibition assessed by VerifyNow. Our results demonstrate a wide variation in PRU for those patients in the study, with both high and low responders seen within the study population. Both the studied regimens of ticagrelor achieved greater and generally more consistent platelet inhibition than clopidogrel, even in patients who did not carry a LOF *CYP2C19* allele. The LOF *CYP2C19*\*2 allele is found in 25-35% of the Caucasian population and 55-70% of the Asian population, with other LOF alleles being rare, and so, not surprisingly, only a small number of LOF allele carriers were found in STEEL PCI (62). Whilst clopidogrel non-responsiveness is clinically relevant and related to recurrent ischaemic events, including stent thrombosis, we did not see any cases of stent thrombosis in STEEL PCI. As discussed earlier, this is likely due, at least in part, to advances in stent design and manufacture and procedural optimization techniques.

The largest single study to assess the influence of *CYP2C19* genotype on clinical outcomes was the PLATO genetic substudy (69). The PLATO substudy findings were also confirmed by the smaller TRITON-TIMI 38 trial that also demonstrated a higher incidence of the primary endpoint in clopidogrel-treated LOF allele carriers (70). However, these studies looked at patients with ACS and not patients with stable CAD.

As expected, there was no influence of *CYP2C19* genotype in the ticagrelor groups, either after a loading dose or during maintenance treatment. The results of STEEL PCI are concordant with the ONSET/OFFSET and RESPOND Genotype study that investigated the effect of *CYP2C19* status on platelet reactivity in stable CAD patients taking clopidogrel or ticagrelor (71). This study also demonstrated that ticagrelor provided greater platelet inhibition than clopidogrel, even in those patients that were GOF allele carriers.

Genetic variants that have been shown to affect ticagrelor and AR-C124910XX levels were studied but only the variant of *SLCO1B1* (rs4149056) was found in the STEEL PCI population whereas the variants of *UGT2B7* and *CYP3A4* were not detected, consistent with their rarity and the relatively small sample sizes of the ticagrelor groups. Whereas Varenhorst et al demonstrated modestly higher ticagrelor and AR-C124910XX levels in carriers of the minor allele of rs4149056, we did not detect any such effect (75). However, the STEEL PCI study was limited by insufficient sample size for conducting genetic analyses compared to that by Varenhorst *et al* in which there were over one-thousand carriers of the minor allele and so only serves to reinforce the message from Varenhorst *et al*’s work that there are no common genetic variants that lead to any clinically-relevant variation in the pharmacodynamic response to ticagrelor.

Overall, the results of the genetic variation in the activity of key CYP enzymes did not influence the relationship between the pharmacodynamic effects of clopidogrel compared with ticagrelor. Following standard loading regimens or maintenance doses of clopidogrel or ticagrelor, the presence or absence of LOF or GOF alleles had no effect on the significant difference in the levels of platelet inhibition assessed by VerifyNow with clopidogrel compared with either regimen of ticagrelor. There were no incidences of recurrent ischaemic events or ST in any group but particularly no events in the clopidogrel group. Therefore, in this setting of patients with stable angina undergoing elective PCI, there is currently no indication for screening of LOF carriers and subsequent treatment with ticagrelor, which would lead to higher levels of platelet inhibition.

# CHAPTER 8: DISCUSSION

Aspirin and clopidogrel have been the mainstay of dual anti-platelet treatment facilitating PCI for many years. The variability in response to clopidogrel is well recognized as a potential risk for stent thrombosis with genetic factors playing an important role. Predictably, STEEL PCI confirms the variable response to clopidogrel with poor response to clopidogrel being common. The results of STEEL PCI also demonstrate that ticagrelor, including the new lower dose of 60mg twice daily, provides higher P2Y12 inhibition compared to clopidogrel. Of course, STEEL PCI was a relatively small study of patients undergoing elective PCI, with only 60 patients recruited to each of the three anti-platelet regimens. However, the results of the PFT used are similar to those seen in other larger studies.

In combination with data from PEGASUS TIMI 54, in patients with prior MI, these data reinforce the major advantage of ticagrelor in terms of platelet inhibition at maintenance doses of 60mg or 90mg twice daily. It is notable that there were no thrombotic events in the clopidogrel group despite evidence of poor response to clopidogrel treatment. The STEEL PCI study was not powered to look at clinical outcomes and these data only hint at what has been recognised in registries. Despite evidence of poor response to clopidogrel, as a consequence of new stent design and implantation techniques the rates of stent thrombosis are very low in modern practice. Consequently, despite the pharmacodynamic and pharmacokinetic advantages of ticagrelor over clopidogrel, the routine use of ticagrelor in patients undergoing PCI for stable CAD cannot be supported without further clinical trials to demonstrate favourable efficacy. However, the very low rates of thrombotic events in stable CAD treated with PCI means it is almost impossible to sufficiently power such a study for thrombotic events.

The current ESC guidelines have taken a pragmatic approach to this conundrum by supporting the use of ticagrelor (or prasugrel) compared to clopidogrel in patients in whom there is particular concern about stent thrombosis risk, such as those patients undergoing PCI for LMS disease or those who have a history of stent thrombosis on clopidogrel (216) (141). This guidance was given a class II level C recommendation and was based on expert consensus rather than clinical trials.

It may be that the routine use of ticagrelor, perhaps at the lower dose of 60mg twice daily, would be appropriate for some patients, such as those with DM or multi-vessel disease. However, further studies looking at longer-term outcomes in these populations in the setting of stable CAD would be required. Of particular interest would be the prolonged use of lower-dose ticagrelor in this setting. Whilst data are available for patients taking DAPT with ticagrelor 60mg bd for up to three years following previous PCI for an ACS, no data in stable CAD are currently available. A larger, long-term study to assess the rates of target vessel revascularisation and ischaemic events in the stable CAD would be needed.

The STEEL PCI data supports the use of ticagrelor 60mg bd as an alternative to ticagrelor 90mg bd when used in such an unlicensed indication, particularly as dyspnoea was less common with the lower dose of ticagrelor. Use of the lower ticagrelor dose may be advantageous when introducing ticagrelor therapy for the first time in low risk patients.

One study participant was excluded from the main analyses as they were subsequently found to be taking a strong inducer of CYP3A4 and so were included in the study in error since this was an exclusion criterion. This patient was randomised to ticagrelor 60mg bd. After a standard ticagrelor 180mg loading dose, and at one month pre and post maintenance dose, the results of the PFTs demonstrated low levels of platelet P2Y12 inhibition. Corresponding to these low levels of platelet P2Y12 inhibition, plasma levels of ticagrelor and its active metabolite ARC124910XX were also low, indicating ultra-rapid metabolism of ticagrelor and its active metabolite. These findings illustrate the importance of checking patients’ concomitant medication and avoiding the use of ticagrelor in patients receiving strong CYP3A inducers.

The PLATO study raised great hopes for the pleiotropic effects of ticagrelor given the great magnitude in reduction of ischaemic events when clopidogrel was compared to ticagrelor (45). In particular, cause of death analysis showed a reduction in sudden cardiac death as well as death due to infection with ticagrelor versus clopidogrel which stimulated reverse transitional work to assess potential mechanisms unrelated to P2Y12 inhibition. *In vitro* studies clearly indicated that ticagrelor was an antagonist of ENT-1 preventing cellular re-uptake of adenosine from plasma. However, ticagrelor is a weak antagonist in contrast to the licensed drug dipyridamole, which is a potent ENT-1 antagonist. Consequently, there has been some doubt whether therapeutic doses of ticagrelor achieve sufficient levels of ticagrelor and its active metabolite to impact on ENT-1 function. Initial preliminary studies by Bonello *et al* suggested that therapeutic doses of ticagrelor do significantly impair ENT-1 function leading to higher circulating levels of adenosine. This was reinforced by observations from studies where ticagrelor was co-administered with intravenous adenosine demonstrating increased levels of dyspnoea with ticagrelor compared to intravenous adenosine alone (92).

The STEEL PCI data refute the suggestion that therapeutic levels of ticagrelor and AR-C124910XX, the active metabolite, are sufficient to antagonise ENT-1 to a sufficient extent to impede cellular adenosine reuptake. Along with this, we saw no impact on plasma adenosine concentration at any time point, either following a standard loading dose or after one month of maintenance therapy. The WB adenosine reuptake assay demonstrated the greatest reduction in residual adenosine concentration occurred between 0 and 15 seconds. This was expected since circulating adenosine is rapidly taken up by erythrocytes and other cells in whole blood. Reassuringly the residual adenosine concentration in the whole-blood uptake assay at 0 seconds was consistently around 1μmol/L, reflecting accurate measurement of the added concentration of adenosine in addition to the much lower concentrations found in circulating blood. These findings also suggests that the stop solution was highly effective in preventing metabolism of the added adenosine.

The reasons for these apparently negative results are unclear. The data clearly show that adenosine uptake over 1 minute in WB samples was assessed accurately. The WB assay results also demonstrated almost complete adenosine uptake at 1 minute, which indicates efficacy of the stop solution in preventing further adenosine generation. One possible hypothesis for the difference in results seen in the two studies may be due to the different composition of pharmacological stop solution utilized, with Bonello et al potentially using less effective stop solution although the details are unclear in their paper. Despite the possible differences in composition of the stop solution compared to the Bonello *et al* study, a more recent study was also in agreement with our findings (206). Another study, on a different cohort of patients but using the same methodology as STEEL PCI, also demonstrated no impact of ticagrelor on plasma adenosine concentration in patients with acute coronary syndrome who were awaiting coronary artery bypass graft surgery (207). Further work would be required to explore whether these differences in stop solution may provide adequate explanation for the conflicting study findings. Whether more subtle effects of ticagrelor on adenosine metabolism could be detected by other assays remains to be explored. Whilst data remain inconclusive, our findings suggest that adenosine is less relevant to ticagrelor’s clinical effects and make it more likely that the benefits seen with ticagrelor relate simply to improved P2Y12 platelet inhibition. One important limitation of the STEEL PCI study is that it only included patients with stable CAD and we have not looked at ticagrelor on adenosine uptake in ACS patients. However, our preliminary data in ACS patients awaiting CABG surgery and treated with ticagrelor, as previously discussed, also showed no impact of ticagrelor on adenosine uptake (207).

Although the mechanism for ticagrelor-induced dyspnoea has been strongly suggested to relate to ENT-1 antagonism, it has also been noted that cangrelor causes the same type of dyspnoea but has no impact on adenosine uptake. This helps to support the current hypothesis that ticagrelor does not impact on adenosine metabolism sufficiently to have any clinical adverse effects. However, current studies, including STEEL PCI, leave uncertainty on the mechanism of dyspnoea in ticagrelor treated patients and further work is still required to address this.

One limitation of ticagrelor’s use is its effect on haemostasis. Ticagrelor’s efficacy in cases of previous stent thrombosis and MI comes with an important disadvantage when used in addition to aspirin. Certainly, bleeding risk can be reduced by giving ticagrelor as monotherapy without aspirin. However, the Global Leaders study suggests ticagrelor monotherapy is not superior to aspirin monotherapy in long-term treatment following PCI, whereas adding ticagrelor to aspirin does have proven benefits in terms of decreasing ischaemic events despite increased risk of bleeding. Further work will address whether the duration of DAPT can be shortened compared to the current practice of using DAPT for six months in stable patients with CAD undergoing PCI. In this regard, the Global Leaders data were encouraging, suggesting that ticagrelor monotherapy for one month after PCI was adequate. However, whether it is safe to consider aspirin monotherapy after one month is uncertain.

In view of the bleeding risk with DAPT, there remains interest in targeting other pathways in patients with CAD. This is more relevant for patients remote from their PCI procedure since DAPT is well established for covering the period of stent thrombosis risk. For example, the COMPASS study used the factor-Xa inhibitor rivaroxaban 2.5mg bd with aspirin in patients at increased risk of ischaemic events, including those with stable multi-vessel CAD (217). In this study, the combination of aspirin and rivaroxaban proved more efficacious than aspirin alone in preventing ischaemic events. The combination was associated with increased bleeding but the relative risk was somewhat less than observed with aspirin and ticagrelor versus aspirin alone. However, there were numerical trends suggesting more haemorrhagic CVA with aspirin and rivaroxaban, which led to caution. The search for alternative targets that allow prevention of arterial thrombotic events without major effects on haemostasis is on-going.

The role of antiplatelet therapy to facilitate PCI in both the setting of elective stable CAD and in the treatment of ACS will continue to be explored. The balance of the requirement of a high level of platelet P2Y12 inhibition against the risk of bleeding events will drive further research looking at lower-dose ticagrelor at different durations. Tailored antiplatelet therapy in certain high-risk groups, such as those patients with DM, will also need further exploration and will provide the basis for further studies outside the scope of STEEL PCI.

**Appendix**

Figure 1: Individual VerifyNow P2Y12 assay results expressed as VerifyNow percentage inhibition following a standard loading regimen of clopidogrel or 180mg loading dose of ticagrelor.

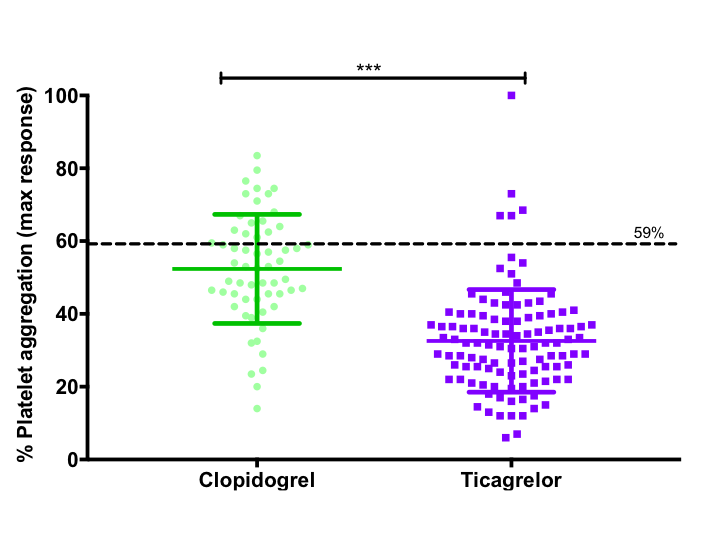


Figure 2. Individual VerifyNow P2Y12 assay results expressed as P2Y12 reaction units following a standard loading regimen of clopidogrel or 180mg loading dose of ticagrelor.



The **dashed line**indicates a level of 208 PRU as a previously-established threshold for high platelet reactivity. **Solid lines with error bars**indicate mean ± SD. \* P < 0.01; \*\*\* P < 0.0001.

Figure 3. Individual results for the platelet aggregation measured by light transmittance aggregometry in response to ADP 20 μmol/L following a standard loading regimen of clopidogrel or 180mg loading dose of ticagrelor.



The dashed lines indicate a level of 59% as a threshold value for high platelet reactivity. Solid lines with error bars indicate mean ± SD. \*\*\* P < 0.0001.

Figure 4. Individual results for plasma concentrations of ticagrelor and active metabolite AR-C124910XX following a standard loading dose of ticagrelor. Solid lines with error bars indicate mean ± SD.



Figure 5. Individual results for plasma concentrations of ticagrelor 60mg and PRU following a maintenance loading dose of ticagrelor.



PLATO Defined major and Minor Bleeding

Major Bleeding

Fatal bleeding

Intrapericardial bleeding with cardiac tamponade

Intracranial bleeding

Severe hypotension, or hypovolemic shock due to bleeding and requiring either vasopressors or surgical intervention

A drop in haemoglobin of 5.0 g/dL or more or the need for transfusion of four or more units of packed red blood cells

An event that led to clinically significant disability or bleeding with an associated drop in haemoglobin of at least 3.0 g/dL but <5.0 g/dL or requiring a 2–3 unit red blood cell transfusion.

Minor bleeding

Any bleeding event requiring medical intervention but not meeting the criteria for major bleeding.

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